

Lactational oestrus in sows

Follicle growth, hormone profiles and early pregnancy in sows
subjected to Intermittent Suckling

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Abstract

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Weaning of piglets at a relatively young age (3 to 4 weeks) can compromise health and welfare. A possible way to increase piglet welfare is to extend lactation length, but this is economically undesirable due to lactational anoestrus of the sow. Thus, an extension of lactation would reduce the number of litters per sow per year. Intermittent Suckling (IS), a management system in which the lactating sow is separated from her litter for a fixed period of the day, is proposed as method to extend lactation length without compromising sow reproductive performance. The aims of this thesis were to study if by application of IS, lactational oestrus and ovulation could be induced in a large proportion of the sows and to examine the quality of such a lactational oestrus by studying hormone levels and pregnancy parameters. Within a first study, sows were separated from their piglets for either 12h continuously or at 6h intervals from d14 of lactation onwards. In a control group, weaning occurred at d21 of lactation. Lactational oestrus was induced in more than 80% of the sows. The pre-ovulatory LH surge, progesterone (P_4) levels and the number of ovulating sows were negatively affected by IS and embryo development was negatively affected by the regimen of IS (6h). Low P_4 levels have been related to a low embryo survival and one factor known to affect P_4 levels was feeding level. Therefore, the aim of a second study was to examine the effect of the high lactational feeding level of IS sows on P_4 levels. Multiparous sows, subjected to IS daily for 12h continuously, were fed at a high ($\pm 6.5\text{kg/day}$) or low ($\pm 4\text{kg/day}$) feeding level from ovulation to 6 days after ovulation. Results of this study indicated that P_4 levels were not affected by high lactational feeding levels and that P_4 levels were comparable to levels found in the first study. In a third study two other factors, possibly involved in the low P_4 levels, were studied: timing of start of IS and continuance of IS during early pregnancy. Multiparous sows were subjected to IS for 12h continuously per day from d14 or d21 of lactation onwards. Weaning occurred either at ovulation or day 20 after ovulation. An early start of IS (d14) did not significantly affect the pre-ovulatory LH surge, but resulted in lower P_4 levels at d7 after ovulation. Continuance of IS after ovulation resulted in lower P_4 levels after ovulation and also negatively affected embryo development. Thus, lactational factors such as suckling related hormones or the metabolic state of the sow may caused the low P_4 levels in IS sows. In general, a high proportion of IS sows developed cystic ovaries. In a final study, reproductive parameters were examined in sows developing cysts. Sows developing cysts lacked an LH surge. A dysfunction in oestradiol feedback seems the underlying mechanism responsible for the lack of the LH surge and may be related with stress or the metabolic state of IS sows. In conclusion, it is possible by means of IS to induce lactational oestrus and ovulation. The rate of success, however, is dependent on several factors such as the breed of the sow and the timing of start of IS. The quality of lactational oestrus (hormone levels, embryo survival) seems comparable to weaned sows when start of IS is not too early after farrowing and IS is not continued during early pregnancy.

Keywords: sow; Intermittent Suckling; oestrus; lactation; oestradiol; LH; progesterone, embryo survival, embryo development, cystic ovaries.

Voorwoord

Hora est... Na vier jaar hard werken is de tijd gekomen om mijn proefschrift af te ronden en te verdedigen. Een verdediging van een proefschrift betekent een afronding van vier jaar onderzoek waarin je als AIO beoordeeld wordt op je kunnen door de aanwezigen op het podium waaronder kritische opponenten, de co-promotoren en de promotor. Gelukkig kan je als AIO op het moment suprême rekenen op steun uit de zaal maar ook van de (co) promotoren op het podium. Deze steun en assistentie is niet alleen op deze dag aanwezig en belangrijk maar heeft ook een grote rol gespeeld gedurende de vier voorafgaande jaren aan deze verdediging. Je kan immers niet alles alleen. Ik ben daarom ook zeer dankbaar dat er gedurende mijn AIO periode veel mensen zijn geweest die geholpen hebben om dit promotieonderzoek tot een goed einde te brengen. Ik wil iedereen bedanken die op welke manier dan ook heeft bijgedragen aan mijn promotieonderzoek met een aantal personen in het bijzonder.

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Hora est!

Rosemarijn

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I

General introduction

Introduction

Over the years, lactation length in pigs has been reduced. In Europe, it is forbidden by law to have lactation lengths shorter than 21 days, and on many farms average lactation length is not longer. The pig husbandry aims to produce a high number of litters per sow per year and as a sow is normally anoestrus during lactation (e.g. Armstrong et al., 1988), a reduction in lactation length was a way to increase production per sow. Lactation in the modern pig husbandry differs greatly from nature, where weaning is a gradual process and occurs when piglets have fully switched from a milk to a non-milk diet at 8 to 12 weeks of age (Miller and Slade, 2003). In pig husbandry systems, weaning is abrupt and piglets almost instantly have to switch from milk to a non-milk diet. This relatively early and abrupt weaning results in weaning problems reducing piglet welfare, such as a reduced growth and even body weight loss, reduced feed intake and diarrhoea (van Beers-Schreurs et al., 1992). Studies have shown that an extension of lactation length (thus increasing weaning age), increases post-weaning growth, reduces mucosal dysfunction of the intestinal tract (Moeser et al., 2007), and reduces weaning stress (Mason et al., 2003). For the wellbeing of the piglets around weaning, it would be better to extend lactation length, but also to stimulate creep feed intake during lactation. In practice, however, this will not be favourable, because a sow is normally anoestrus during lactation (e.g. Armstrong et al., 1988) and an extension of lactation length will thus reduce the yearly production of the sow. The question is if it is possible to induce lactational oestrus. A possible way to do this is to use Intermittent Suckling or limited nursing. This means that during part of lactation sow and piglets are separated for a certain period of the day. Using this system, creep feed intake was stimulated (Kuller et al., 2004) and a number of sows showed lactation oestrus (Matte et al., 1992; Kuller et al., 2004). The question is whether such a system can be a solution to overcome weaning problems in piglets without compromising sow reproductive performance. To find answers to this question a project was set up to investigate the effects of Intermittent Suckling on piglet and sow performance during lactation. In this thesis the effects of Intermittent Suckling on sow reproductive performance will be studied. Before the aims and outline of this thesis will be described, the next paragraphs will give background information about lactational anoestrus and induction of lactational oestrus in sows.

Lactational anoestrus in the sow

For oestrus and ovulation to occur it is necessary that follicles develop into pre-ovulatory follicles. For development to pre-ovulatory size, follicles need to be selected from the present follicle pool. This is initiated by a change in GnRH/LH pulsatility: immediately at weaning, GnRH/LH pulsatility shifts from a low frequency, high amplitude to a high frequency with low amplitude (Shaw and Foxcroft, 1985). In response, follicles will develop and sows will

show oestrus within 5 days after weaning (Steverink et al., 1999).

During lactation, a sow is considered to be anoestrus. GnRH/LH pulsatility during lactation is low (Cox and Britt, 1982) and consequently follicle development is inhibited and oestrus and ovulation do not occur. Studies have shown that the inhibition of the GnRH/LH pulsatility is related to the suckling stimulus of the piglets (Stevenson et al., 1981; De Rensis et al., 1999b) and the metabolic state of the sow (for review see Prunier et al., 2003).

During suckling, prolactin, oxytocin and endogenous opioids are released and have been examined as potential inhibitors of GnRH/LH pulsatility and thus the development of follicles. Prolactin is a hormone released during teat massage and nuzzling before milk let down and peak levels of prolactin are reached approximately 15 min later (Varley and Foxcroft, 1990). As a result of a high suckling frequency, prolactin levels are chronically elevated during lactation (Varley and Foxcroft, 1990) and therefore prolactin has been put forward as a candidate involved in the mechanism inhibiting LH secretion during lactation. Inhibition of prolactin secretion during lactation, however, did not result in an increase in LH pulse frequency (Bevers et al., 1985). Also, prolactin did not affect LH secretion in the pituitary gland in sheep (Gregory et al., 2004). Thus, prolactin does not seem to be the inhibitor of LH secretion during lactation.

Oxytocin is also suckling-induced and is released before milk let down in response to nuzzling and teat massage (Varley and Foxcroft, 1990). It is currently not clear how oxytocin levels and LH secretion are related (Evans, 1996).

The suckling stimulus does not only result in the release of prolactin and oxytocin, but also endogenous opioid peptides (EOPs) are released (Cox et al., 1988). Infusion of an EOP antagonist (naloxone), increased LH secretion and reduced prolactin secretion during lactation (Armstrong et al., 1988; De Rensis and Foxcroft, 1999). The inhibition of LH secretion early post partum (42 h- 72 h post partum) could not be prevented by infusion of naloxone (De Rensis et al., 1993). Thus, inhibition of LH secretion during early lactation is not related to EOPs, but during established lactation, EOPs suppress LH secretion and increase prolactin secretion.

Also, the metabolic state of the sow is related to the inhibition of LH pulsatility during lactation. Milk production of the sow is high and feed intake is often not sufficient for both milk production and maintenance of the sow resulting in a catabolic state. This catabolic state has been found to suppress LH pulsatility (Zak et al., 1998). Thus, a catabolic state during lactation can affect LH pulsatility and consequently follicle development (for review see Prunier et al., 2003).

As lactation progresses a gradual increase in LH pulsatility is observed (Quesnel and Prunier, 1995), due to a less pronounced inhibition of LH secretion (Edwards and Foxcroft, 1983) and a decrease in suckling frequency (Kirkwood et al., 1984). This slight increase in LH pulsatility can result in follicle growth, but not of pre-ovulatory size as long as LH pulsatility does not shift from low to high frequency and from a high to low amplitude.

Also the response of LH to exogenous GnRH (Bevers et al., 1981; Cox et al., 1988; Sesti and Britt 1993b) and oestradiol benzoate (Elsaesser and Parvizi, 1980; Sesti and Britt 1993a) increase as lactation progresses. Oestradiol benzoate resulted in LH surges during late lactation (day 28), but not during early lactation (day 14), indicating that the positive feedback mechanism of oestradiol and GnRH matures as lactation progresses (Sesti and Britt, 1993b). In most cases, the observed LH surges in response to oestradiol benzoate were not accompanied by ovulation. Thus, the hypothalamus-pituitary axis is capable of responding to oestradiol by releasing GnRH and LH, but follicles have not developed to pre-ovulatory size because of the low LH pulsatility during lactation.

In summary, sows are anoestrus during lactation as a result of inhibition of LH pulsatility by the suckling stimulus of the piglets. As lactation progresses, a slight increase in LH secretion is observed and the hypothalamus-pituitary axis increases in capability to respond to exogenous hormones. Normally, however, no lactational ovulation is observed as follicles do not develop to pre-ovulatory size until LH pulsatility switches from the low frequency, high amplitude observed during lactation to high frequency, low amplitude normally observed at weaning.

Occurrence and induction of lactational oestrus

As described earlier, as lactation progresses sows can sequentially escape from the inhibition of LH pulsatility. Normally, only a slight increase is observed in the frequency of LH pulses as lactation progresses and this increase is much lower than the frequency of LH pulses observed after weaning (Quesnel and Prunier, 1995). Sometimes, however, sows do show LH pulse frequencies comparable to weaned sows and lactational oestrus is observed (e.g. 7% vs. 2% of the sows showed lactational oestrus during a lactation length of 19 days (Soede et al., 1995a; Soede et al., 1995b). In practice the percentage of sows with lactational oestrus has increased over the years, probably related with the reduced weaning-to-oestrus interval (Prunier et al., 1993). There are several factors which may play a role in the occurrence of spontaneous lactational oestrus such as parity, breed, litter size and suckling frequency.

In weaned sows, the interval from weaning to oestrus is longer in primiparous than in multiparous sows (e.g. Vesseur et al., 1994). This delay in return to oestrus in primiparous sows has been related to their metabolic state (for review: Prunier et al., 2003). As described above, the restore of LH pulsatility can be delayed when sows are in a catabolic state (Zak et al., 1998). Primiparous, lactating sows need nutrients for milk production, but also for their own growth as they have not reached a mature body weight (Van den Brand, 2000). Feed intake during lactation in these sows is not sufficient, resulting in a severe catabolic state. In multiparous sows, feed intake during lactation is higher (Britt, 1986) and sows suffer less from this severe catabolic state. In summary, in primiparous sows LH pulsatility is inhibited by suckling and the metabolic state, which is less pronounced in multiparous sows.

Consequently, lactational oestrus is more likely to occur in multiparous sows.

Breed is another factor which may affect the occurrence of lactational oestrus, since the weaning-to-oestrus interval has also been known to differ between breeds (Vesseur et al., 1994). No studies have been performed in comparing different breeds and the occurrence of lactational oestrus within one study, but responses to induction of lactational oestrus differed between studies using different breeds. For example, litter separation for 12 h daily resulted in lactational oestrus in 3% of the Large White* Landrace sows (Henderson and Hughes, 1984) and 50% in Yorkshire* Duroc sows (Stevenson and Davis 1984).

A reduction in litter size from 10 to 5 piglets increased LH pulsatility during lactation (Varley and Foxcroft, 1990). Also, coverage of the three pairs of anterior teats during day 14 to day 21 of lactation increased LH pulsatility and resulted in enhanced follicle growth at day 21 of lactation (Varley and Foxcroft, 1990), indicating that the number of teats used during lactation can affect the inhibition of LH pulsatility. A small litter will most likely affect suckling intensity and also the number of teats used, increasing the chance of an escape from LH inhibition and consequently lactational oestrus. Thus, a small litter size can result in spontaneous lactational oestrus in sows.

Suckling frequency reduces as lactation progresses (Puppe and Tuchscherer, 2000) and subsequently sows sequentially can escape from the suckling inhibition on LH pulsatility. Also a reduction of suckling frequency by preventing suckling for 8 h resulted in an increase in LH pulsatility (Armstrong et al., 1988). It is possible that in for example older sows, suffering less from a catabolic state, a reduction in suckling frequency results in spontaneous lactational oestrus.

The knowledge that a reduction in suckling frequency can result in spontaneous lactational oestrus can be helpful when one wants to induce lactational oestrus. Reducing suckling frequency without reducing the number of suckling piglets can be accomplished by reducing the time per day that the piglets can suckle at the udder. This is called limited nursing or Intermittent Suckling. Intermittent Suckling regimens, in which sows and piglets are separated for a number of hours per day have been studied in the 1970's and 1980's and are summarized in Table 1. Separation of the sow from her litter for an 8 h period immediately increased LH pulsatility and average LH concentrations (Armstrong et al., 1988). When sow and piglets were reunited, LH levels decreased as a result of suckling but remained higher when compared to LH levels during continuous suckling. Thus, separation results in increased LH pulsatility during lactation and continuance of periods of separation for more days can result in lactational oestrus (e.g. Newton et al., 1987a). Nevertheless, the response of sows to daily separation from their litters was highly variable and ranged from 0% (Cole et al., 1972; Thompson et al., 1981; Grinwich and McKay, 1985; Newton et al., 1987b) to 100% (Newton et al., 1987b). The variation in response to daily separation in the different studies can be explained by several factors, such as the timing of initiation of separation relative to parturition, the hours of separation per day and the number of days of separation. For example, initiation of separation early post partum (day 10 of lactation) resulted in a low

percentage of lactational oestrus (3%; Henderson and Hughes, 1984) compared to the response when separation was initiated at day 20 of lactation (100%; Newton et al., 1987b). The number of hours of separation per day also greatly influences the response of sows as 3 h separation resulted in 0% lactational oestrus (Grinwich and McKay, 1985) whilst in the same study with 22 h of separation 73% of the sows showed lactational oestrus. Combinations of different factors can increase the response of sows to daily separation as for example in general the percentage of multiparous sows responding to separation with lactational oestrus is higher (on average 30%) than primiparous sows (on average 18%). The response of primiparous sows, however, is higher (60%) when the number of hours of daily separation is increased to 22 h per day (Grinwich and McKay, 1985).

Another factor, which may stimulate the occurrence of lactational oestrus is boar contact. Boar contact after weaning stimulates the onset of oestrus in sows (Langendijk et al., 2000a) and Boar contact during lactation resulted in a shorter weaning-to-oestrus interval in sows (Walton, 1986). Furthermore, exposure to a boar led to mating during lactation in 48% of sows grouped at day 14 of lactation (Petchey and Jolly, 1979).

In summary, lactational oestrus can be induced by separation of sow and her litter for several hours per day, but the response is highly variable and is related with factors such as timing of initiation and duration of separation.

Establishment of pregnancy in sows with lactational oestrus

Studies have shown that sows can express oestrus during lactation but the question is if ovulation and pregnancy during lactation will result in high pregnancy rates and good embryo survival rates. Studies examining pregnancy parameters in sows with lactational ovulation either during continuous lactation (Gaustad-Aas et al., 2004) or as a result of litter separation are limited (Table 1). Furthermore, in the studies using litter separation and examining pregnancy rates, presented data are often pooled data of sows with lactational ovulation and sows ovulating after weaning in which the majority of the sows ovulated after final weaning (Thompson et al., 1981; Grinwich and McKay, 1985).

Gaustad-Aas et al. (2004) examined farrowing rates in sows with lactational oestrus during continuous lactation and that were inseminated between 8 to 14 days after farrowing. Farrowing rates in sows inseminated during lactational oestrus were comparable to farrowing rates of sows inseminated during a post-weaning oestrus (47% vs. 50%). These results indicate that mating during lactation does not necessarily negatively affects farrowing rates. In both groups, however, farrowing rates were low, probably related with a suboptimal uterine environment caused by the early insemination relative to farrowing (Gaustad-Aas et al., 2004). It is possible that insemination of sows during their lactational oestrus results in low pregnancy rates by factors associated with lactation such as suckling related factors (prolactin,

oxytocin and endogenous opioids) or factors related to the metabolic state of the lactating sow (insulin, IGF-1).

In summary, not many studies have been performed in which the establishment of pregnancy during lactation was examined. There are, however, indications that pregnancy can be established during lactation, but it is unclear how lactation affects embryo survival and embryo development.

Table 1. Overview of methods of Intermittent Suckling or limited nursing used and the percentage of response.

Parity	Start IS (day of lactation)	Number of days of IS	Separation per day (h)	Lactational oestrus (%)	Pregnancy rate (%)	Reference
Primiparous	20	8	6	0	-	Newton et al., 1987b
	21	7	22/ 22.5 ²	0	-	Thompson et al., 1981
	21	12	22	13	63 ¹	Thompson et al., 1981
	21	14	22	60	ND	Grinwich and McKay, 1985
Multiparous	10	25	12	3	100	Henderson and Hughes, 1984
	14	11	12	22	ND	Kuller et al., 2004
	20	8	6	100	ND	Newton et al., 1987b
	20	10	12	0	-	Cole et al., 1972
	21	7	22/22.5 ²	15	77 ¹	Thompson et al., 1981
	21	10	12	20	100	Crighton, 1970
	21	12	22	39	65 ¹	Thompson et al., 1981
	21	14	3	0	63 ¹	Grinwich and McKay, 1985
	21	14	22	73	70 ¹	Grinwich and McKay, 1985
Mixed	13	7	3	65	ND	Newton et al., 1987a
	13	7	6	79	ND	Newton et al., 1987a
	13	7	6	16	ND	Newton et al., 1987a
	14	14	6	65	ND	Stevenson and Davis, 1984
	14	14	12	50	ND	Stevenson and Davis, 1984

¹The number of pregnant sows is based on all inseminated sows; not only sows with lactational oestrus. ² Piglets were limited to 3 or 4 nursings per day. ND = not determined.

Aims of this thesis

For Intermittent Suckling (IS) to be a successful management system from the sow reproductive point of view, several criteria must be met: (1) sows need to respond to IS by showing normal follicle growth, and ovulation with limited variation in timing, (2) sows should show oestrous behaviour, (3) sows should become pregnant without compromising subsequent litter size. As described above, the percentage of sows responding to litter separation by showing lactational oestrus is highly variable (see Table 1). These studies date back to the 1970's and 1980's in which the average weaning-to-oestrus interval was 18.7 days (Fahmy et al., 1979). Over the years sows have been selected for a shorter weaning-to-oestrus interval resulting in an average of 5.8 days in 1995 (Vesseur, 1997). Thus, sows have changed greatly over the years and the question arises if, with these changes, the number of sows with lactation oestrus in response to Intermittent Suckling is higher in modern sow lines. Therefore, the first aim of this thesis is to study if by means of IS it is possible, in modern sows, to induce lactational oestrus and ovulation in a high proportion of the animals.

If lactational oestrus can be induced it is not only of great importance that the sows show oestrous behaviour, but it is also important that follicle development and ovulation are of a good quality. Therefore, follicle development and peri-ovulatory hormone profiles are studied and compared with a post-weaning oestrus. For IS to be successful in practice it is also important that after ovulation, sows become and remain pregnant with a good embryo survival. Therefore, pregnancy rate, embryo survival and embryo development are studied in sows subjected to Intermittent Suckling. The second aim of this thesis is, therefore, to examine the quality of lactational oestrus and subsequent pregnancy as induced by IS in comparison with a post-weaning oestrus and pregnancy.

Outline of the thesis

The aim of a first experiment was to examine if by means of Intermittent Suckling lactational oestrus could be induced in a large proportion of the sows. A second aim was to examine if separation for either for 12 continuous h per day or at 6 h intervals would influence the occurrence of lactational oestrus. Levels of oestradiol, LH and progesterone were measured and sows were inseminated and slaughtered at day 23 after ovulation. The results of this experiment are described in Chapters II and III.

One of the results from the first experiment was that low post-ovulatory progesterone levels were found in Intermittently Suckled sows. Progesterone levels have been associated with embryo survival and it was therefore important to clarify why progesterone levels were low. Since high feeding levels have been associated with low progesterone levels we examined if the low progesterone levels were related to the high lactational feeding levels of Intermittently Suckled sows. The results of this experiment are described in Chapter IV.

Not only progesterone levels were found to be low during the first experiment, but also the pre-ovulatory LH surge was lower and embryo development was retarded in Intermittently Suckled sows. This might be related to either effects of lactation or the timing of start of Intermittent Suckling relative to farrowing. To study the possible involvement of these factors, a third experiment was carried out. In this experiment, start of Intermittent Suckling was initiated at two different stages of lactation (day 14 or day 21 of lactation) to study possible effects of timing on oestrus and pregnancy parameters. Furthermore, to study possible effects of lactation on early pregnancy, Intermittent Suckling either ended at ovulation or continued during early pregnancy. The results of this experiment are described in detail in Chapter V.

In the three experiments, sows responded differently to the IS treatments in terms of follicle growth and ovulation and relatively many sows developed cystic follicles. Data of these sows were pooled to study the development of cystic ovaries, related hormone profiles and oestrous expression, compared to sows that normally ovulated. These analyses are described in Chapter VI.

Finally in the General Discussion (Chapter VII), results of the experiments are combined and different categories of response in follicle growth and factors related to this response are discussed. Furthermore, the findings with regard to oestrus, ovulation and pregnancy parameters are discussed with regard to practical implication.

II

Peri-oestrus hormone profiles and follicle growth in lactating sows with oestrus induced by Intermittent Suckling

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Abstract

This study describes follicle dynamics and endocrine profiles in multiparous sows with lactational oestrus compared to conventionally weaned sows (C). Lactational oestrus was induced by Intermittent Suckling (IS) with separation of sows and piglets for either 12 consecutive h per day (IS12, n=14) or twice per day for 6 h per occasion (IS6, n=13) from day 14 of lactation onwards. Control sows (n=23) were weaned at day 21 of lactation. Pre-ovulatory follicles (≥ 6 mm) were observed in 100% of IS12, 92% of IS6 and 26% of C sows before day 21 of lactation and in the remaining 74% C sows within 7 days after weaning. All sows with pre-ovulatory follicles showed oestrus, but not all sows showed ovulation. Four IS6 sows and one IS12 sow developed cystic follicles of which two IS6 sows partially ovulated. Follicle growth, ovulation rate, and time of ovulation were similar. E₂ levels tended to be higher in IS sows (P=0.06), the pre-ovulatory LH surge tended to be lower in IS12 (5.1 ± 1.7 ng/ml) than in C sows (8.4 ± 5.0 ng/ml; P=0.08) and P₄ levels were lower in IS12 and IS6 than in C sows (at 75 h after ovulation: 8.8 ± 2.4 ng/ml vs. 7.0 ± 1.4 ng/ml vs. 17.1 ± 4.4 ng/ml; P<0.01). In conclusion, sows with lactational oestrus induced by IS are similar to weaned sows in the timing of oestrus, early follicle development and ovulation rates, but the pre-ovulatory LH surge and post-ovulatory P₄ increase are lower.

Introduction

Normally during lactation, a sow will remain anoestrus; the suckling stimulus of the piglets triggers the release of endogenous opioid peptides in the brain, which suppresses pulsatile LH secretion (Armstrong et al., 1988; Barb et al., 1986; De Rensis et al., 1999b), follicle development and oestrus. After weaning, secretion of pulsatile LH shifts to high frequency, low amplitude LH pulses (Shaw and Foxcroft, 1985) and as a result follicles develop, and sows show oestrous behaviour within approximately 5 days (Steverink et al., 1999). Reduction of the suckling stimulus during lactation by reducing the number of piglets from 10 to 5 also results in increased LH secretion (Varley and Foxcroft, 1990). Similarly, temporary separation of sow and piglets for a number of hours increases LH pulsatility (Armstrong et al., 1988). When separation of sow and piglets is repeated daily [limited nursing or Intermittent Suckling (IS)], lactational oestrus can be induced (Newton et al., 1987; Stevenson et al., 1981; Stevenson et al., 1984). By inducing oestrus during lactation, it is possible to prolong lactation to increase piglet welfare without decreasing the number of litters per sow per year. It is still not clear whether a lactational oestrus is similar to a post-lactational oestrus in terms of peri-oestrus hormone profiles (oestradiol- 17β , LH and progesterone) and processes like follicle development and ovulation. It is also not clear whether the lactational oestrus is affected by the method of induction (e.g frequency and duration of separation). The objective of this study was to examine follicle dynamics and endocrine profiles related to follicle development, ovulation and luteinisation in sows with lactational oestrus induced by two different regimes of separation in comparison with sows that ovulate after conventional weaning.

Materials and Methods

General design

The experiment was approved by the Ethical Committee for animal experiments of Wageningen University.

A total of 56 multiparous Topigs40 sows (Topigs, Vught, The Netherlands) were used in three batches. Parity ranged from 3 to 10 (5.8 ± 0.3 on average; mean \pm SD). Before parturition, sows were assigned to one of three treatments based on parity, weight and back fat measured at the P2 site at three months of gestation. Weight and back fat at three months of gestation was 273 ± 20 kg and 17.8 ± 3.0 mm (mean \pm SD). Treatments were: 1. Control (C); continuous lactation with weaning at day 21 of lactation; 2. Intermittent Suckling 12 (IS12); 3. Intermittent Suckling 6 (IS6). In IS12 and IS6, piglets and sows were separated for 12 h in total each day from day 14 of lactation until sows were slaughtered (day 41 to 45 of lactation). For the IS12 treatment, separation of sows and piglets occurred from 8:00 h to 20:00 h and for the IS6 treatment from 8:00 to 14:00 h and from 20:00 to 02:00 h. From day 7 of lactation onwards, creep feed was provided for the piglets. Blood samples were collected every 6 h. To

study follicle development, the ovaries were examined daily by means of transrectal ultrasonography. Sows were slaughtered to assess ovulation rate at weaning of the IS piglets (day 41 to 45 of lactation), which was at d23 after ovulation. Control sows were also slaughtered at day 23 after ovulation.

Animals and housing

For frequent blood sampling, sows from batch one ($n=15$) were fitted with a permanent ear catheter at day 13 or day 12 of lactation for IS sows or at day of weaning (day 21 of lactation) for C sows, as described by Peacock (1991) using anaesthetics: 0.5 mg/kg Dormicum (Roche Nederland BV, The Netherlands) and 1.0 mg/kg Propofol (Fresenius Kabi Nederland BV, The Netherlands). Sows from batch two and three were surgically fitted with a permanent jugular vein catheter 11 days (range 5-15) before parturition as described by Soede et al. (1997).

All sows were moved to farrowing crates one week before expected farrowing (batch one) or one week before surgery (batch two and three). Each treatment group was housed in a different unit. Control sows remained in farrowing crates until weaning and were housed in individual crates thereafter. IS sows were also housed in farrowing crates but from day 14 onwards sows were moved to a unit with individual crates during the hours of separation from the piglets. In this unit, auditory, visual and olfactory stimuli of the piglets were absent. After each separation period the sows returned to the piglets in the farrowing crate.

From day 1 to day 10 after farrowing, sows were fed three times daily with increasing amounts of a commercial lactational feed (12.8 MJ/kg ME, 145 g/kg CP) until the maximum allowance of 1% of body weight plus 0.5 kg per piglet was reached at day 11. After weaning C sows were fed to maintenance with a commercial gestation feed (12.45 MJ/kg ME, 140 g/kg CP) twice per day. Sows had ad libitum access to drinking water at all times. Within 72 h after farrowing litter size was standardized. Due to the low number of total live born piglets, three C sows were weaned within 72 h after parturition and their piglets were placed with other sows. One C sow suffered from agalactia, one IS6 sow became ill before start IS and one C sow became ill after weaning. All of the above mentioned sows were excluded from data analyses, resulting in 50 sows present at day 14 of lactation. The number of piglets per sow was 9.8 ± 0.8 piglets at day 14 of lactation. Data on litter size, growth and feed intake of the piglets are published elsewhere (Berkeveld et al., 2007). Sows were weighed and back-fat was measured on day 1 (day after farrowing) and every week of lactation. Average weights on the day after farrowing (day 1) was 283 kg (SD 22 kg; Range 239-324 kg) and at the day before start of IS (day 13) it was 273 kg (SD 25 kg; Range 225-326 kg) and similar for the three treatments.

Blood sampling and hormone analyses

From 48 h after start of IS, or 24 h after weaning for C sows, blood samples were collected at 6 h intervals until 24 h after ovulation. From 24 h until 96 h after ovulation, blood samples were collected at 12 h intervals. Blood samples were collected in tubes containing 100 μ l of

EDTA solution, placed on ice immediately after collection and centrifuged at 3600 rpm for 10 min at 4°C. Plasma was stored at -20°C until analyses. Blood samples were analyzed for oestradiol-17 β (E₂), LH and progesterone (P₄).

Concentrations of LH were determined in duplicate aliquots of 100 μ l plasma by a homologous radio immuno assay (RIA) as validated for pig plasma (Van den Brand et al., 2000a). Porcine LH-LER 778.4 (kindly supplied by Dr. L.E. Reichert, Tucker Endocrine Research Institute LLC, Atlanta, GA) was used for iodination and standards, and rabbit anti-pLH (UCB A528; Campro Scientific, Veenendaal, The Netherlands) as antiserum. Specificity of the RIA was high as indicated by low cross-reactivity for other pituitary hormones (Van den Brand et al., 2000a) and by the observed parallelism. The limit of quantitation was 0.2 ng/ml for LH.

Concentrations of progesterone and oestradiol-17 β were determined by solid-phase ¹²⁵I RIA methods (Coat-A-Count TKPG and TKE, respectively; Diagnostic Products Corporation, Los Angeles, CA) as validated for cow plasma (Dieleman and Bevers, 1987). Briefly, duplicate aliquots of 100 μ l plasma were directly used in the RIA for progesterone, while for oestradiol 1 ml plasma was extracted with 2 ml diethyl ether. After evaporation of the diethyl ether, the residues were dissolved in 250 μ l borate buffer, and duplicate aliquots of 100 μ l were used in the RIA. Extraction efficiency was determined in parallel samples with tritiated steroid. Specificity of both RIAs was high as indicated by low cross-reactivity for other steroid hormones (Dieleman and Bevers, 1987) and by the observed parallelism. The limits of quantitation were 0.1 ng/ml and 2 pg/ml for progesterone and oestradiol, respectively. Calculation of all results was done applying the spline approximation for the standard series from RIASmart (Packard Instruments Company, Meriden, CT, U.S.A.). The calculated doses were <4 % different from the defined doses over the entire range. In general, the intra- and inter-assay coefficients of variation were <10 and <15% for all assays, respectively.

The pre-ovulatory peak concentration of E₂ was defined as the moment after which a consistent decrease occurred in E₂ concentration. The return to basal E₂ level was defined as the first sample at which the concentration of E₂ was lower than 7.5 pg/ml.

Onset of the LH surge was defined as the last sample before a consistent increase in LH concentration occurred and end of the LH surge was defined as the first sample after a consistent decrease in LH concentration.

Progesterone (P₄) concentrations were measured in samples collected from 24 h before ovulation until 96 h after ovulation. The basal concentration of P₄ was calculated per sow as the average of three values before ovulation. The start of P₄ increase was defined as the time at which the concentration of P₄ rose >1 ng/ml above basal concentrations.

As a result of catheter obstruction, blood samples could not be taken for all animals at all times. As a result, the number of profiles for E₂ and LH was 16 for C, 12 for IS12 and 7 for IS6 and for progesterone 12 for C, 11 for IS12 and 7 for IS6.

Oestrus detection, follicle development and ovulation

Oestrus detection was carried out every 6 h, starting at day 16 of lactation (2 days after start of IS) or one day after weaning for C sows, by performing a Back-Pressure-Test (BPT) in absence of a boar. A sow was regarded to be in oestrus when she reacted with a frozen posture and arched back to the BPT. Onset of oestrus was defined as the first time a sow exhibited a standing response minus 3 h (half the time since the former oestrus check). End of oestrus was defined as the last time a sow exhibited a standing response plus 3 h.

To study follicle development, the ovaries were examined daily by means of rectal ultrasonography using a 7.5 MHz annular array sector probe and ultrasound scanner (Scanner 200, Pie Medical, Maastricht, The Netherlands), starting one day before start of IS or one day before weaning for C sows. The diameters of the three largest follicles on the ovary were measured. When the average follicle diameter of the three largest follicles was \geq than 6.0 mm, ultrasound was performed every 6 h to establish time of ovulation. Time of ovulation was defined as the time when no follicles could be detected minus 3 h (half the time since the former scan). When follicles were still observed but the number of follicles was considerably lower than at the former scan, that time was defined as start of ovulation. During a scan 6 h later, ovulation was confirmed. For two sows that partially ovulated, time of ovulation was defined as the first time that the number of follicles on the ovary was consistently lower than the former scan, minus 3 h.

Sows were slaughtered at d23 after ovulation and the number of Corpora Lutea and abnormalities including cysts present on the ovaries were examined. For four C sows, time of ovulation was not established; therefore these animals were excluded for analyses of ovulation parameters.

Statistical analyses

Data were analyzed using SAS (SAS institute, Cary, NC, USA). Differences between treatments in the percentage of sows showing oestrous behaviour and ovulation were analyzed using the Fisher's exact test. To analyze effects of treatment in oestrous parameters, follicle development and intervals between parameters, the following model was used in a PROC GLM: $Y_{ijk} = \mu + T_i + B_j + T_i * B_j + e_{ijk}$, with Y_{ij} the dependent variable, μ the mean, T_i treatment ($i=C, IS12, IS6$), B_j batch ($j=1, 2, 3$), $T_i * B_j$ the interaction between treatment and batch. When the interaction between treatment and batch and a batch effect were absent, they were omitted from the model ($P>0.05$). Hormones were analyzed using the same GLM model as mentioned above. For overall profiles of E_2 , data of IS12 and IS6 were pooled (IS) and analyzed in PROC GLM with the nested model: $Y_{ij} = \mu + T_i + sow_j(T_i) + S_k + (T * S)_{ik} + e_{ijk}$ with Y_{ij} the dependent variable, μ the mean, T_i treatment ($i=C, IS$), $sow_j(T_i)$ =the effect of sow_j ($j=1, \dots, 54$) nested within treatment, S_k =the time of sampling ($k=-30, -24, -18, -12, -6, 0$ hours relative to time of LH surge), $T_i * S_k$ =the interaction between treatment and time of sampling. Treatment was tested against sow nested within treatment. For overall profiles of LH and P_4 , the latter model was used with time of sampling for LH is $k= -30, -24, -20, \dots, 24$,

30 relative to time of LH surge and for P₄ k= -9, -3, 3, 9..., 96 h after ovulation. Relationships between parameters were tested in a GLM procedure with variables as a covariate. Relationships were calculated per treatment when the interaction between treatment and covariate was significant (P<0.10). Correlations were tested in the PROC CORR procedure of SAS. Values presented in the present study are means with standard deviation (SD) unless stated otherwise.

Results

Follicle development, ovulation and oestrous behaviour

All IS12 sows and 92% of the IS6 sows showed follicle growth up to pre-ovulatory size (≥ 6 mm) during lactation and 74% of the C sows showed follicle growth up to pre-ovulatory size after weaning (Table 1). Of the C sows without follicle growth after weaning (26%) five had CLs, indicating lactational ovulation and one sow had cysts on the ovaries at weaning. From weaning (C) or start of IS (IS6 and IS12) to follicle diameter ≥ 6 mm and ovulation, follicle development appeared similar among treatments (P>0.10; Table 2). Ovulation rate was 25, 26 and 27 for C, IS12, and IS6 sows.

All C sows, showing follicle growth after weaning, ovulated and 93% of the IS12 sows and 83% of the IS6 sows with follicle growth ovulated during lactation (Table 1; P>0.10). Two IS6 sows that ovulated had partial ovulation; the remaining follicles became cystic. In animals (one IS12 and two IS6 sow) in which no ovulation occurred, cystic follicles were observed.

Table 1. Number of sows showing (lactational) oestrus and ovulation

	C	IS12	IS6
Number of sows in experiment at start IS/ weaning	23	14	13
Reproductive parameters during lactation			
- Lactational oestrus	ND ¹	14	12
- ovulation	5	13	10 ²
- cystic ovaries	1	1	4 ²
Reproductive parameters after weaning			
- Post-lactational oestrus	17	-	-
- ovulation	17		
- cystic ovaries	0		

¹ Not Determined ² Two sows partially ovulated and developed cystic ovaries and therefore appear double in this table

Table 2. Follicle development, oestrus and ovulation in sows per treatment

Parameter	C		IS12		IS6	
	mean	SD	mean	SD	mean	SD
Follicle development						
Follicle diameter day 13 (IS) and day 20 (C) (mm)	3.3	1.1	3.0	0.8	2.8	0.7
Follicle diameter day 2 after start IS/weaning (mm)	5.7	0.5	5.9	0.8	5.6	0.4
Follicle diameter day 4 after start IS/weaning (mm)	7.1	0.5	7.2	0.5	7.0	0.4
Follicle diameter at ovulation (mm) ¹	7.5	0.7	8.0	0.8	7.8	0.4
Ovulation rate	25	5	26 ²	3	27	6
Interval start IS/ weaning to follicle diameter \geq 6 mm (h)	68	16	61	24	70	20
Interval follicle diameter \geq 6 mm to ovulation (h) ¹	61	21	74	20	80	26
Oestrus						
Interval start IS/ weaning to onset oestrus (h)	100	22	101	21	117	18
Duration of oestrus (h)	71 ^a	18	63 ^{a,b}	22	48 ^b	18
Ovulation time						
Interval start IS/ weaning to ovulation (h) ¹	131 ^c	10	135 ^{c,d}	23	149 ^d	21
Interval from onset of oestrus to ovulation (h) ¹	40	14	34	14	32	15

Unless stated otherwise, the number of observations are 17 for C, 13 for IS12 and 8 for IS6. ¹ The number of observations for ovulation parameters is 13 for C, 12 for IS12 and 7 for IS6. ² For one sow ovulation rate was not measured because new follicles were found on the ovary, and the corpora albucantia were not counted. ^{a,b} Different superscripts within one row indicate significant differences ($P<0.05$). ^{c,d} Different superscripts within one row indicate differences ($P=0.10$)

IS6 sows tended to ovulate later after the start of IS than C sows after weaning ($P = 0.10$; see Table 2). Follicle size at the start of IS or weaning was not related to the time of ovulation ($r = 0.13$; $P > 0.10$). Further, time of ovulation after start of IS or weaning was not correlated with follicle size around ovulation ($r = 0.07$; $P > 0.10$), or with ovulation rate ($r = -0.04$; $P > 0.10$).

Oestrous behaviour was shown by all sows that showed follicle growth up to pre-ovulatory size during Intermittent Suckling and all C sows that showed follicle development after weaning (Table 1). The interval from either start of IS or from weaning to onset of oestrus was similar for the different treatments and thus not affected by lactation (Table 2). However, duration of oestrus was shorter in IS6 sows than C sows ($P < 0.05$). IS12 sows were intermediate.

Peri-ovulatory hormone profiles

The regimen of IS did not affect the pattern of change within time or levels of E_2 , LH and progesterone as no differences were found between IS12 and IS6 sows (Figure 1, Figure 2 and Table 3; $P > 0.10$).

When IS12 and IS6 sows were pooled and compared with C sows, the overall profile of E_2 tended to be higher in IS sows ($P = 0.06$). The concentration of E_2 at 30 h and 24 h before the LH surge, tended to be higher in IS12 than C sows ($P < 0.10$), but was not different between C and IS6 or IS12 and IS6 sows ($P > 0.10$) as illustrated in Figure 1.

For LH, the mean LH levels between -6 h and +6 h from peak LH levels were lower for IS sows (3.8 ± 0.3 ng/ml) than for C sows (5.8 ± 0.3 ng/ml; $P = 0.01$). Furthermore, the peak LH concentration of IS12 sows tended to be lower than the peak LH concentration of C sows with LH peak concentration of IS6 sows being intermediate (see Figure 1 and Table 3). In the mean of the LH peak concentration of C sows two sows were included with extremely high LH peak values (13.5 ng/ml and 25.2 ng/ml respectively). When these sows were excluded, the difference between IS12 and C sows was still present ($P = 0.07$).

The levels of P_4 were also affected by Intermittent Suckling as from 21 h after ovulation onwards higher P_4 levels were found in C sows than IS12 and IS6 sows (see Figure 2). At 75 h after ovulation the concentration of P_4 in C sows was 1.9 and 2.4 times higher than in IS12 and IS6 sows respectively ($P < 0.05$; Table 3). These differences in P_4 levels were not related to the differences in peak LH levels; C sows with comparable levels of peak LH to IS sows had considerably higher P_4 levels (see Figure 3). Nevertheless, within IS sows peak LH levels were correlated with P_4 levels ($r = 0.64$, $P < 0.05$).

Relative timing of endocrine events, behavioural oestrus and ovulation are presented in Table 3. IS6 sows needed more time after start IS to reach the pre-ovulatory drop in E_2 , onset of the LH surge, and peak LH levels than C sows after weaning ($P < 0.05$), whilst IS12 sows were intermediate. Intervals between hormonal events like peak E_2 to onset of the LH surge were not affected ($P > 0.10$).

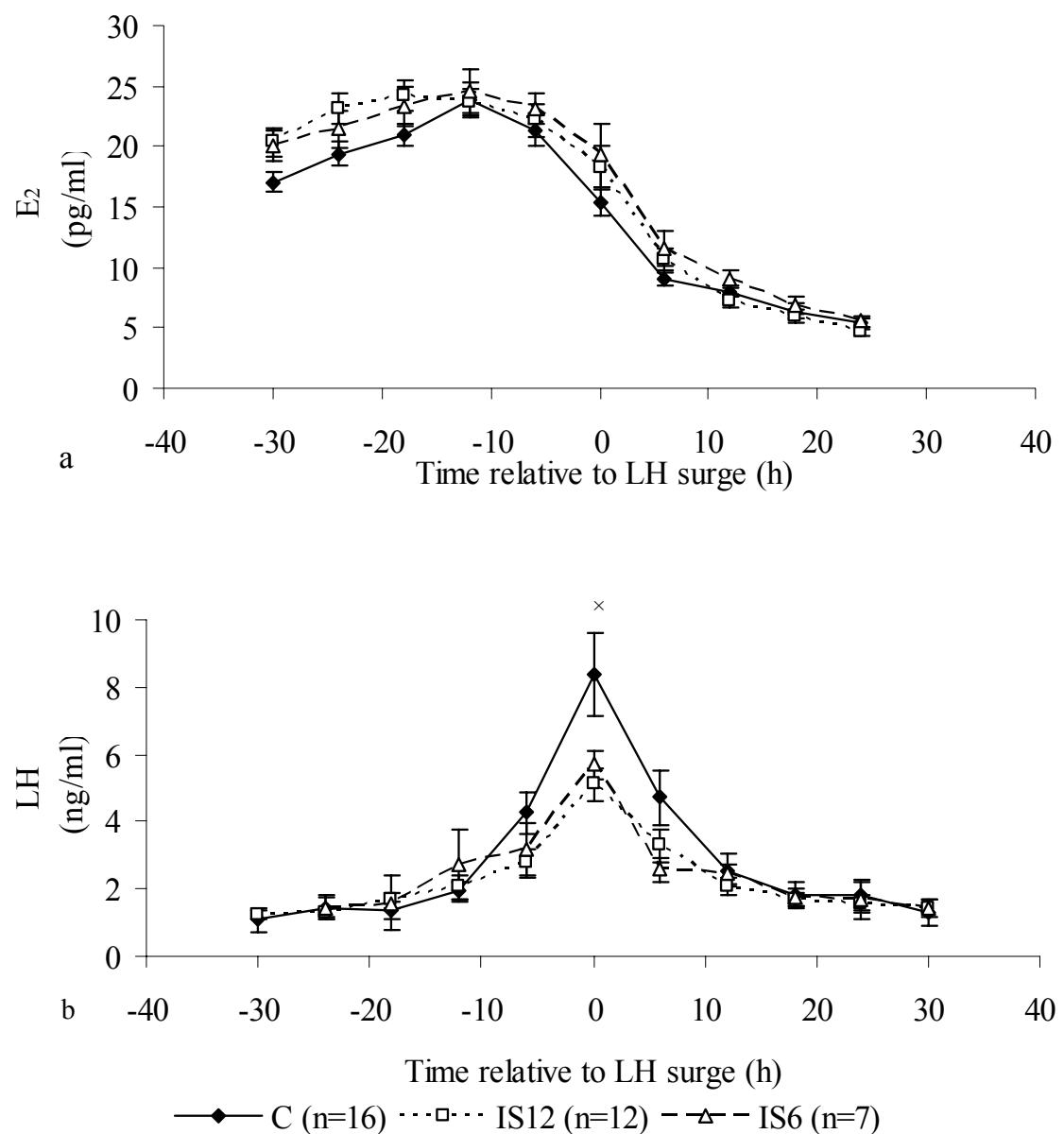


Figure 1. Hormone profiles of (a) oestradiol-17 β (E₂) and (b) LH per treatment and relative to the time of the LH surge. Number of observations for oestradiol and LH profiles was 16 for C, 12 for IS12 and 7 for IS6. (* = P<0.10 for C vs IS12). The LH profile for C sows includes two sows with extremely high peak LH levels of 13.5 ng/ml and 25.2 ng/ml.

Non-ovulating sows

During the experiment, five sows developed cystic ovaries (four IS6 and one IS12) of which two partially ovulated (ovulation rate of 8 and 21 respectively). All (partially) cystic sows showed oestrous behaviour during lactation with duration of oestrus between 24 h and 90 h. Up to day 5 after start of IS, follicle development in four out of the five cystic sows was similar to ovulating IS sows. For two sows the diameter of the cystic follicles remained similar (11 and 13 mm) during the 2nd and 3rd week after the start of IS, but decreased at slaughter. In a third sow, the diameter of the cystic follicles increased during the 2nd (15 mm) and 3rd (20 mm) week after the start of IS and was 24 mm at slaughter. In the fourth and fifth sow (partially ovulated) the cysts were still present at slaughter (day 23 after ovulation).

For the two sows with partial ovulation hormone profiles were assessed. The E₂ profiles were similar to other IS sows, but their LH profiles were different. No LH surge was visible and the highest levels of LH measured were 2.7 ng/ml and 2.4 ng/ml respectively. One sow that partially ovulated had a P₄ profile comparable with the other IS sows, with a P₄ concentration of 8.16 ng/ml at 75 h after ovulation and an ovulation rate of 21, but the other sow had a very low P₄ increase with a P₄ concentration of 3.09 ng/ml at 75 h after ovulation and an ovulation rate of 8.

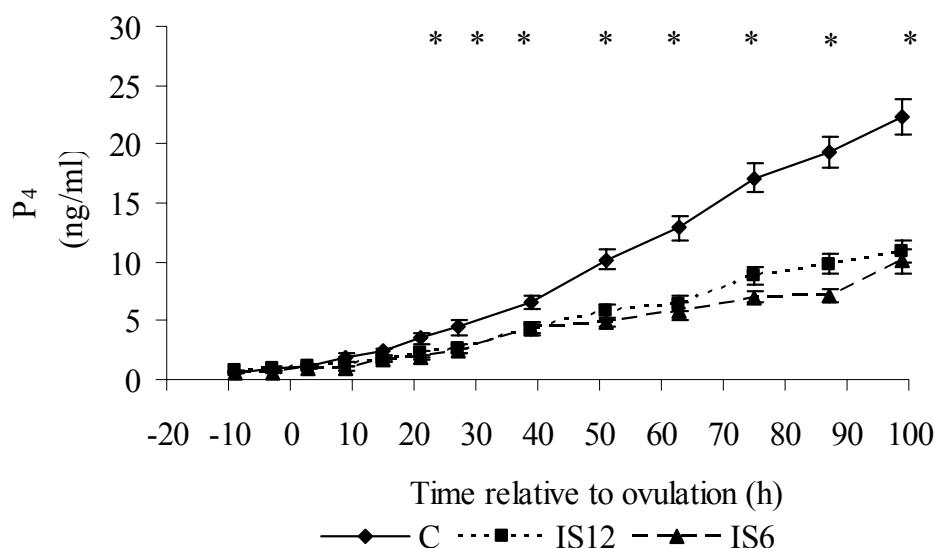


Figure 2. Hormone profiles of progesterone (P₄) per treatment and relative to the time of ovulation in sows. Number of observations for P₄ was 12 for C, 11 for IS12 and 7 for IS6. (* = P<0.05 for C vs. IS12 and IS6)

Table 3. Parameters of hormone profiles of sows and intervals between parameters per treatment

Parameter	C		IS12		IS6	
	mean	SD	mean	SD	mean	SD
E₂						
Peak E ₂ concentration (pg/ml)	25.0	4.8	26.5	3.7	25.7	4.2
LH						
Peak LH concentration (ng/ml)•	8.5 ^c	5.0	5.2 ^d	1.7	5.7 ^{c,d}	2.2
Duration of LH surge (h) [*]	34	4	32	8	30	9
Relative timing of peak during LH surge (%) [*]	42	10	49	12	52	17
P₄						
P ₄ concentration at 75h after ovulation (ng/ml)	17.1 ^a	4.4	8.8 ^b	2.4	7.0 ^b	1.4
Intervals						
Start IS/weaning- peak E ₂ (h)	80 ^a	10	86 ^{a,b}	28	108 ^b	18
Start IS/weaning- end of E ₂ (h)	107 ^a	13	116 ^{a,b}	29	136 ^b	13
Peak E ₂ – onset oestrus (h)	21	27	17	24	12	16
Peak E ₂ – peak LH (h)	14	6	15	7	10	6
Peak E ₂ – ovulation (h)	49	9	49	15	46	2
Peak E ₂ – basal E ₂ (h)	27	11	30	7	28	8
Start IS/weaning – onset LH surge (h)**	78 ^a	9	88 ^{a,b}	27	102 ^b	16
Start IS/weaning – peak LH (h)	94 ^a	10	101 ^{a,b}	28	118 ^b	15
Onset oestrus – onset LH surge (h)**	-23	28	-15	21	-18	16
Onset oestrus – peak LH surge (h)	-8	27	-2	22	-5	10
Onset LH surge- ovulation (h)	51	8	47	10	52	9
Peak LH- ovulation (h)	36	9	34	12	36	5
Onset oestrus – initial rise P ₄ (h)	48	8	58	23	56	11
End of oestrus – initial rise P ₄ (h)	-16 ^a	18	-2 ^{a,b}	16	6 ^b	12
Peak E ₂ – initial rise P ₄ (h)	64	9	75	18	64	8
Peak LH – initial rise P ₄ (h)	50	8	59	15	58	9
Ovulation – initial rise P ₄ (h)	15	5	24	11	23	9

Unless stated otherwise, the number of observations for E₂ and LH parameters is 16 for C, 12 for IS12 and 7 for IS6. The number of observations for P₄ is 12 for C, 11 for IS12 and 7 for IS6. The number of observations for ovulation parameters is 12 for C, 12 for IS12 and 7 for IS6. *The number of observations 14 for C, 10 for IS12 and 6 for IS6. ** The number of observations 16 for C, 11 for IS12 and 7 for IS6. • In C treatment for LH peak two sows are included with extremely high peak levels. ^{a,b} Different superscripts within one row indicate significant differences (P<0.05). ^{c,d} Different superscripts within one row indicate significant differences (P<0.10)

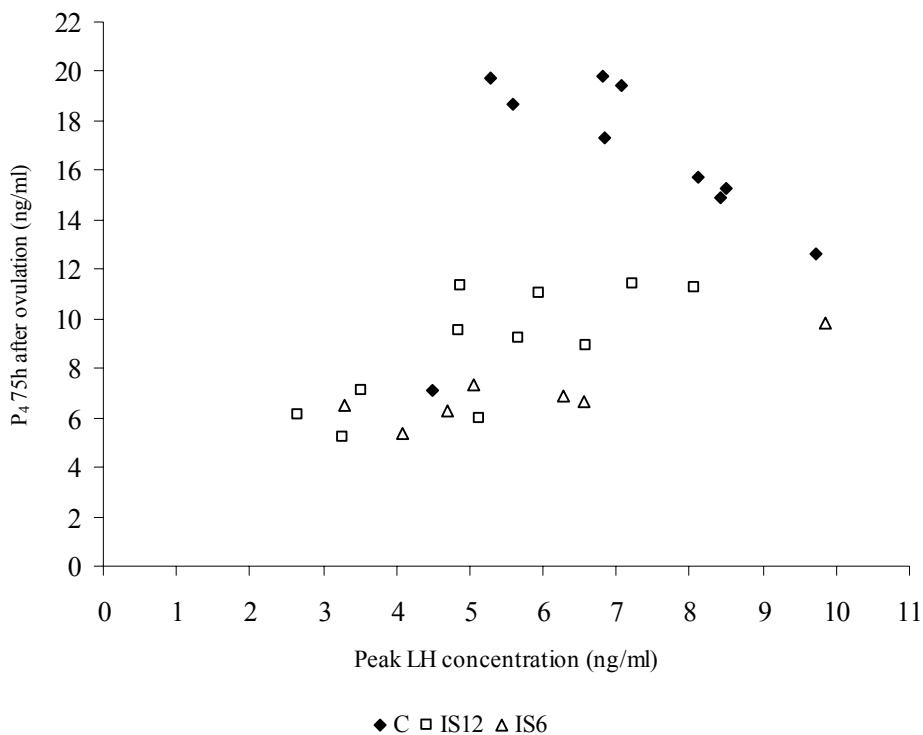


Figure 3. The relation between peak LH concentration and P₄ at 75 h after ovulation. Two sows with extremely high peak LH concentrations are excluded.

Suckling/non-suckling periods

Weaned C sows had no particular period of the day during which the onset of the LH surge started, at which peak levels of LH were reached, or when ovulation occurred. For IS sows onset of the LH surge was equally distributed over the day, and did not seem to occur specifically during suckling (S) or non-suckling (NS) periods for IS12 (7 NS vs. 4 S) or IS6 sows (3 NS vs. 4 S). Ovulation in IS6 sows, however, occurred during suckling periods ($n = 7$) rather than during non-suckling periods (7 S vs. 1 NS); this distribution tended to differ from that in IS12 sows (6 S vs. 7 NS; $P < 0.10$).

Discussion

In this study, follicle development, ovulation and hormone profiles were examined during lactational oestrus induced by two methods of Intermittent Suckling (IS) in comparison with oestrus after conventional weaning. IS led to follicle growth up to pre-ovulatory size ($\geq 6\text{mm}$) in nearly 100% of the sows and to ovulation in 90% of the sows. The proportion of IS sows showing follicle growth and ovulation was not different from sows weaned at day 21 of lactation. A number of C sows already ovulated (22%) or developed cystic ovaries (5%) during lactation, indicating that in the commercial sow line used in the present experiment

follicle development is easily induced during lactation. It is possible therefore, that some of the sows subjected to IS would also have shown oestrus when Intermittent Suckling would not have been applied. IS, however, resulted in nearly 100% lactational oestrus and was therefore a strong stimulus to induce lactational oestrus.

Sows subjected to IS6 or IS12 in the present study, showed the same interval from start of IS to follicle size of 6 mm as C sows from weaning. In weaned sows, pulsatile LH secretion increases and is important for follicle growth and for the interval from weaning to oestrus (Shaw and Foxcroft, 1985). LH pulsatility was not measured during the present experiment, but LH pulsatility has been shown to increase immediately when sows and piglets were separated for 8h (Armstrong et al., 1988). Apparently, the increase in LH during the periods of non-suckling resulted in similar follicle development and follicle function up to pre-ovulatory size as in weaned sows. Besides LH, also FSH is of importance as follicles can grow up to 4-6mm in pigs (Guthrie et al., 1990) and 2-3mm in goats (Gonzalez-Bulnes et al., 2005) under FSH (porcine FSH) stimulation only. No effect of IS was found on the number of follicles selected as no differences were found in ovulation rate between treatments. This seems to indicate that there was no deficiency in either FSH or LH during the period of follicle growth up to 6mm in the limited nursed sows.

Follicles of IS sows were able to produce similar amounts of E₂ as weaned sows and E₂ even tended to be higher in IS sows. In mammals (Clarke, 1995) including pigs (Kraeling and Barb, 1990), E₂ triggers the pre-ovulatory LH surge which is necessary for the process of ovulation to occur. Despite the higher E₂ levels, a lower LH surge was seen in IS sows. The lower LH surge might be related to the stage of lactation at which the LH surge occurred: on average at day 18 after parturition compared to day 25 after parturition for the weaned sows. Lower LH surges were found during lactation periods of 21 days vs. 35 days (Edwards and Foxcroft, 1983), 10 days vs. 35 days (Kirkwood et al., 1984) and 14 days vs. 35 days (Willis et al., 2003). Edwards and Foxcroft (1983) and Kirkwood et al. (1984) found that E₂ levels seemed higher in early weaned sows, although not significant. After a short lactation, the readily releasable pool of LH might be low because the LH response increases as lactation progresses (Bevers et al., 1981; Cox et al., 1988; Sesti and Britt, 1993b; Sesti and Britt 1993c; Stevenson et al., 1981). Administration of Oestradiol Benzoate was found not to induce LH surges in early lactation (Cox et al., 1988; Sesti and Britt, 1993a) but did during later lactation with (Elsaesser and Parvizi, 1980; day 35) or without ovulation (Sesti and Britt, 1993a; day 28).

Alternative mechanisms which might cause a low LH surge and affect ovulation during early lactation might be endogenous opioid peptides which are produced during suckling (Cox et al., 1988) and stress. If endogenous opioid peptides released during the suckling periods would inhibit the release of LH, then the onset of the LH surge would not occur during a suckling period. However, the onset of the LH surge appeared to be evenly distributed over suckling and non-suckling periods. Endogenous opioid peptides could possibly also directly affect the magnitude of the LH surge but seems unlikely as in rats an

opioid agonist either abolished the LH surge completely (high levels) or resulted in a normal LH surge (medium levels) (Pang et al., 1977). This suggests that suckling was not an explanation for the lower LH surge in IS sows.

LH release has been found to be affected by cortisol in humans (Sakakura et al., 1975), heifers (Stoebel and Moberg, 1981), and (prepubertal) gilts (Hennessy and Williamson, 1983; Pearce et al., 1988). In IS sows, a rise in cortisol was found around time of separation (unpublished results). Sows undergoing Intermittent Suckling at 6 h intervals (IS6) were moved four times a day and thus possibly experienced a rise in cortisol four times a day, resulting in chronic stress. Induction of chronic stress by administration of ACTH resulted in a depressed LH surge in pigs (Hennessy and Williamson, 1983) and a change in timing of the LH surge in both pigs (Hennessy and Williamson, 1983) and heifers (Stoebel and Moberg, 1981). Furthermore, ACTH administration resulted in a delayed E₂ surge, a shorter duration of oestrus (Liptrap, 1970), and inhibition of ovulation and development of cysts in pigs (Liptrap, 1970; Liptrap, 1973). IS6 sows had a shorter duration of oestrus, the interval from start IS until E₂ surge was longer, the LH surge was lower and more sows were observed with cysts than C sows, all possibly related to stress. Thus, the lower LH surge in IS sows might have multiple causes interacting with each other; endogenous opioid peptides released by suckling in combination with stress and the stage of lactation at which Intermittent Suckling is initiated.

After ovulation, IS sows had low P₄ levels. The height of the LH surge was found to affect luteinisation in pigs (Einarsson and Rojkittikhun, 1993) and thus might result in lower P₄ levels. Another explanation for the lower P₄ in IS sows may be that as the animals were fed a lactational regime (6.5–7 kg), they may have had a higher metabolic clearance rate and as a result a lower P₄ level in the blood as lower P₄ levels were found in studies examining high feeding levels in gilts (Jindal et al., 1996), sows (Virolainen et al., 2005), and sheep (Parr et al., 1987). In sheep, feeding level was directly related to the mean rate blood flow in the portal vein (Parr et al., 1993) and in gilts a higher metabolic clearance rate was found at a high feeding level (Miller et al., 1999). Lactation length was found not to affect P₄ levels after ovulation in sows (Belstra et al., 2002; Edwards and Foxcroft, 1983; Willis et al., 2003). It is unknown whether in pigs lactation itself can affect P₄ levels but in cows P₄ levels were not affected by suckling (Henao et al., 2000). In women, however, long term opioid treatment resulted in low levels of P₄ and their menstrual cycle was disrupted (Abs et al., 2000) possibly caused by the effect of endogenous opioid peptides on LH pulsatility. In pigs, the CL functions independently of LH during the first twelve days after ovulation (Brinkley et al., 1964). Therefore it seems unlikely that endogenous opioid peptides affected P₄ production in limited nursed sows in this study. Thus, the low P₄ levels found in IS sows could be a result of the high feeding level or a consequence of the low LH surge which results in reduced luteinisation.

In conclusion, sows subjected to IS are similar to weaned sows in timing of onset of oestrus, early follicle development and ovulation rates, but the pre-ovulatory LH surge and post-

ovulatory progesterone increase are lower. These effects of IS may be due to effects of lactation itself, to separation stress or the stage of lactation at which Intermittent Suckling is initiated. The reason for the differences between the two IS regimes (IS12 vs. IS6) remains unclear.

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III

Early embryo survival and development in sows with lactational ovulation

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Reproduction in Domestic Animals (in press)

Abstract

During lactation, daily separation of sow and piglets, Intermittent Suckling (IS), can induce lactational oestrus and ovulation. This study examined effects of IS on subsequent early embryo survival and development. Multiparous Topigs40 sows were separated from their piglets for either 12 consecutive h per day (IS12, n=13) or two times for 6 h per day (IS6, n=10) from day 14 of lactation onwards until 23 days after ovulation. Control sows (C, n=17) were weaned at day 21 of lactation. Oestrus was shown in all treatments within five days after the start of treatment. Sows were inseminated each day of oestrus and slaughtered at D23 after ovulation. IS did not significantly affect pregnancy rates of sows (75% IS12 vs 78% IS6 vs 94% C; P>0.10). Embryo survival was not significantly affected by IS (IS12: 57%; IS6: 51%; P>0.10) although it seemed to be lower than in C sows (70%). Some parameters of embryo, placental and uterine development were affected by IS, especially in the IS6 group. IS6 embryos had shorter placentas (17.5 ± 1.2 cm; P<0.05) than C (20.3 ± 1.4 cm) and IS12 sows (20.9 ± 0.7 cm) were smaller and less developed than C sows (P<0.05). In conclusion, embryo survival does not seem significantly affected by Intermittent Suckling although numerical differences were great. Embryo development, however, was negatively affected in IS6 sows possibly due to a combination of high milk production, stress and lactational effects on uterine development.

Introduction

Daily separation of sow and piglets during lactation by means of Intermittent Suckling regimens (IS) can induce lactational oestrus and ovulation (e.g. Stevenson et al., 1981; Newton et al., 1987). The percentage of oestrous and ovulating sows during the first week after start IS may even be comparable to that of conventionally weaned sows (C) in the first week after weaning (oestrus: 96% IS vs. 100% C; Chapter II). It is not clear if these sows ovulating during IS have a normal fertility. First, in Chapter II was found that the LH surge and post-ovulatory plasma progesterone (P_4) levels are significantly lower in IS sows. Since high levels of P_4 have been found to positively affect embryo survival in pigs (Jindal et al., 1997) the low P_4 levels in IS sows might have consequences for uterine development and embryo development and survival. Second, the metabolic state of the sow during lactation has been found to affect the development and quality of oocytes that ovulate during the subsequent post-weaning oestrus (Zak et al., 1997). Thus, ongoing lactation during early pregnancy as is the case during IS, may affect subsequent embryo survival due to these metabolic effects. Therefore, the aim of this study is to examine the effects of intermittent suckling on pregnancy rate, embryo survival and embryo development.

Materials and Methods

Experimental design

The experiment was approved by the Ethics Committee for Animal Experiments of Wageningen University. Treatments were: 1. Control (C); continuous lactation with weaning at day 21 of lactation; 2. Intermittent Suckling 12 (IS12); 3. Intermittent Suckling 6 (IS6). In IS12 and IS6, piglets and sows were separated for 12 h in total each day from day 14 of lactation until 23 days after ovulation, when sows were slaughtered (day 41 to day 45 of lactation). For the IS12 treatment, separation of sows and piglets occurred from 8:00 h to 20:00 h and for the IS6 treatment from 8:00 to 14:00 h and from 20:00 to 02:00 h. From day 7 of lactation onwards, piglets were provided with creep feed. Each treatment group was housed in a different farrowing unit to prevent possible effects of separation on other treatments. Control sows remained in farrowing crates until weaning and were housed in individual crates thereafter. IS sows were also housed in farrowing crates but from day 14 onwards sows were moved to a unit with individual crates during the hours of separation from the piglets. In this unit, auditory, visual and olfactory stimuli of the piglets were absent. After each separation period the sows returned to the piglets in the farrowing crate.

During lactation sows were fed three times per day (08:00, 14:00, 20:00 h) a commercial feed (12.8 MJ/kg ME, 145 g/kg CP) which was stepwise increased from 3.0 kg at day of farrowing until the maximum daily allowance of 1% of body weight plus 0.5 kg per piglet at day 11. After weaning C sows were fed to maintenance (1% of body weight) with a

commercial gestation feed (12.45 MJ/kg ME, 140 g/kg CP) twice per day (08:00 and 20:00 h). IS sows were kept on the lactation feeding regime until the day before slaughter. When feeding times were similar to times of separation or return to the piglets, the sows were fed after relocation. Sows were fed for the last time at 2000 h on the day before slaughter. Sows had ad libitum access to drinking water at all times.

For frequent blood sampling, sows were fitted with permanent jugular catheters before farrowing (Chapter II). Blood samples were taken every 6 h blood samples from 24 h before ovulation until 21 h after ovulation and every 12 h from 21 h until 96 h after ovulation. Plasma progesterone (P_4) concentrations were measured as described in Chapter II. Three batches of in total fifty multiparous Topigs40 sows (Topigs, Vught, The Netherlands) were available at day 14 of lactation (C: n=23; IS12: n=14; IS6: n=13). For the present study only data were used of sows with ovulation during IS (IS12: n=13 and IS6: n=10) or after conventional weaning (C: n=17) (see Chapter II). Detailed information on oestrous behaviour, hormone profiles, follicle development and ovulation has been published in a different paper (Chapter II).

Ovulation and insemination

Time of ovulation was established by means of transrectal ultrasonography performed every 6 h using a 7.5 MHz annular array sector probe and ultrasound scanner (Scanner 200, Pie Medical, Maastricht, The Netherlands). Time of ovulation was defined as the time when no follicles could be detected minus 3 h (half the time since the former scan). When a sow was ovulating during an ultrasound session that time was defined as time of ovulation. During a scan 6 h later, the sow was checked for to confirm ovulation.

Control sows were inseminated at post-weaning oestrus and IS sows at lactational oestrus. Sows were inseminated with a commercial AI dose every day of oestrus or when follicle diameter was $\geq 7\text{mm}$ for two days in sows not showing oestrous behaviour. All sows were slaughtered at day 23 of gestation.

Uterus, placenta and embryo analyses

At day 23 after ovulation, sows were slaughtered and their reproductive tracts were collected and kept on ice until analyses. Ovaries were removed and the numbers of corpora lutea and, after dissection, luteal weight was determined. Allantoic fluid samples were taken and after classification of viable and non-viable embryos, fluid was pooled per sow from 3 viable embryos per horn, and stored at -20°C until analyses. After removal of the mesometrium and separation of the uterine horns, the horns were opened at the antimesometrial side. The number of embryos was counted and each embryo was classified as viable or non-viable based on signs of degeneration of the embryo such as colour and size of the embryo but also colour and development of the placenta (necrosis of placenta). Embryos and placentas were removed from the uterine horn and separated from each other. Length of the individual

placentas was measured immediately and weight was determined after the placentas were freeze-dried. The length and weight of the empty uterine horns were determined, as well as the number, length and width of the placental-attachment sites. After removal of the amnion, all embryos from one horn were placed together in Bouin Reagents for 24 h, followed by two times 24 h in alcohol. After this procedure each embryo was weighed and digitally photographed. Head size, trunk length and eye diameter of each individually photographed embryo were measured by means of the software program analySIS (Version 3.1, 2001; Soft-Imaging, Germany). Head size was defined as the length from mouth to the neck and trunk length was defined as the length from the neck to the tail bone as illustrated in Figure 1. Variation in embryo development was measured by calculating the coefficient of variance for embryo weight and head size per litter.

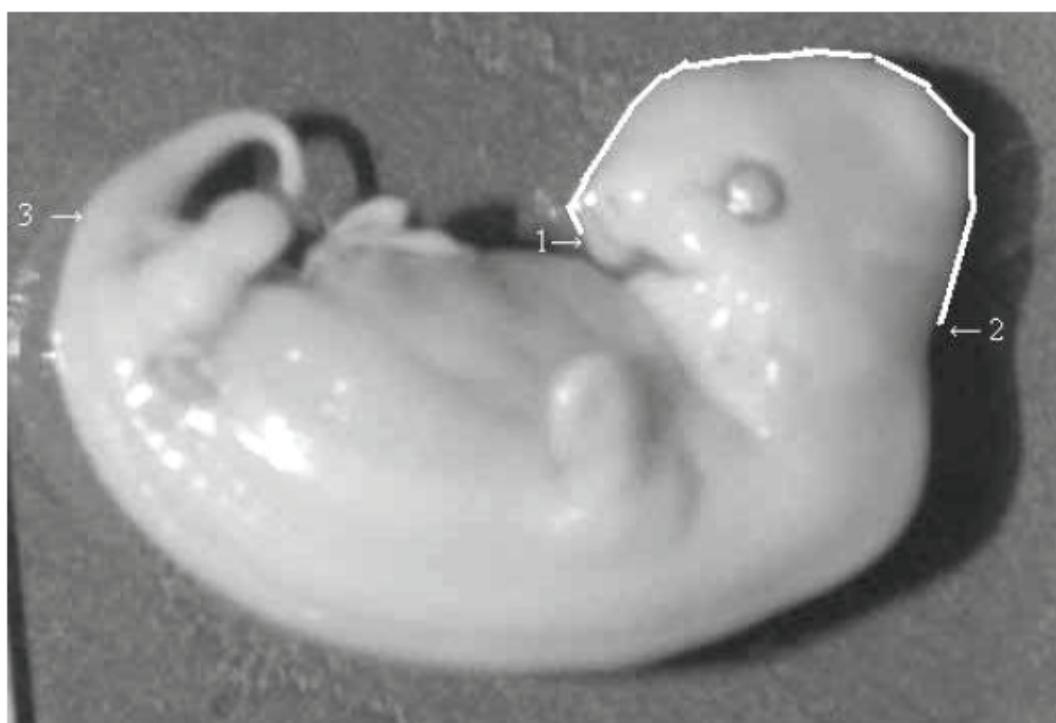


Figure 1. Embryo measurement. Head size was measured as the circumference from mouth (1) to neck (2) and trunk size was measured as the circumference from neck (2) to tailbone (3)

In the allantoic fluid samples and sow plasma collected at slaughter, glucose concentrations were determined spectrophotometrically by means of a commercial kit (GOD-PAP kit, Roche Diagnostics GmbH, Germany).

Sows were identified as pregnant at day 23 when at least one viable embryo was present. When placental attachment sites were observed and/or nonviable embryos were found, sows were considered to have been pregnant before day 23. The percentage of initially pregnant sows was defined as all sows having at least one placental attachment site and/or (non) viable embryos divided by the total number of inseminated sows. The percentage of

sows pregnant at day 23 was defined as the number of sows pregnant at day 23 divided by the total number of sows inseminated. Four C sows were omitted for analysis of embryo development, uterine and placental characteristics because timing of ovulation was not established accurately, resulting in 12 sows for these parameters in the C group.

Weight, back-fat, and body fat/protein mass

Sows were weighed and P2 back-fat was measured at D1 (D0 = day of farrowing) after farrowing and once every week of lactation thereafter (D6, D13, D20, D27, D34, D41). Control sows were weighed until weaning at day 21. Sow back-fat was measured at two points established at the last rib and at 6 cm from the midline. Sow body fat and protein mass were calculated as described by Whittemore and Yang (1989) by using weight and back-fat data.

Statistical Analyses

Data were analyzed using SAS (SAS institute, Cary, NC, USA). Differences between treatments in initial and day 23 pregnancy rates were analyzed using Fischer's exact test. Embryo and placental parameters were averaged per sow and then analyzed. All parameters were analyzed in PROC GLM after a normality test with PROC UNIVARIATE. Time of ovulation, ovulation rate, embryo survival, the total number of embryos and glucose parameters were analyzed in a PROC GLM: $Y_{ijk} = \mu + T_i + B_j + T_i * B_j + e_{ijk}$, with Y_{ij} the dependent variable, μ the mean, T_i treatment ($i=C, IS12, IS6$), B_j batch ($j=1, 2, 3$), and $T_i * B_j$ the interaction between treatment and batch. When the interaction or batch effects were absent ($P>0.05$) they were omitted from the final model. Luteal weight was analyzed in a covariate model with ovulation rate as covariate. The number of viable, nonviable embryos, the number of placentas, placental attachment sites, and uterine parameters were analyzed in the covariate model with the total number of embryos as a covariate. For embryo development parameters the total number of embryos as covariate in the model was not significant and omitted from the model. Sow weight and back-fat were analyzed in a covariate model with weight or back-fat at farrowing as covariate. Sow protein and fat loss during lactation were analyzed in a model with sow protein mass and fat mass at farrowing as a covariate. Sow body weight loss and back-fat loss during the weeks of lactation were tested in a simple GLM-model with treatment, as sow body weight at day 1 as covariate appeared not significant. Correlations were tested in the PROC CORR procedure of SAS. Relationships were calculated per treatment when the interaction between treatment and covariate was significant ($P<0.10$). Values presented are means with standard error (se) unless stated otherwise.

Results

Timing of ovulation and insemination

Timing of ovulation relative to start IS at day 14 or weaning at day 21 for C sows tended to be later in IS6 (149 ± 7 h, n=6) than C sows (131 ± 3 h, n=13; P=0.10) and IS12 sows were intermediate (135 ± 6 h, n=12). On average time of ovulation relative to day of farrowing was 19.5 ± 0.4 days for IS12, 20.3 ± 0.3 days for IS6 and 26.6 ± 0.3 days for C sows.

Pregnancy rate and early embryo survival

Initial pregnancy rate was not affected by Intermittent Suckling and the regime of Intermittent Suckling (IS12 or IS6), (85% IS12 vs 100% IS6 vs 94% C; P>0.10) as shown in Table 1. Although 1 out of 10 IS12 sows and 2 out of 9 IS6 sows lost their initially present embryos, pregnancy rate at day 23 was not affected by treatment either (C: 94%; IS12: 75%; IS6: 78%; P>0.10). Ovulation rate of day 23 pregnant sows (25.9; SD 4.7) and the number of viable embryos (16.0; SD 5.7), were not affected by treatment (P>0.10; Table 2). The number of nonviable embryos at day 23, however, tended to be affected by the regime of IS as IS6 sows had 3.4 nonviable embryos on average and IS12 sows only had 1.4 nonviable embryos (P<0.10). Embryo survival at day 23 after ovulation was higher in C sows (70%) but not significantly different from IS12 (57%) and IS6 sows (51%; P>0.10). No relationship was found between plasma P₄ levels at 75 h after ovulation and embryo survival (P>0.10).

Table 1. Pregnancy rates per treatment

	C	IS12	IS6
Initially pregnant sows ¹	16/17	11/13	9/9
Day 23 pregnant sows	16/17	9/12 ²	7/9

¹ Initially pregnant sows were defined as sows with placental attachment sites at D23 and for IS12 one sow culled before day 23, with embryos. ² One sow was culled at day 8 of pregnancy, therefore number of sows pregnant at D23 is based on 12 sows

Uterine and placental characteristics

Although treatment did not affect the number of embryos at day 23 after ovulation, uterine horn weight was affected; IS12 and IS6 sows had lighter uterine horns than C sows (Table 3; P<0.01). Furthermore, the regime of IS affected placental parameters; placental attachment site area and placental length were negatively affected by IS6 (Table 3).

Table 2. Number of embryos and placentas and embryonic survival per treatment at D23 after ovulation

Parameter	C		IS12		IS6	
	Mean	se	Mean	se	Mean	se
Slaughter day after ovulation (d)	23.0	0.07	23.0	0.09	22.9	0.09
Ovary						
Ovulation rate	24.9	1.2	27.0	1.0	26.6	2.5
Luteal weight (g)	10.5 ^a	0.5	12.9 ^b	0.5	12.0 ^{a,b}	0.6
Number of embryos						
Total	19.3	1.4	16.7	1.4	17.4	3.4
Viable	17.3	1.4	15.2	1.4	14.0	3.0
Nonviable	1.9 ^{c,d}	0.4	1.4 ^c	0.5	3.4 ^d	0.8
Placentas						
Total number of placentas	19.4	1.3	16.8	1.4	17.3	1.2
Placental attachment sites	19.6	1.3	17.1	1.2	17.7	3.2
Embryo survival						
Viable embryos/CL (%)	70	4	57	5	51	9
Total embryos/ CL (%)	78	5	62	5	65	10

^{a,b} Different superscripts within one row indicate P<0.05. ^{c,d} Different superscripts within one row indicate P<0.10

Early embryo development

At day 23 after ovulation, development of C embryos was enhanced when compared to that of IS6 sows (Table 3). C embryo weight was greater (0.20 ± 0.01 g vs. 0.14 ± 0.01 g; P<0.05), C eye diameter was larger (0.80 ± 0.01 mm vs. 0.70 ± 0.02 mm; P<0.05) and C head size was larger (10.5 ± 0.2 mm vs. 9.4 ± 0.3 mm) than that of IS6 embryos. Embryos of IS12 sows were intermediate for these parameters. Rump size was similar between the treatments (P>0.10). The variation in embryo eye diameter was significantly lower in C than IS6 sows (P<0.05) and variation in embryo weight tended to be smaller (P<0.10) in C than IS6 sows. For head size and trunk length no differences in variation were found (P>0.10; Table 3). No relationships were found between plasma P₄ levels at 75 h after ovulation and embryo development or survival (P>0.10). For the two IS treatments no relations were found between sow metabolic parameters during IS (weight loss, fat and protein losses) and embryo development at day 23 of gestation (P>0.10).

Table 3. Uterine and placental characteristics of weaned (C) and limited nursed sows (IS12 and IS6) at day 23 after ovulation

Parameter	C (n=12)		IS12 (n=9)		IS6 (n=7)	
	Mean	se	Mean	se	Mean	se
Uterine characteristics						
Length horns(cm)	353	9.6	350	9.2	320	14.6
Weight horns(g)	2420 ^a	133	1780 ^b	85	1495 ^b	126
Placental attachment sites						
Length (cm)	10.5	0.9	12.4	0.7	11.0	1.0
Area (cm ²)	121 ^a	8	117 ^a	9	92 ^b	10
Placenta						
Length (cm)	20.3 ^a	1.4	20.9 ^a	0.7	17.5 ^b	1.2
Weight (g)	0.19 ^a	0.01	0.15 ^{a,b}	0.02	0.13 ^b	0.02
Embryo						
Weight (g)	0.20 ^a	0.01	0.18 ^{a,b}	0.02	0.14 ^b	0.01
Head size (mm)	10.5 ^a	0.2	9.9 ^{a,b}	0.2	9.4 ^b	0.3
Rump length (mm)	19.5	0.4	20.2	0.5	20.1	0.5
Eye diameter (mm)	0.80 ^a	0.01	0.75 ^{a,b}	0.02	0.70 ^b	0.02
Variation in embryo development						
CV ¹ eye diameter (%)	8.3 ^a	0.8	11.7 ^{a,b}	1.1	13.9 ^b	2.0
CV head size (%)	10.1	1.6	9.2	0.9	9.3	0.9
CV trunk length (%)	5.9	0.9	6.4	1.0	7.1	0.9
CV weight (%)	16.0 ^c	1.9	18.6 ^{c,d}	2.5	27.5 ^d	7.1

^{a,b} Different superscripts within one row indicate P<0.05. ^{c,d} Different superscripts within one row indicate P=0.10. ¹ CV: coefficient of variance.

Glucose levels in sow plasma and allantoic fluid

The regimens of Intermittent Suckling did not affect glucose concentrations in the allantoic fluid and sow plasma at day 23 after ovulation, nor the sow/allantoic fluid concentration ratio (Table 4). As no differences were found between IS12 and IS6 sows, data were pooled. Concentrations of glucose in the allantoic fluid and sow plasma of IS sows (IS12 and IS6) were similar to the concentrations of C sows. The plasma/allantoic fluid glucose ratio, however, was higher in weaned C sows than IS sows (2.9 ± 0.2 vs. 2.4 ± 0.2 ; P=0.05). The levels of glucose found in the allantois at day 23 of gestation were not related to maternal P₄ levels at 75 h after ovulation or embryo survival or embryo development at day 23 of gestation (P>0.10).

Table 4. Glucose concentrations in pregnant C, IS12 and IS6 sows at day 23 after ovulation

Parameter	C		IS12		IS6	
	Mean	se	Mean	se	Mean	se
Glucose concentration in allantoic fluid (mg/dl) ¹	27.6	1.1	30.7	1.8	29.6	2.0
Glucose concentration in sow plasma at D23 after ovulation (mg/dl) ¹	80.1	4.6	75.2	4.4	68.0	6.8
Sow plasma glucose concentration/allantoic plasma glucose concentration ²	2.9	0.2	2.5	0.2	2.3	0.2

C n=16 IS12 n=9 IS6 n=7.¹ Sows were slaughtered in the morning at day 23 after ovulation and had received their last feed the day before slaughter at 20:00 h. ² The sow plasma glucose concentration/allantoic plasma concentration was higher for C (2.9) compared to IS sows (2.4); P=0.05

Sow body weight and back-fat thickness

The change in sow body weight during lactation (21 days in C sows and about 41 days in IS sows) is illustrated in Figure 2. Weight change was similar among the three treatments during the first three weeks of lactation, i.e. including the first week with Intermittent Suckling (P>0.10). During week 5 of lactation IS12 sows lost less weight than IS6 sows (P<0.05). During week 6 of lactation IS12 sows did not loose weight but remained at the same weight whilst IS6 sows still lost weight (P<0.10). Back-fat at farrowing (C: 18.3 ± 0.9 mm vs. IS12: 18.2 ± 0.8 mm vs. IS6: 18.1 ± 0.9 mm) and its change within the first three weeks of lactation (C: -2.3 ± 0.5 mm vs. IS12: -1.8 ± 0.5 mm vs. IS6: -1.8 ± 0.6 mm) were similar between treatments (P>0.10). During the period of IS (day 13- day 41), IS12 sows lost less back-fat (-0.75 ± 0.5 mm) than IS6 sows (-2.7 ± 0.5 mm; P<0.05). At day 1 of lactation (day after farrowing), fat (C 63.7 ± 1.7 kg vs. IS12 64.9 ± 2.3 kg vs. IS6 65.5 ± 2.2 kg and protein (C 45.0 ± 1.0 kg vs. IS12 46.8 ± 1.0 kg vs. IS6 47.5 ± 1.3 kg) mass were similar between the three treatments groups. There were also no differences in fat loss (C 9.7 ± 0.7 kg vs. IS12 9.5 ± 0.9 kg vs. IS6 10.4 ± 1.4 kg; P>0.10) and protein loss (C 3.7 ± 0.3 kg vs. IS12 3.5 ± 0.4 kg vs. IS6 3.9 ± 0.6 kg; P>0.10) during the first three weeks of lactation. During the entire IS period (day 13 to day 41), fat loss (IS6 8.0 ± 1.0 kg vs. IS12 3.9 ± 0.8 kg) and protein loss (IS6 2.2 ± 0.4 kg vs. IS12 0.4 ± 0.4 kg) were significantly greater in IS6 than IS12 sows (P<0.05).

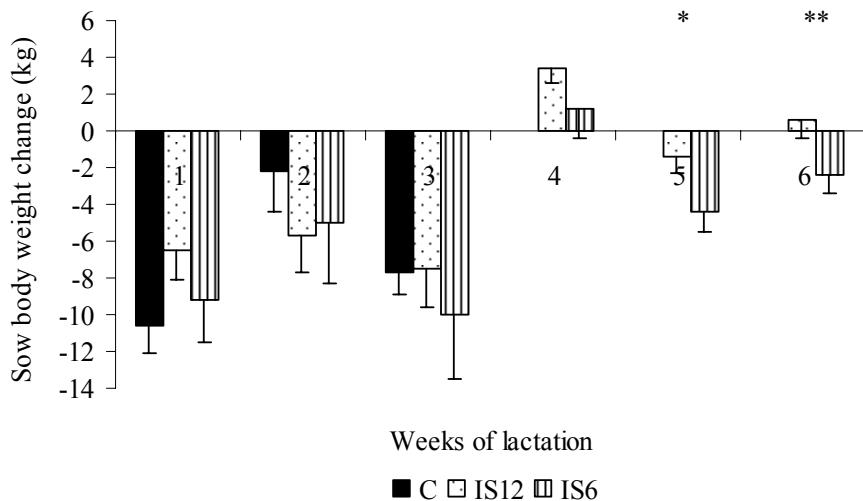


Figure 2. Sow body weight change \pm se during weeks of lactation for C, IS12 and IS6 sows. The number of observations was 17 for C, 13 for IS12 and 9 for IS6. Week 1 is day 0 – day 6, week 2 is day 6 – day 13, week 3 is day 13 – day 20, week 4 is day 20 – day 27, week 5 is day 27 – day 34 and week 6 is day 34 – day 41. (* = $P < 0.05$ and ** = $P < 0.10$ for IS12 vs. IS6)

Discussion

In this study we examined effects of Intermittent Suckling (IS) on pregnancy rates, embryo survival and embryo development at day 23 after ovulation; ovulation, the rise in progesterone, fertilization, and embryo development all occurred during lactation. Other studies in which Intermittent Suckling or limited nursing was used and pregnancy rates or embryo survival were examined, IS was initiated only 48 h before weaning and ovulation occurred after weaning (Britt and Levis, 1982), or IS was applied for a longer period but the majority of ovulations occurred after weaning (Thompson et al., 1981; Grinwich and McKay, 1985). In these studies no effects of IS were found on conception rates (Britt and Levis, 1982; Thompson et al., 1981) and embryo survival rate (Grinwich and McKay, 1985), but for the majority of sows, conception and embryo development occurred in a weaned animal and were therefore hardly comparable to our study.

Although pregnancy and embryo survival rates were not significantly lower in sows undergoing IS, the rates were substantially lower when compared to weaned sows and might have not been significant due to low numbers of animals in the IS treatments (IS12: n=9; IS6 n=7). In IS sows conception and embryo development occurred during lactation but conception also occurred early post-partum (about day 20 pp). So, either one of these factors or the combination of the two might have affected pregnancy rates and embryo survival. For example, Gaustad-Aas et al. (2004) found that serving lactating sows before 3 weeks post-partum results in compromised farrowing rates (8-14 days 50%; 15-21 days 59.5%) when

compared to sows served during lactation at 22-28 days post partum (74.5%). Litter size was not affected. Thus, Gaustad-Aas et al. (2004) also showed that when sows were served during lactation, timing of service post-partum was just as important as it was for service of weaned sows (Varley and Cole, 1976; Belstra et al., 2002). The study of Gaustad-Aas et al. (2004) did not specifically compare farrowing rates between lactating and weaned sows, thus it is still unclear whether and how lactation itself affects farrowing rate. However, in their study, weaned sows served 8-14 days post partum and lactating sows served 8-14 days post partum had similar farrowing rates (47% vs. 50%). This, together with the results of the present study, indicates that sows served during lactation could perform similarly to weaned sows.

A low pregnancy rate and retarded embryo development in sows bred early after farrowing can be caused by incomplete involution of the uterus or incomplete recovery of the hypothalamic-pituitary-ovarian axis. In early weaned sows LH surges were lower than in sows weaned later (10 vs. 35 days; Kirkwood et al., 1984; 21 vs. 35 days; Edwards and Foxcroft, 1983) which was also seen in the IS sows in the present study (Chapter II). The amplitude of the pre-ovulatory LH surge has been found to affect the levels of P₄ after ovulation (Einarsson and Rojkittikhun, 1994) and relations have been found between P₄ levels and embryo parameters (Jindal et al., 1996; Van den Brand et al., 2000b). Therefore it is possible that low LH surges found in early weaned and IS sows indirectly affected embryo survival. In the present study, P₄ levels in IS sows were lower than in conventionally weaned sows but no relation was found between embryo survival and development and P₄ levels at 75 h after ovulation. Therefore it seems unlikely that low levels of P₄ caused the smaller embryos in the IS group directly. However, the low plasma P₄ levels may have an indirect negative effect on embryo survival. P₄ levels are thought to positively influence the number of LH/hCG receptors (Ziecik et al., 1992) which were found in the porcine uterus (Ziecik et al., 1986). These LH/hCG receptors are thought to affect uterine blood flow which could be important for uterine development. Therefore, the low plasma P₄ levels in our study may result in an insufficient increase in LH/hCG receptors in the IS sows, and consequently an incompetent uterus. In other research it was found that incompetence of the uterus to respond to embryo signals (Belstra et al., 2002) or incomplete involution (Svajgr et al., 1974) might negatively affect embryo survival and/ or embryo development. In the present study, uterine weights of the IS sows were low at day 23 after ovulation, which is about day 44 after farrowing and a time at which involution has been completed. These low uterine weights may be an indirect result of low plasma P₄ levels and could be a reflection of a less favourable environment for embryo development. Plasma P₄ levels could therefore be involved in retarded embryo development.

Body weight loss during lactation (e.g. by means of restricted feeding) has been found to negatively affect embryo survival rate and development in primiparous sows bred after weaning (e.g. Zak et al., 1997; Vinsky et al., 2006). Effects of lactation weight loss on embryo parameters, however, were only apparent when weight loss exceeded 10% (Thaker and Bilkei, 2005). All IS sows were fed according to lactational requirements and each

individual sow received a feeding level to fulfil their individual requirements. Yet, IS6 sows lost weight till the end of the experiment whilst IS12 sows gained weight at that time. Body weight loss of IS6 sows can possibly be explained by the level of milk production. It has been reported that creep feed intake of IS6 litters was lower than in IS12 litter whilst their growth was similar, indicating that milk was a more important source of energy for the IS6 litters than creep feed (Berkeveld et al., 2007). This higher milk production in the IS6 sows resulted in a higher body weight loss and as these sows still used more energy from the feed for the production of milk during the follicular phase, and during early pregnancy, this might have affected oocyte and embryo quality (e.g. Zak et al., 1997). However in the present study, no relations were found between weight loss parameters and embryo parameters at day 23 of pregnancy. It is therefore unclear, if the metabolic state of the IS6 sows can be an explanation for the retarded embryo and placental development.

The retarded embryo development in IS6 sows might also have been related to stress. IS6 sows were separated from their litters twice per day whilst for IS12 sows this occurred only once daily. This process of separation may be stressful for the sows and stress is assumed to negatively influence embryo survival (e.g. review Einarsson et al., 1996). Induced stress, mimicked by means repeated ATCH injections, negatively affected embryo development within 48 h after ovulation (Razdan et al., 2002) and negatively affected oestrogen production of embryos by day 20 when the induced stress occurred at day 13 and day 14 of pregnancy (Razdan et al., 2004). However, the induced stress did not result in different embryo development at day 30 (Razdan et al., 2004). Also Soede et al. (2006) did not find effects of stress induced by weekly re-grouping and feed competition up to day 15 of pregnancy on embryo survival at day 35 after insemination. Therefore, effects of stress on embryo survival and development seem controversial and it remains to be investigated if stress is a factor involved in the lower embryo development found in the IS6 sows.

In conclusion, conception rate and embryo survival are not significantly affected by Intermittent Suckling although the numerical differences with weaned sows were present. Embryo and placental development were negatively affected by the regime of IS and this may be due to a combination of high milk production, low plasma P₄ levels, stress, and lactational effects on uterine development. IS12 was intermediate for most embryo parameters at day 23 after ovulation but the question remains how these embryos will develop during later stages of gestation. Furthermore, it remains to be explored whether the negative effects found on embryo and placental development are a result of early ovulation post partum and/ or the fact that the sows are lactating during the follicular phase and early pregnancy.

Acknowledgements

The authors wish to thank Bjorge Laurensen, Frits Rietveld and all the MSc students for their help with the practical work.

IV

Feeding level does not affect progesterone levels in Intermittently Suckled sows with lactational ovulation

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Abstract

The aim of this study was to examine whether the low post-ovulatory plasma P₄ levels found in Intermittently Suckled (IS) sows are related to the high feeding level during intermittent lactation. Multiparous sows (n=21) were separated from their piglets for 12 h per day starting at day 14 of lactation until 6 days after ovulation. At day 28 of lactation, 9 sows had ovulated (spontaneous) and the remaining 12 sows were treated with PG600 (PG600-induced). At ovulation sows were allocated to either a high (H) feeding level (on average 6.5 kg) or a low (L) feeding level (high feeding level minus 2.5 kg) until 6 days after ovulation. Plasma P₄ levels were not affected by feeding level or type of ovulation ($P>0.10$), and neither were CL parameters, embryo survival rate and embryo development at day 30 of gestation. In conclusion, low levels of plasma P₄ were found in IS sows with lactational ovulation but these were not affected by feeding level during the 1st week after ovulation. Further studies are needed to investigate which factors cause the lower plasma P₄ levels in these sows.

Introduction

In a previous experiment, Intermittent Suckling (IS) resulted in lactational ovulation, but post-ovulatory plasma progesterone (P_4) levels were lower in IS sows compared to sows that ovulated after weaning (Chapter II). Several factors could have influenced the plasma P_4 levels in IS sows. For example, in the previous experiment, IS sows had a lower LH surge than weaned sows and the height of the LH surge has been known to influence luteinisation (Einarsson and Rojkittikhun, 1993), consequently affecting P_4 levels. Further, IS sows were fed at a lactational feeding level (6-7 kg/day) whilst weaned sows were fed at maintenance (2.5 kg/day). High feeding levels reduced P_4 levels in gilts (Jindal et al., 1996) and sheep (Parr et al., 1987) and tended to reduce P_4 levels in sows (Virolainen et al., 2005) due to a higher metabolic clearance rate of P_4 (Miller et al., 1999). Thus, low P_4 levels in IS sows could be a result of the high lactational feeding level. The aim of this study therefore is to examine whether P_4 levels in IS sows are affected by post-ovulatory feeding levels.

Materials and Methods

Animals and treatment

The experiment was approved by the Ethics Committee for animal experiments of Wageningen University. The present experiment was part of a larger experiment in which Topigs20 (Topigs, Vught, The Netherlands) sows were used. For the aim of the experiment, only the sows with lactational ovulation were used ($n=21$) of parity 5-9 (6.9 ± 1.1). For frequent blood sampling, sows were fitted with a permanent catheter in the jugular vein on average 23 days (range 17-29 days) before parturition as described in Chapter II. Within 48 h after farrowing, litters were standardized to 11.8 ± 1.1 piglets per litter. From 7 days after farrowing onwards, piglets were provided with creep feed. At day 14 of lactation IS was initiated and sows were removed from their piglets for a continuous period of 12 h per day. At day 28 of lactation, sows that had not ovulated were treated with PG600 (Intervet, Boxmeer, The Netherlands). Sows with lactational ovulation as a result of IS only are referred to as 'spontaneous' sows and sows that ovulated after treatment with PG600 are referred to as 'PG600-induced' sows.

Feeding of the sows during lactation and after final weaning was carried out as described in Chapter II. At ovulation, sows were allocated to either a high (H) feeding level (1% of body weight plus 0.5 kg per piglet) or a low (L) feeding level (H - 2.5 kg) until 6 days after ovulation. H fed sows were fed 6.5 kg daily on average (actual intake: 6.2 ± 0.3 kg/day) and L fed sows received 4.0 kg daily on average (actual intake: 3.9 ± 0.4 kg/day). IS continued until piglets were fully weaned at 6 days after ovulation. Transrectal ultrasonography was performed every 12 h to establish time of ovulation by using a 7.5 MHz

annular array sector probe and ultrasound scanner (Scanner 200, Pie Medical, Maastricht, The Netherlands).

Blood sampling

From ovulation onwards, blood samples were taken every 12 h before feeding until 8 days after ovulation. Blood samples were collected, stored and analyzed for P₄ as described in Chapter II. As a result of catheter obstruction, blood samples could not be taken for all animals at all times and therefore the number of animals used for P₄ analyses is seventeen.

Uterus, placenta and embryo analyses

At day 30 after ovulation (30 ± 0.2 days), reproductive tracts of all sows were collected and analyzed. Numbers of corpora lutea, and numbers of viable and non-viable embryos were determined. Luteal weight, embryo crown-rump length and embryo weight were measured as well as placental length and weight. Sows were identified as pregnant at day 30 when at least one viable embryo was present.

Statistical Analyses

Data were analyzed using SAS (SAS institute, Cary, NC, USA). For all PROC GLM models in which feeding level (high/low) and lactational ovulation (spontaneous/PG600-induced) were included, interactions were tested and omitted when not significant ($P>0.10$). Time of ovulation relative to start IS or PG600-induction was analyzed with type of lactational ovulation as treatment effect. CL parameters, P₄ levels at different time points, embryo survival and development, and uterine and placental parameters were all tested in a covariate model with either the total number of CLs, the total number of embryos or gestation day as covariate. P₄ levels after final weaning were tested in a nested model in which treatment was tested against sow nested within treatment. Differences between treatments in pregnancy rate were analyzed using the Fisher's exact test. Values presented in the results are means with standard errors unless stated otherwise.

Results

Spontaneous sows ovulated at day 21.2 ± 0.8 of lactation on average and PG600-induced sows at day 33.7 ± 0.3 . Ovulation in spontaneously ovulating sows (156.7 ± 6.0 h) occurred later after start IS than in PG600-induced sows relative to PG600 injection (137.0 ± 5.2 h; $P<0.05$).

Ovulation rates were not affected by type of ovulation (Table 1; $P>0.10$). Feeding level tended to affect luteal weight, resulting in a higher luteal weight for H sows ($P<0.10$; Table 1). P₄ levels were not affected by feeding level or type of ovulation (Figure 1). Further, the concentration of P₄ after final weaning (156-192 h), remained similar between H or L

sows and spontaneous or PG600-induced sows ($P>0.10$). No correlation was found between actual individual feed intake and P_4 at any time or luteal weight and P_4 ($P>0.10$). Ovulation rate did not affect the P_4 concentration ($P>0.10$).

Day 30 pregnancy rates and embryo survival rate did not differ between H and L sows or between spontaneous and PG600-induced sows ($P>0.10$; Table 1). Type of lactational ovulation did not affect embryo development ($P>0.10$) but embryo weight at day 30 tended to be higher in H (1.51 ± 0.11 g) than in L (1.48 ± 0.05 g; $P<0.10$) sows. Placenta development was not affected by feeding level but placentas of sows with a spontaneous lactational ovulation were smaller (36.8 ± 1.2 cm) and weighed less (19.9 ± 1.5 g) than those of PG600-induced sows (46.2 ± 1.9 cm and 31.3 ± 3.1 g; $P<0.05$). Embryo survival and embryo development were not related to the level of P_4 at any time ($P>0.10$).

Table 1. Corpora Lutea parameters at day 30 of pregnancy and P_4 levels in early pregnancy for the variables feeding level during early pregnancy and lactational ovulation (spontaneously vs. PG600-induced)

	Feeding Level		Lactational Ovulation	
	H	L	Spontaneous	PG600-Induced
	n=10	n=11	n=9	n=12
Ovulation rate	28.9±2.3	26.3±1.5	27.8±2.3	27.8±2.0
Luteal weight (g)	9.5±0.6 ^c	7.7±0.7 ^d	9.3±1.0	8.5±0.6
P ₄ concentration (ng/ml) at 72 h after ovulation	8.1±1.5	8.1±0.8	8.4±1.0	7.9±1.3
Day 30 pregnancy rate	80 (8 /10)	67 (6/9)	71 (5/7)	75 (9/12)
Embryo survival (%)	65±4	55±10	57±8	62±6

Different superscripts ^(c,d) within one row indicate a tendency for a difference between the variables feeding level or lactational ovulation ($P<0.10$). Values presented are means ± se

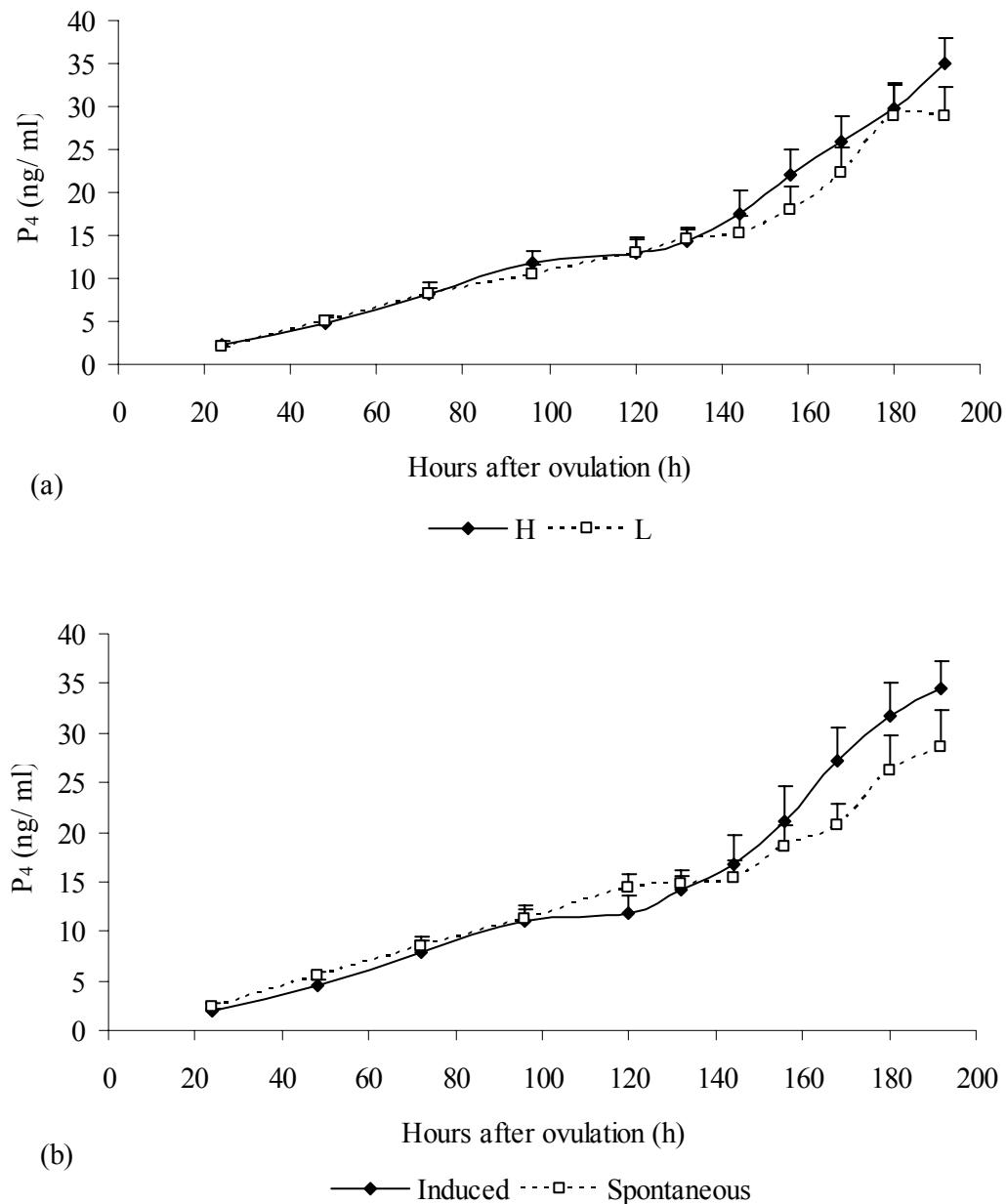


Figure 1. Profiles of progesterone (P_4) relative to the time of ovulation for (a) Feeding level (H/L) and (b) Lactational ovulation (spontaneous/PG600-induced). Litters were fully weaned at 144 h after ovulation of the sow. The number of observations was 8 for H, 9 for L, 8 for spontaneous and 9 for induced. (Means + se).

Discussion

In the present study, plasma P₄ levels during the first week after lactational ovulation were not affected by feeding level in that week. In previous work we found that IS sows fed at a lactational feeding level had low plasma P₄ levels in comparison to weaned control sows fed at maintenance level (Chapter II). The difference in feeding level could have been the cause of this difference in P₄ levels as high feeding levels have been found to result in low plasma P₄ levels during early pregnancy in sheep (Parr et al., 1987), gilts (e.g. Jindal et al., 1996), and in multiparous sows ($P<0.10$; Virolainen et al., 2005). Animals fed at high feeding levels have an increased blood flow through the liver and a higher metabolic clearance rate (Parr et al., 1993; Miller et al., 1999), resulting in a higher clearance of P₄ (Parr et al., 1993; Sangsritavong et al., 2002). The high feeding level in the current experiment did not seem to have this effect, as P₄ levels were comparable between the H and L feeding level. Furthermore, the plasma P₄ levels of the present experiment are comparable to the plasma levels of P₄ found for IS sows in our earlier study. The difference in feeding level in the current experiment (H 6.5 kg vs. L 4 kg) should be sufficient in inducing differences in P₄, when compared to the differences between high and low fed animals in other experiments, e.g. 0.7 kg in gilts (2.6 kg vs. 1.9 kg; Jindal et al., 1996) or 2 kg in multiparous sows (4 kg vs. 2 kg; Virolainen et al., 2005). As no difference in P₄ was found between feeding levels, there must be other factors that cause low P₄ levels in IS sows.

In the former experiment, control sows (with high P₄ levels) were weaned and IS sows (with low P₄ levels) were lactating (Chapter II). Lactation itself may be one of the factors affecting P₄ levels, since in cows basal liver blood flow was greater during lactation, presumably related with the high feeding levels (Sangsritavong et al., 2002). Possibly, basal liver blood flow and metabolic clearance rate are already high in IS sows due to lactation, which may override effects of different feeding levels during IS.

Another possible reason for the low P₄ levels may be insufficient luteinisation as a result of follicle development and ovulation during lactation. In IS sows, the height of the LH surge tended to be lower than in weaned control sows and within IS sows a correlation was found between the height of the LH surge and P₄ levels (Chapter II). Proliferation and luteinisation of granulosa cells have been found to be influenced by the height of the LH surge (Einarsson and Rojkittikhun, 1993) and when proliferation is insufficient, a reduction of the number of luteal cells is the result (Smith, 1986). It is therefore possible that also in these sows a low LH surge resulted in poor luteinisation and thus in low P₄ levels.

P₄ levels may have also been reduced due to metabolic factors such as insulin (Smith et al., 2006) or IGF-1 (Webb et al., 2002) but these have not been measured in the current experiment.

Embryo survival was not affected by feeding level. In gilts, embryo survival was reduced due to a high feeding level, which was related to the P₄ concentrations during early

pregnancy (Jindal et al., 1996). In the present study no relations were found between P₄ concentrations and embryo survival at day 30 after ovulation. In conclusion, in IS sows, feeding level in the first week after ovulation did not affect plasma P₄ levels or embryo survival; the cause of the low P₄ levels in IS sows remains unclear.

In the present study, ovulation occurred either spontaneously as a result of IS or was induced by PG600 during IS. Levels of P₄ did not differ between spontaneous and induced sows although the PG600-induced sows ovulated later post partum (about 11 days later) than the spontaneously ovulating sows. Other studies examining the effect of a short lactation length did not find effects on P₄ (Belstra et al., 2002; Willis et al., 2003) which is concurrent with the results of the present study.

Conclusion

In sows subjected to IS, levels of plasma P₄ were low, but not affected by feeding level during the first week after ovulation. Whether the low plasma P₄ levels in IS sows are caused by lactation (high liver blood flow, metabolic status) or insufficient luteinisation needs to be further investigated.

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V

Hormone profiles and establishment of pregnancy in sows in an Intermittent Suckling regimen with start at day 14 or 21 of lactation and continued up to or after ovulation

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Submitted

Abstract

Earlier work has shown that LH and P₄ levels were low and embryo development was retarded when Intermittent Suckling (IS) was initiated at day 14 of lactation and continued till day 23 after ovulation. Therefore the aims of the present study were to examine effects of timing of start of IS, to compare IS sows with weaned control sows and to examine effects of continuation of IS after ovulation on hormone profiles and embryo survival. Multiparous Topigs40 sows (n=95) were weaned at day 21 (C) or subjected to IS; IS starting at day 14 (IS14) or 21 (IS21) of lactation and separation for 12h daily. IS sows were weaned at ovulation (W_{ov}) or 20 days after ovulation (W_{ov+20}). Numbers of sows ovulating within 8 days after start of treatment were similar between treatments ($P>0.10$). Early start of IS resulted in a shorter duration of oestrus ($P<0.05$), lower P₄ levels at day 7 (IS14: 25±2 ng/ml vs. IS21: 28±2 ng/ml; $P<0.10$) and embryo survival at day 30 (IS14: 50±4 % vs. IS21: 61±5 %; $P<0.10$). IS21 sows ovulated later than C sows and oestrous duration was longer ($P<0.05$). Continuation of IS during pregnancy (W_{ov+20}) resulted in lower P₄ levels at day 7 and 12 (W_{ov}: 46±2 ng/ml vs. W_{ov+20}: 28±2 ng/ml; $P<0.05$), a similar embryo survival, but negatively affected embryo development ($P<0.10$). Start of IS at day 21 of lactation and weaning at ovulation seems to be the best option when conception is to be achieved during lactation.

Introduction

Weaning of piglets at a relatively young age is often accompanied by a reduced growth and feed intake (Van Beers-Schreurs et al., 1992) compromising piglet welfare. Extending lactation would increase piglet welfare but reduces the number of litters per sow per year due to the anoestrus block during lactation e.g. (Armstrong et al., 1988). Separation of sows and piglets for a period of the day, Intermittent Suckling (IS), has been found to increase piglet creep feed intake during lactation (Kuller et al., 2004; Berkeveld et al., 2007) and resulted in oestrus and ovulation (Chapter II) and establishment of pregnancy during lactation (Chapter III). However, despite a normal follicle development, oestradiol concentration and ovulation, peak plasma LH levels and post-ovulatory progesterone (P_4) levels were lower in IS sows than sows weaned after 21 days of lactation (Chapter II). Furthermore, embryo survival was numerically lower and embryo development was negatively affected (Chapter III). In this previous experiment IS was initiated at day 14 of lactation and all sows ovulated within 7 days after start of IS (around day 21 post partum), whilst the control sows ovulated within 7 days after weaning (around day 28 post partum), so timing of ovulation occurred at two different stages post partum. In other studies in which sows were weaned early post partum, lower peak LH levels (Edwards and Foxcroft, 1983; Willis et al., 2003) and farrowing rates were found (Gaustad-Aas et al., 2004). Furthermore, a relation has been found between peak LH levels and luteinisation (Einarsson and Rojkittikhun, 1993) and this might explain the lower P_4 levels found in IS sows. It is still not known to what extend the timing of start of IS (day 14 vs. day 21 of lactation affects peak LH levels and P_4 levels and consequently reproductive performance in terms of oestrus and embryo survival.

As the IS sows in our previous experiment (Chapter II) were lactating during the follicular phase, the low peak LH levels could also be caused by factors associated with lactation such as release of endogenous opioids released by the suckling stimulus or stress due to separation (Chapter II). In this respect one might investigate this possibility comparing LH secretion between IS sows and weaned control sows where treatment (IS or weaning) starts for both groups on day 21 of lactation.

Lactation during the follicular phase and after ovulation can also have affected P_4 levels through its effects on follicular granulosa cell quality with subsequent effects on CL quality (Smith, 1986) or through high liver blood flow (Sangsrivatavong et al., 2002). If IS will be used in combination with extended lactation, sows will be pregnant during lactation and one may ask whether the low P_4 levels and differences found in embryo parameters are a result of the continuation of lactation post ovulation or that lactation during the follicular phase affects follicle quality. This could be studied by weaning IS sows either at ovulation (follicular development during lactation) or at 20 days after ovulation (follicle development and early pregnancy during lactation) and comparing P_4 levels and embryo survival and embryo development.

The present study therefore has the following three aims: 1) to examine effects of timing of start of IS (day 14 vs. day 21 of lactation) on follicle development, hormone profiles and day 30 pregnancy parameters, 2) to compare reproductive parameters of IS sows with weaned control sows where day 21 of lactation is start of IS or weaning, 3) to examine effects of continuance of IS during pregnancy by weaning sows at ovulation (follicular development during lactation) or 20 days after ovulation (follicle development and early pregnancy during lactation) on progesterone levels and day 30 embryo survival and embryo development parameters.

Materials and Methods

Experimental design

The experiment was approved by the Ethics Committee for animal experiments of Wageningen University. Five batches of in total 95 multiparous Topigs40 sows (Topigs, Vught, The Netherlands) were used in the period March 2006-March 2007. Before parturition, sows were assigned to one of three treatments based on parity (4.9 ± 0.2 ; mean \pm se), body weight (260 ± 2.3 kg) and back fat measured at the P2 site (15 ± 0.3 mm) at three months of gestation. Sows were selected to farrow within a range of 3 to 4 days. When sows did not show any signs of farrowing within the range, the sows were induced to farrow at their due date or the day after by means of 2 ml of Dinolytic i.m. (Pfizer bv, Capelle a/d IJssel, The Netherlands). Litters were standardized within 3 days after farrowing (on average 10.9 piglets).

Treatments

Sows were assigned to either a control (C) group in which sows underwent continuous lactation until final weaning at day 21 of lactation (n=30), or to one of two Intermittent Suckling (IS) groups with start of IS at day 14 (IS14; n=32) or day 21 (IS21; n=33) of lactation. Sows in either one of the IS groups were separated from their litters for 12 consecutive hours per day from 7:45 h to 19:45 h. Within the two IS groups, sows were allotted to one of two weaning times; either weaning immediately after ovulation (W_{ov}) or weaning 20 days after ovulation (W_{ov+20}).

Each treatment group was housed in a different farrowing unit to prevent possible effects on other treatments. When the sows were with their litter, sows were housed in farrowing crates. During the period of separation and after final weaning sows were housed in a unit with individual crates. In this unit, auditory, visual and olfactory stimuli of the piglets were absent.

Housing and feeding

An easily digestible creep feed (11.4 MJ/kg Metabolizable Energy (ME), 17.8% Crude Protein (CP)) was provided for the litters from one week before the start of IS or weaning of the C sows onwards. During lactation sows were fed three times daily with a commercial lactational feed (12.8 MJ/kg ME, 145 g/kg CP), the daily allowance increasing stepwise from 3.5 kg at farrowing to 1% of body weight plus 0.5 kg per piglet at day 10. After final weaning, sows received 2.5 kg of a commercial gestation feed (12.45 MJ/kg ME, 140 g/kg CP). From ovulation onwards, lactating sows (W_{ov+20}) received their maximum lactational allowance minus 1 kg. Sows had ad libitum access to drinking water at all times.

Blood sampling and hormone analyses

For frequent blood sampling sows from batch 1 and 2 were surgically fitted with a permanent jugular vein catheter 14.2 days (range 11 to 21 days) before parturition as described by Soede et al. (1997). From 48 h after start of IS or weaning of C sows, blood samples were collected every 3 h until time of ovulation. From ovulation until day 7 after ovulation, blood samples were collected every 12 h and an additional blood sample was taken at day 12 after ovulation. For batches 3 to 5, blood samples were taken from the jugular vein at day 7 and day 12 after ovulation. For all pregnant sows from all batches, blood samples were taken at time of slaughter at day 30 after ovulation. Blood samples were collected in tubes containing 100 µl of EDTA solution, placed on ice immediately after collection and centrifuged at 2000g for 10min at -4°C. Plasma was stored at -20°C until analyses.

Concentrations of LH and progesterone were determined as described in Chapter II. Specificity of the RIA was high for LH, as indicated by low cross-reactivity for other pituitary hormones (Van den Brand et al., 2000a), and for progesterone, as indicated by low cross-reactivity for other steroid hormones (Dieleman and Bevers, 1987) and by the observed parallelism. The limit of quantitation was 0.2 ng/ml for LH and 0.1 ng/ml for progesterone.

In general, the intra- and inter-assay coefficients of variation were <10 and <15% for both assays, respectively.

LH and P₄ profiles were analyzed for animals with ovulation within 8 days after start of the treatment only. Due to catheter obstruction, blood samples could not be taken for all animals at all times. The number of LH profiles obtained was 7 for C, 8 for IS14 and 8 for IS21. The number of P₄ profiles obtained from ovulation to 168 h after ovulation was 7 for C, 4 for IS14W_{ov}, IS14W_{ov+20}, and IS21W_{ov} and 3 for IS21W_{ov+20}. For analysis of P₄ on days 7 and day 12, the numbers of samples available were 16, 12, 11, 9 and 9, for groups C, IS14W_{ov}, IS14W_{ov+20}, IS21W_{ov} and IS21W_{ov+20} respectively.

Oestrus detection, follicle development, time of ovulation and insemination

Oestrus detection was carried out every 12 h, starting 2 days after start of IS or weaning (C sows), by performing a Back-Pressure-Test (BPT) in absence of a boar. A sow was regarded to be in oestrus when she reacted with a frozen posture and arched back to the BPT. Onset of oestrus was defined as the first time a sow exhibited a standing response minus 6 h (half the time since the former oestrus check). End of oestrus was defined as the last time a sow exhibited a standing response plus 6 h.

To study follicle development, the ovaries were examined daily by means of rectal ultrasonography using a 7.5 MHz annular array sector probe and ultrasound scanner (Scanner 200, Pie Medical, Maastricht, The Netherlands), starting at the day of start of IS and at day of weaning (C sows). The diameters of the four largest follicles on the ovary were measured. When the average follicle diameter of the four largest follicles was greater than 6.0 mm or when the sow was in oestrus, ultrasound scanning was performed every 12 h to establish time of ovulation. Time of ovulation was defined as the time when no follicles could be detected minus 6 h (half the time elapsed since the former scan). During a scan 12 h later, ovulation was confirmed. Sows were inseminated on each day of standing oestrus or when follicle size was greater than 7.0 mm. On average sows were inseminated 2.3 times (SD 0.6).

Embryo analyses

At day 30 after ovulation, sows were slaughtered and their reproductive tracts were collected and kept on ice until analyses. Sows were classified as pregnant at day 30 when at least one viable embryo was present at day 30. Sows were classified as initially pregnant when at least one placental attachment site and/or (non) viable embryos were present at day 30. Ovaries were removed and the number of corpora lutea and, after dissection, luteal weight was determined. After removal of the mesometrium and separation of the uterine horns, the horns were opened at the antimesometrial side. The number of embryos was counted and each embryo was classified as viable or non-viable based on signs of degeneration of the embryo such as color and size of the embryo. Placental viability was also determined based on color and development of the placenta (necrosis of placenta). Embryos and placentas were removed from the uterine horn and separated from each other. Length of the individual placentas was measured immediately and weight was determined after the placentas were freeze-dried. The length and weight of the empty uterine horns were determined, as well as the number, length and width of the placental-attachment sites. Individual viable embryos were weighed and their crown-to-rump length was measured.

Statistical Analyses

Data were analyzed using SAS (SAS institute, Cary, NC, USA). Only sows with ovulation within 8 days after the start of treatment were used for analyses. One IS14 sow ovulated but with an ovulation rate of 4 and very low P₄ levels and was removed from analyses of LH, P₄,

ovulation and pregnancy rate data. Fisher's exact test was used to test for effects of timing of start IS (IS14 vs. IS21) on the number of sows expressing oestrous behaviour, ovulating and becoming pregnant. Effects of timing of start IS on oestrus parameters, follicle development , ovulation rate and peak LH levels were tested in the following model with the GLM procedure of SAS: $Y_{ij} = \mu + T_i + B_j + T_i * B_j + e_{ijk}$ with Y_{ij} the dependent variable, μ the mean, T_i treatment ($i=IS14, IS21$, B_j batch ($j=1,2,3,4,5$) and $T_i * B_j$ the interaction between treatment and batch. When interactions and batch effects were absent, they were omitted from the final model. Levels of LH over time were tested with the nested model: $Y_{ij} = \mu + T_i + sow_j(T_i) + S_k + (T * S)_{ik} + e_{ijk}$ with Y_{ij} being the LH level, μ the mean, T_i treatment ($i=IS14, IS21$), $sow_j(T_i)$ = the effect of sow_j ($j=1, \dots, 22$) nested within treatment, S_k =the time of sampling ($k=-27$ to -21, or 21 - 27 hours relative to peak LH), $T_i * S_k$ = the interaction between treatment and time of sampling. Effect of treatment was tested against sow nested within treatment. The above described models were also used to compare IS21 sows with the control group (C) for the measured parameters. After ovulation, the effect of timing of start IS and the effect of continuation of IS on embryo, placental and uterine parameters, P₄ at day 7, 12 and 30, were tested in the model: $Y_{ij} = \mu + P_i + W_j + P_i * W_j + e_{ijk}$ with P_i the start of IS relative to parturition (IS14, IS21), W_j the time of weaning relative to ovulation (W_{ov}, W_{ov+20}), $P_i * W_j$ the interaction between the start of IS and time of weaning. Furthermore, to study differences between sows weaned after a 21 day lactation (C) and the combination of IS (IS14, IS21) and time of weaning (W_{ov}, W_{ov+20}) a model was run for all post-ovulatory parameters in which five treatments were used in a covariate model: $Y_{ij} = \mu + T_i + B_j + b_1 X_{ijk} + e_{ijk}$ with Y_{ij} the dependent variable, μ the mean, T_i treatment ($i=IS14W_{ov}, IS14W_{ov+20}, IS121W_{ov}, IS21W_{ov+20}, C$), B_j batch ($j=1,2,3,4,5$), X_{ijk} the covariate. The covariate for the embryo, placental and uterine parameters was the total number of embryos and was ovulation rate for P₄ and luteal weight.

P₄ levels over time were run in the same model as for LH but with T_i treatment ($i = IS14W_{ov}, IS14W_{ov+20}, IS121W_{ov}, IS21W_{ov+20}, C$) and S_k =the time of sampling ($k=$ from 0 h to 24 h, or from 144 to 168 h relative to ovulation). Relations between parameters were tested by calculating Pearson correlation coefficients. All values presented are means \pm se unless stated otherwise.

Results

Follicle development, oestrus and ovulation

Timing of start of IS relative to parturition did not influence the numbers of sows with follicle diameters reaching pre-ovulatory size (>6mm) within 8 days after start of IS (87% IS14 vs. 100% IS21; $P>0.10$). Furthermore, IS initiated at day 21 of lactation resulted in a similar number of sows with pre-ovulatory follicles within 8 days as control (C) sows weaned at day 21 of lactation (100% vs. 100%). Of the sows with follicle growth up to pre-ovulatory size,

73% of the IS14 (22/30), 87% of the IS21 (20/23) and 90% of the C (17/19) sows ovulated (Table 1; $P>0.10$). The three non-ovulating IS21 sows showed oestrous behaviour but the follicles regressed and new follicles developed within the next 7 days. In the non-ovulating IS14 and C sows, cystic follicles were found on the ovaries. Early start of IS (IS14) was accompanied by a smaller follicle diameter at start of IS ($P<0.05$; Table 2) and follicle size during the first four days of IS remained smaller than in IS21 sows ($P<0.05$). When correcting for follicle diameter at start of IS, no differences were found in follicle growth between IS14 and IS21 sows ($P>0.10$). IS21 sows had a similar follicle diameter as C sows at day 21 and did not differ in follicle growth up to ovulation ($P>0.10$) but the interval from peri-ovulatory size to ovulation was longer for 15 h longer for IS21 (62 ± 6 h) than C sows (47 ± 4 h; $P<0.05$).

The interval from start of IS to onset of oestrus did not differ between the two IS treatments ($P>0.10$) but oestrus duration tended to be longer in IS21 than in IS14 sows ($P<0.10$) and was also longer than for C sows ($P<0.05$; Table 2). Timing of ovulation relative to start of IS was not affected by stage of lactation ($P>0.10$) but C sows ovulated 19 h earlier after weaning than IS21 sows after start of IS ($P<0.05$; Table 2).

Table 1. Follicle development, oestrus and ovulation within the first 8 days after start of IS/weaning

	IS14	IS21	C
Number of sows			
- Number of sows excluded at start of IS/weaning ¹	1/31	10/33	11/30
- Number of sows included in the treatments	30	23	19
Follicle development < 6.0 mm			
- No pre-ovulatory follicles (< 6mm)	4 [0]	0 [0]	0 [0]
Follicle development > 6.0 mm			
- Ovulation within 8 days after start of IS/ weaning	22 [20]	20 [19]	17 [17]
- No ovulation			
○ Regression of follicles ²	1 ³ [0]	3 [3]	0 [0]
○ Cystic follicles	3 [2]	0 [0]	2 [2]

¹These sows were in oestrus or had ovulated at start of IS or weaning at day 21 and were excluded from the treatments.

²This group represents sows with follicle growth up to pre-ovulatory size without ovulation and subsequent follicle regression.

³This sow showed follicle growth up to pre-ovulatory size in response to IS but the follicles regressed. Newly developed follicles ovulated 10 days after start IS. [] between brackets: the number of sows showing oestrous behaviour.

Peri-ovulatory LH levels

Basal LH levels before the LH surge (-27 to -21 h before peak LH levels) were not affected by timing of start of IS and were not different between IS21 or C sows ($P>0.10$). Peak LH levels were numerically (but not significantly) lower when start of IS was early (IS14; Figure 1 and Table 2; $P>0.10$). After the LH peak (21 to 27 h after peak LH levels), no differences in

LH levels were found between the two IS groups ($P>0.10$) but IS21 sows tended to have higher LH levels than C sows ($P<0.10$). The interval from start IS/weaning to peak LH levels was greater in IS21 (126 ± 8 h) than in C sows (99 ± 8 h; $P<0.05$; Table 2).

Table 2. Oestrus, follicle development and LH parameters of sows with ovulation within 8 days after start IS/ weaning

Parameter	IS14	IS21	C
Follicle development			
Follicle diameter at start of treatment (mm)	2.7 ± 0.13^a	3.5 ± 0.12^b	3.5 ± 0.12
Follicle diameter at day 4 after start IS/weaning (mm)	6.1 ± 0.13^a	6.6 ± 0.11^b	6.6 ± 0.12
Follicle diameter at ovulation (mm)	7.2 ± 0.10	7.4 ± 0.08	7.2 ± 0.07
Ovulation rate	26 ± 2	27 ± 1	25 ± 2
Oestrus			
Duration of oestrus (h)	48 ± 5^c	$63 \pm 5^{d,x}$	49 ± 4^y
LH			
Peak LH (ng/ml)	4.3 ± 0.3	5.0 ± 0.6	5.2 ± 0.4
Intervals			
Interval start IS/weaning to follicle diameter ≥ 6 mm (h)	99 ± 4	91 ± 3	90 ± 4
Interval start IS/weaning to onset oestrus (h)	112 ± 6	105 ± 3	100 ± 6
Start IS/weaning- peak LH (h)	120 ± 6	126 ± 8^x	99 ± 8^y
Interval start IS/weaning to ovulation (h)	154 ± 5	151 ± 4^x	135 ± 4^y

^{a,b}Different superscripts within one row indicate $P<0.05$ between IS14 and IS21 sows. ^{c,d}Different superscripts within one row indicate $P<0.10$ between IS14 and IS21 sows. ^{x,y}Different superscripts within one row indicate $P<0.05$ between IS21 and C sows.

Post-ovulatory P_4 levels

Plasma P_4 profiles during the first 7 days after ovulation were assessed in the first two batches (see Figure 2). During the early P_4 rise after ovulation (0 to 24 h after ovulation) no effects were found stage of lactation, continuation of IS during pregnancy, nor were there significant differences between the five treatments ($P>0.10$). Between day 6 and 7 after ovulation, continuation of IS during pregnancy affected P_4 levels; weaning at day 20 after ovulation (W_{ov+20}) resulted in lower P_4 levels ($P<0.01$). When analyzing the combinations of timing of start of IS relative to parturition and continuation of IS during pregnancy in a model with C sows, P_4 levels were also lower in sows lactating after ovulation (IS14 W_{ov+20} and IS21 W_{ov+20}) than sows weaned at ovulation (IS14 W_{ov} and IS21 W_{ov}) or weaned control sows (C; $P<0.05$). Furthermore P_4 levels between day 6 and 7 were affected by timing of start of IS relative to

parturition, within sows weaned at ovulation (W_{ov}), as P_4 levels were higher in IS21 than IS14 sows ($P<0.05$). In samples taken at day 7 and 12 in all sows, timing of start of IS relative to parturition tended to affect P_4 levels at day 7 (IS21: 28 ± 2 ng/ml vs. IS14: 25 ± 2 ng/ml; $P<0.10$) but not at day 12 ($P>0.10$). Continuation of IS affected P_4 levels at both day 7 and 12 ($P<0.05$); P_4 levels were lower in sows with continued lactation after ovulation (IS14 W_{ov+20} and IS21 W_{ov+20} ; on average 21 ± 2 ng/ml at day 7) than sows weaned at ovulation (IS14 W_{ov} and IS21 W_{ov} ; on average 32 ± 2 ng/ml at day 7) or C sows ($P<0.05$; Table 3), but at day 30 after ovulation all differences had disappeared ($P>0.10$; Table 3). Peak LH levels were not related to P_4 levels at 72 h, day 7, day 12 or day 30 after ovulation ($P>0.10$).

Table 3. Progesterone parameters of sows ovulating within 8 days after start of IS/ weaning

P_4 parameter	IS14		IS21		C
	W_{ov}^1 (n=11)	W_{ov+20} (n=11)	W_{ov} (n=9)	W_{ov+20} (n=10)	(n=16)
P_4 at day 7 after ovulation	31 ± 2^a	21 ± 2^b	36 ± 2^a	21 ± 2^b	33 ± 2^a
P_4 at day 12 after ovulation	47 ± 2^a	27 ± 3^b	47 ± 3^a	30 ± 2^b	44 ± 3^a
P_4 at day 30 after ovulation ²	36 ± 2	33 ± 2	33 ± 2	36 ± 2	33 ± 2

¹One IS14 W_{ov} ovulated within 8 days after start IS but had an ovulation rate of 4 and was therefore excluded from P_4 analyses. ²The number of observations at day 30 after ovulation is 10 for IS14 W_{ov} , 8 for IS14 W_{ov+20} , 9 for IS21 W_{ov} , 8 for IS21 W_{ov+20} and 12 for C. P_4 at day 7 after ovulation: IS14 sows (25 ± 2 ng/ml) vs. IS21 (28 ± 2 ng/ml; $P<0.10$) and W_{ov} (33 ± 2 ng/ml) vs. W_{ov+20} (21 ± 1 ng/ml; $P<0.05$). P_4 at day 12 after ovulation W_{ov} (47 ± 2 ng/ml) vs. W_{ov+20} (28 ± 2 ng/ml; $P<0.05$). ^{a,b} Different superscripts within one row indicate $P<0.05$ for analysis of five treatments.

CL parameters and pregnancy rates

Initial pregnancy rates varied between 82% and 100% and day 30 pregnancy rates between 76% and 100% and these were not affected by timing of start of IS or continuation of IS during pregnancy and did not differ from weaned C sows ($P>0.10$; Table 4). Also ovulation rates and luteal weights were similar between treatments ($P>0.10$; Table 5).

Table 4. Pregnancy rates per treatment group

	IS14 ¹		IS21 ²		C
	W_{ov}	W_{ov+20}	W_{ov}	W_{ov+20}	
Initially pregnant ³	10/10	9/11	9/9	9/10	16/17
Pregnancy day 30	10/10	9/11	9/9	8/10	13/17

¹Pregnancy rates of IS14 were based on 10 and 11 animals as one IS14 W_{ov} sow only had an ovulation rate of 4 and was therefore removed from analyses. ²One IS21 W_{ov+20} sow with ovulation could not be inseminated and was therefore not used for pregnancy rates. ³Initially pregnant was defined as sows with placental attachment sites at day 30 or sows not showing estrus behavior within 3 weeks after insemination

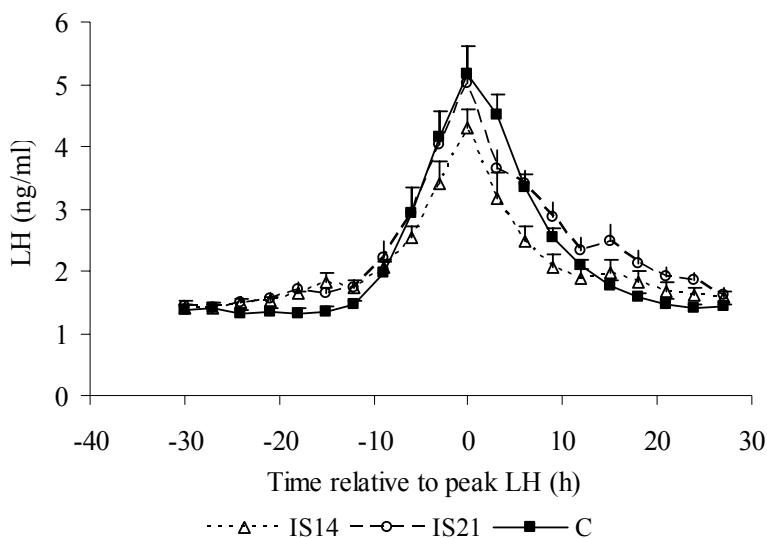


Figure 1. Pre-ovulatory LH surge levels per treatment relative to the time of the LH surge. Number of observations was 7 for IS14, 8 for IS21 and 7 for C sows. No differences were found between treatments in peak LH levels ($P>0.10$). One IS14 sow had an LH surge with a peak level of 1.69 ng/ml and an ovulation rate of 4 and was excluded from LH analyses. Values are means \pm se.

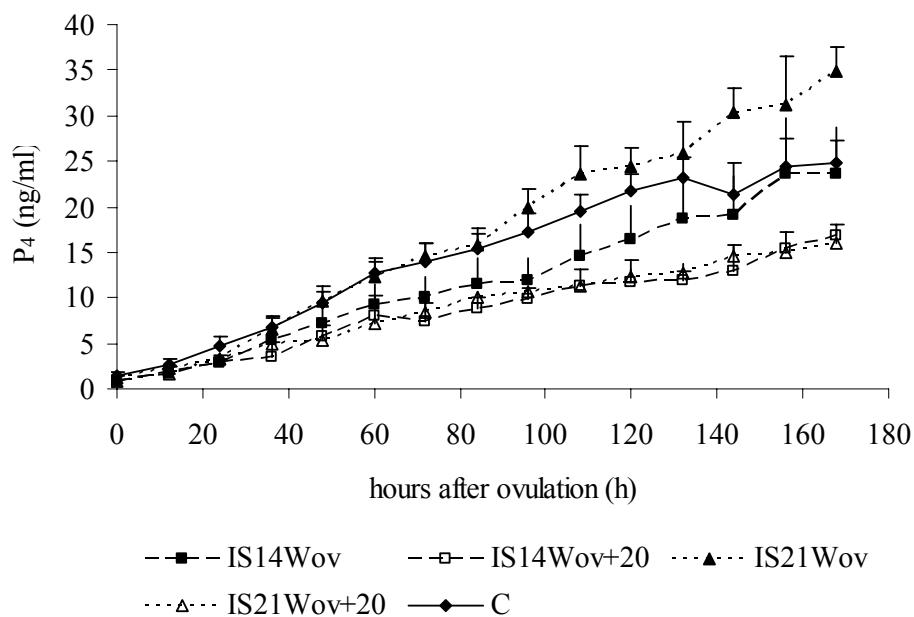


Figure 2. Post-ovulatory progesterone (P_4) levels per treatment relative to the time of ovulation in sows. Number of observations for P_4 was 4 for all IS treatments and 7 for C. One IS14W_{ov} sow had an ovulation rate of 4 and was therefore excluded from P_4 analyses. In the period from 144-168 h after ovulation, P_4 levels were lower in W_{ov+20} sows than W_{ov} and C sows ($P<0.05$). Within W_{ov} sows, IS21 sows had higher P_4 levels than IS14 sows ($P<0.05$) but also higher than C sows ($P<0.05$). Values are means \pm se.

Embryo survival and embryo, placental and uterine development

Embryo survival tended to be influenced by timing of start of IS relative to parturition (IS14: $50 \pm 4\%$ vs. IS21: $61 \pm 5\%$; $P < 0.10$) but not by continuation of IS during pregnancy (W_{ov} : $57 \pm 4\%$ vs. W_{ov+20} $54 \pm 6\%$; $P > 0.10$) and the IS treatments did not differ from the C sows ($P > 0.10$; Table 5). Numbers of total, viable and non-viable embryos were not affected by timing of start of IS relative to parturition or continuation of IS during pregnancy ($P > 0.10$) and were not different between IS sows or C sows ($P > 0.10$; Table 5). Continuation of IS during pregnancy (W_{ov+20}) tended to result in lower embryo weight at day 30 of pregnancy (W_{ov} : 1.65 ± 0.05 g vs. W_{ov+20} 1.55 ± 0.07 g; $P < 0.10$), but was not found to be affected by timing of start of IS relative to parturition ($P > 0.10$). No differences were found in placental and uterine development when analyzed for timing of start of IS relative to parturition or continuation of IS during pregnancy, but the combination of early stage of lactation with continuation of IS for 20 days after ovulation (IS14 W_{ov+20}) tended to result in lower placental weight than in IS14 W_{ov} sows ($P < 0.10$) and a lower uterine weight than C sows ($P < 0.10$; Table 5). P_4 levels at day 7 after ovulation were related to embryo survival at day 30 in IS14 sows ($r = 0.47$, $P < 0.05$) but not in IS21 sows ($r = 0.002$, $P > 0.10$) or C sows ($r = 0.05$; $P > 0.10$). At day 12 and day 30 no relation was found between embryo survival and P_4 levels for any of the treatments ($P > 0.10$).

Discussion

In a previous experiment (Chapter II) peak LH levels and P_4 levels were lower in sows subjected to an intermittent suckling (IS) regime. The IS regime, however, commenced at day 14 of lactation whereas control sows were weaned at day 21 of lactation. Also, IS sows continued with IS until day 23 after ovulation and embryo development was negatively affected and embryo survival was numerically lower (Chapter III). To investigate if these differences were a result of the early start of IS after parturition, the first aim of the present study was to examine effects of timing of start of IS relative to parturition (day 14 vs. day 21 of lactation). The number of sows showing follicle growth and ovulation did not differ between sows with initiation of IS at day 14 or day 21 of lactation. For ovulating sows, timing of start of IS relative to parturition did affect duration of oestrus; a later start of IS (day 21) resulted in a longer duration of oestrus. In a former study, IS initiated at day 14 of lactation resulted in a duration of oestrus similar to weaned C sows (Chapter II) and in early weaned sows duration of oestrus was similar (Belstra et al., 2002) or even tended to be longer than in sows weaned at day 24 (Willis et al., 2003). Thus, it remains unclear why duration of oestrus in IS21 sows was longer.

Table 5. Embryo, placental and uterine parameters

	IS14 W _{ov} (n=10)	IS14 W _{ov+20} (n=9)	IS21 W _{ov} (n=9)	IS21 W _{ov+20} (n=8)	C (n=13)
Ovary					
Ovulation rate	26.9±1.2	26.9±2.1	26.2±1.5	26.7±2.1	27.8± .1
Luteal weight (g)	9.7±0.5	9.6±0.6	9.6±0.6	9.8±0.5	9.0±0.8
Number of embryos					
Total	19.5±1.7	17.9±1.3	19.6±1.0	19.2±2.1	19.4± .7
Viable	14.0±1.7	12.9±1.9	16.0±1.3	15.0±2.4	15.8± .9
ESR (%) ¹	52±6	48±7	63±6	59±9	58±5
Embryo development					
Length (cm)	2.79±0.06	2.73±0.09	2.76±0.04	2.72±0.05	2.80±0.04
Weight (g) ²	1.69±0.09	1.54±0.13	1.61±0.04	1.57±0.07	1.68±0.04
Placental characteristics					
Length (cm)	40.5±1.8	35.7±3.4	38.4±1.5	38.5±2.5	40.5±1.4
Weight (g)	1.04±0.07 ^c	0.82±0.12	0.94±0.04 ^{c,d}	0.92±0.08 ^{c,d}	0.99±0.03 ^{c,d}
Uterine characteristics					
Length horns (cm)	461±30	435±27	457±29	416±31	439±16
Weight horns (g)	3703±234 ^{c,d}	3303± 33 ^d	3918± 87 ^{c,d}	3890±352 ^{c,d}	4181±187 ^c
Placental attachment site area (cm ²)	219±27	209± 9	226±21	212±17	230±15

¹ IS14 (50 ± 4%) vs. IS21 (61± 5%; P<0.10). ² W_{ov} (1.65 ± 0.05 g) vs. W_{ov+20} (1.55 ± 0.07 g; P<0.10). ^{c,d} different superscripts indicate P<0.10

A lower LH surge has been reported in sows with start IS at day 14 of lactation (Chapter II) or weaned early after parturition (Edwards and Foxcroft, 1983; Cox et al., 1988). In the current study, IS14 sows had a numerically but not significantly lower peak LH level but this was not significant, possibly due to the large variation between animals within treatments. A lower LH surge with early start of IS may be due to low levels of readily releasable pools of LH (Cox et al., 1988).

Studies examining oestrus after early weaning did not find effects on post-ovulatory P₄ levels (Kikrwood et al., 1984; Willis et al., 2003), but in IS sows with start of IS at day 14 of lactation P₄ levels were low (Chapter II). In the present study, P₄ levels were lower for IS14 sows at day 6 to 7 and levels were similar to IS21 sows from day 12 onwards. It seems that sows with an early start of IS are able to produce sufficient levels of P₄ but that the production starts up slower (when weaned at ovulation). Early P₄ levels (72 h post ovulation) have been positively related to embryo survival (Jindal et al., 1997), and indeed embryo survival tended to be lower in IS14 than IS21 sows and within IS14 sows a relation was found between P₄ at day 7 and embryo survival at day 30. P₄ levels are important for secretion of proteins by the uterus (Vallet et al., 1998), and it is possible that in IS14 sows, where uterine involution is not

yet completed, high levels of P₄ are needed for an optimal uterine environment for the embryos to survive. In IS21 sows, involution of the uterus has completed at the time of ovulation (Palmer et al., 1965a; Palmer et al., 1965b), the uterine environment is not suboptimal anymore and possibly high P₄ levels are less essential for the uterine environment to increase embryo survival. This might explain why we found no relationship between early P₄ and embryo survival in IS21 sows. Although in the present study embryo survival tended to be affected by timing of start of IS relative to parturition, placental and embryo development were not affected which is concurrent with results from studies with early weaned sows (Belstra et al., 2002). In conclusion, early start of IS (day 14) affects some sow reproductive parameters; a numerically lower LH surge, a lower embryo survival and a lower level of P₄ early after ovulation (day 6 to 7) were found when IS was initiated early after parturition.

In the previous experiment IS (Chapter II and Chapter III) started at day 14 and sows were lactating during the follicular phase and during pregnancy. Besides the timing of start IS relative to parturition, lactation itself might also be a factor affecting reproductive parameters, therefore in the present study IS sows were compared with weaned control sows where start of treatment for both groups is day 21 of lactation (start of IS vs. weaning). No differences were found in the number of sows with follicle growth, ovulation or oestrous behaviour between weaned (C) or IS sows (IS21) as expected. Duration of oestrus, however, was longer in IS21 than C sows but it is unclear why this occurred. The interval from start of IS to peak LH levels and thus ovulation was prolonged in IS21 sows whilst peak LH levels were similar. Why IS sows need more time to reach peak LH levels is not clear but it might be caused by factors such as suckling (Cox et al., 1988) or possibly enhanced cortisol release during stress of separation due to IS (Chapter II), or changes in metabolic hormones related to lactation (e.g. insulin). Thus factors such as suckling or stress might influence the timing of peak LH levels but not the amplitude of the LH peak.

P₄ levels in IS21 weaned at ovulation did not differ from weaned C at any times. Although granulosa cell quality was not measured in the present study it is unlikely that an insufficient proliferation of granulosa cells caused the low P₄ levels observed in our previous experiment (Chapter II). Embryo survival and embryo development were also not affected by IS. In summary, lactation during the follicular phase does not affect peak LH levels, P₄ levels or embryo survival and development. Timing of the pre-ovulatory LH peak and ovulation seem delayed, but it is unclear which exact factors are causing this delay.

We previously reported that IS sows lactating after ovulation had low P₄ levels (Chapter II) and the present study examined effects of continuance of IS by weaning sows at ovulation or at 20 days after ovulation on P₄ levels. Continuation of lactation during the first 20 days of pregnancy (W_{ov+20}) resulted in lower P₄ levels, but at day 30 P₄ levels were similar in all treatments. This indicates that either P₄ secretion was suppressed during lactation or P₄ secretion was sufficient but was cleared more rapidly due to a high liver blood flow and metabolic clearance rate during lactation (Sangsrivatavong et al., 2002). Even though the fat

content in sow milk is about two times higher than that of cows (7.9 g/kg vs. 3.9 g/kg) (Csapo et al., 1996), it is not likely that drainage of P₄ to milk (like in cows; Jouan et al., 2006) is responsible for the low P₄ levels, because in cows only 0.1% of the produced P₄ is found in milk (Rabiee et al., 2001). It is also possible that P₄ production is suppressed in lactating IS sows by metabolic hormones such as insulin. During continuous lactation the plasma levels of insulin are low in pigs (Prunier et al., 1993; Revell et al., 1998). Possibly, IS sows have lower levels of insulin and consequently low levels of P₄ as infusion of insulin reduced the clearance rate of P₄ in ewes (Smith et al., 2006) and even stimulated P₄ secretion in humans (Willis et al., 1996). After weaning, insulin levels rise quickly (Eriksson et al., 1987) and consequently also P₄, which would explain why at day 30 after ovulation no differences in P₄ were found between our treatments. As insulin was not measured in IS sows, it is not known if and how insulin levels change in an IS regimen during periods of suckling and non-suckling. This needs to be further investigated.

In sows with continuation of IS after ovulation, pregnancy rates at day 30 were 20% lower than in IS sows weaned at ovulation but this difference was possibly not significant due to the low numbers of animals. No differences were found in embryo survival but embryo development was affected. Lactation during the first 20 days of pregnancy resulted in lighter embryos but placental and uterine parameters were not affected by time of weaning. When analyzing combinations of timing of start of IS and continuance of IS, placental and uterine weight were negatively affected in sows with an early start of IS and lactation during pregnancy. Possibly due to the low P₄ levels, uterine protein secretion is lower, resulting in a suboptimal development of the uterus and placenta which may restrict foetal development during later stages of gestation. This suboptimal development could also be an explanation for the relatively low pregnancy rate in sows lactating during the first 20 days of pregnancy. In summary, continuation of IS after ovulation results in low P₄ levels and tends to negatively affect embryo development.

In conclusion, an early start of IS (day 14) results in a numerically, but not significantly lower LH surge and a lower embryo survival. IS during the follicular phase only does not affect peak LH levels, P₄ levels or embryo survival and development. Continuation of lactation during the first 20 days of pregnancy results in lower P₄ levels until final weaning and tends to affect embryo development. This effect on P₄ may be related with metabolic parameters such as insulin but this needs to be further investigated. Furthermore, the combination of an early start of IS and continuance of lactation during early pregnancy affects placental and uterine parameters which may restrict foetal development during later stages of lactation. Start of IS at day 21 of lactation and weaning at ovulation seems to be the best option when conception is to be achieved during lactation.

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VI

Naturally developing cystic ovaries in sows: follicle growth and endocrine profiles

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Submitted

Abstract

The aim of this study is to describe follicle development and hormone levels in sows that naturally developed cystic ovaries and their normally ovulating counterparts. Data come from three experiments using Intermittent Suckling, in which 12 sows developed cystic ovaries. Follicle development, oestrous behaviour, E₂, LH and P₄ profiles were assessed. At days 1 to 4 after start of treatment, follicle diameter of sows that developed cystic ovaries was similar to follicle diameter of sows that ovulated ($P>0.10$), but follicle diameter was greater from day 5 onwards ($P<0.05$). Peak levels of E₂ were similar (27 ± 2.4 pg/ml vs. 26 ± 0.9 pg/ml; $P>0.10$) for the two groups but in sows that developed cysts E₂ levels did not return to basal levels within 48h after peak E₂. LH basal levels were similar, but the pre-ovulatory increase in LH was lower (0.4 ± 0.1 ng/ml vs. 3.6 ± 0.3 ng/ml; $P<0.01$). In sows that developed cystic ovaries, no rise in P₄ was observed ($P<0.05$). Timing of onset of oestrus was similar for sows developing cysts or with ovulation, but duration of oestrus was longer for cystic sows (73 ± 11 h vs. 52 ± 3 h; $P<0.05$). In conclusion, sows that develop cystic ovaries seem to have normal follicle development (both size and E₂ production), but have no apparent pre-ovulatory LH surge. It seems that ovulation most likely does not occur due to dysfunction in the feedback from E₂ to the hypothalamus, possibly related to effects of stress (cortisol) or the metabolic state (insulin levels).

Introduction

Reproductive failure in sows is characterized by factors such as failure to return to oestrus, repeated breeding and small litter size and is the main cause for culling (Heinonen et al., 1998). Studies have shown that about 2.1-5.8% (Geudeke, 1992), 3.1% (Heinonen et al., 1998), 2.4% (Castagna et al., 2004) of sows culled have cystic ovaries. The general belief is that the absence of the pre-ovulatory LH surge causes the formation of cystic ovaries (for review see (Ryan and Raeside, 1991a; Ryan and Raeside, 1991b)). Indeed studies have shown that when the LH surge is blocked by for example ACTH injections, cysts are formed in pigs (e.g. Liptrap, 1970; Liptrap, 1973; Peter and Liptrap, 1985) and cows (Stoebel and Moberg, 1982). In pigs, most studies examining hormone profiles of sows with cystic ovaries used sows with ACTH induced cysts (Liptrap, 1973; Close and Liptrap, 1975; Liptrap and Doble, 1981; Peter and Liptrap, 1985; Babalola and Shapiro, 1990; Fitko et al., 1996). From these studies knowledge has been gained about the endocrinology of established cystic ovaries. The endocrinological profile preceding and during the formation of cysts is not known.

We performed a number of experiments in which we attempted to induce lactational ovulation by separating sows from their piglets for a number of hours daily (Chapter II, Chapter III; Langendijk et al., 2007c). In these studies we repeatedly found sows that developed cystic ovaries and since assessment of follicle development occurred daily (by using ultrasound) and hormone levels (oestradiol, LH, progesterone) were assessed in these sows, these data provide insight in the endocrinology and development of naturally developing cysts. The aim of this study is to describe follicle development and hormone levels in sows that naturally developed cystic ovaries and their normally ovulating counterparts.

Material and Methods

Data from sows described in this paper were derived from three different experiments described in detail in Chapter II, Chapter III, Chapter V and Langendijk et al. (2007c). The experimental designs are described in short below. All three experiments were approved by the Ethics Committee for Animal Experiments of Wageningen University. A sow was defined as cystic when all observed pre-ovulatory follicles failed to ovulate within 8 days after start of start of separation or weaning, follicles remained present on the ovaries and continued to increase in diameter. A sow was defined as partially ovulated when at the expected time of ovulation, a number of follicles ovulated but multiple pre-ovulatory follicles persisted at the ovaries and after 1 week Corpora Lutea (CL) and persistent pre-ovulatory follicles were observed. A sow was defined as having ovulated when pre-ovulatory follicles were no longer present on the ovaries and CLs were observed 1 week later.

Experimental designs

Experiment 1

Multiparous sows from the Topigs40 line (Topigs, Vught, The Netherlands) were used with parity ranging from 3 to 10. Treatments were a control group (C) with weaning at day 21 of lactation or one of two Intermittent Suckling (IS) groups with start of IS at day 14 of lactation and separation for 12 h per day either one period for 12 h (IS12) or two periods of each 6h (IS6). In total 38 sows ovulated (C n=17; IS12 n=13; IS6 n=8) and five sows developed cystic ovaries (IS12 n=1; IS6 n=4) of which two sows partially ovulated.

Experiment 2

For experiment 2, multiparous sows from the Topigs20 line (Topigs, Vught, The Netherlands) were used, with parity ranging from 5 to 9. Sows were assigned to one of two Intermittent Suckling (IS) groups with start of IS at day 14 of lactation and separation for one period of 12 h daily. One IS group received boar contact three times daily (n=16) and the other IS group did not receive boar contact (n=16). In total 9 sows ovulated and two sows developed cystic ovaries.

Experiment 3

Multiparous sows from the Topigs40 line (Topigs, Vught, The Netherlands) were used, with parity ranging from 1 to 8. Treatments were a control group (C) with weaning at day 21 of lactation or one of two Intermittent Suckling (IS) groups with separation for one period of 12 h per day and start of IS at either day 14 of lactation (IS14) or day 21 of lactation (IS21). In total 59 sows ovulated (C n=17; IS14 n=20, IS21 n=22) and five sows developed cystic ovaries (C n=2; IS14 n=3) of which one IS14 sow partially ovulated.

Follicle development

To study follicle development, the ovaries were examined daily by means of transrectal ultrasonography (Scanner 200, Esaote, Maastricht, The Netherlands). The four largest follicles were measured and used to calculate the average follicle diameter. When follicle size was 6 mm or greater, ultrasonography was performed every 6 h for experiment 1 and every 12 h for experiment 2 and 3 to establish the time of ovulation. Sows that developed cystic ovaries were examined again around day 14 (range day 11 to 15) and day 21 (range day 18 to 24) after the start of treatments. Sows of experiment 1 and 3 were slaughtered around day 30 after start of treatment (range day 28 to 36) and the ovaries were examined. Sows of experiment 2 were slaughtered at day 20 after start of treatment.

Oestrus detection

Oestrus detection was carried out by means of a back-pressure-test (BPT) in absence of a boar either every 6 h (exp 1), 8 h (exp 2) or every 12 h (exp 3). In experiment 2, in one treatment group, oestrus detection was carried out with a boar and a BPT.

Hormone assays

Sows were fitted with permanent catheters about 2 weeks before farrowing in all three experiments. Blood samples were collected in tubes containing 100 µl of EDTA solution, placed on ice immediately after collection and centrifuged at 2000g for 10 min at -4 °C. Plasma was stored at -20 °C until analyses. For experiment 1, blood samples for oestradiol (E_2) and LH were collected every 6 h. For experiment 2, daily samples were collected and analyzed for E_2 . For experiment 3, samples for LH were collected every 3 h. For all three experiments, blood samples were collected every 12 h for P_4 analyses. Concentrations of E_2 , LH and P_4 were determined as described in Chapter II. In short, the specificity of the RIA was high for LH as indicated by low cross-reactivity for other pituitary hormones (Van den Brand et al., 2000a) and by the observed parallelism. The limit of quantitation was 0.2 ng/ml for LH. Specificity of E_2 and P_4 RIAs was high as indicated by low cross-reactivity for other steroid hormones (Dieleman and Bevers, 1987) and by the observed parallelism. The limits of quantitation were 0.1 ng/ml and 2 pg/ml for P_4 and E_2 , respectively. Calculation of all results was done applying the spline approximation for the standard series from RIAsmart (Packard Instruments Company, Meriden, CT, U.S.A.). The calculated doses were <4 % different from the defined doses over the entire range. In general, the intra- and inter-assay coefficients of variation were <10 and <15% for all assays, respectively. The number of available hormone profiles for sows that developed cystic ovaries was 7 for E_2 , 9 for LH and 9 for P_4 . For 5 sows that developed cystic ovaries from experiment 1 all three hormones were measured (E_2 , LH and P_4) and for 4 sows that developed cystic ovaries from experiment 3 LH and P_4 . For 2 sows only E_2 was measured (experiment 2). The number of available hormone profiles for E_2 was 19 for experiment 1 and 9 for experiment 2, for LH was 17 for experiment 1, 14 for experiment 3 and for P_4 was 18 for experiment 1 and 11 for experiment 3 for sows that ovulated.

Sow body weight and Back-fat

For all three experiments sow body weight was measured at day after farrowing and every week of lactation.

Statistical Analyses

In total, 12 sows developed cystic ovaries (Exp 1 n=5, Exp 2 n=2, Exp 3 n=5) and in total 69 sows ovulated at on average day 6 after start of treatment (range day 4 to day 8). For all parameters treatment effects were evaluated within experiments. When treatment effects were

absent, data within one experiment were pooled. For all analyses models were run in the PROC GLM procedure of SAS and the residuals of the models were tested for normality. When the residuals were not normally distributed, the parameter was analyzed non-parametrically by using the Kruskal Wallis test (PROC NONPAR, SAS). The general model used in the PROC GLM was $Y_{ij} = \mu + T_i + E_j + e_{ijk}$, with Y_{ij} the dependent variable, μ the mean, T_i cystic ($i=yes, no$), E_j experiment ($j=1,2,3$). For follicle diameter analysis during the first four days after start of treatment, data were used from sows with lactational ovulation from experiment 1 (IS), 2, 3 (IS14) where IS was initiated at day 14 post partum. Follicle diameter at day 5 was tested in the Kruskal Wallis test with cystic (yes or no) as variable. For E_2 , basal levels were defined as the first time E_2 reached a level below 7.5 pg/ml as described in Chapter II. Peak E_2 levels were defined as the time at which a sow had the highest level of E_2 measured. Basal LH levels were defined as the average of LH levels before 12 h before the highest LH levels and from 12 h after the highest LH levels onwards. For P_4 , the level at 66 h after peak LH levels was used as at that time point for most sows samples were available. For analysis of basal and peak E_2 parameters the following GLM was used $Y_{ij} = \mu + T_i + e_{ij}$, with Y_{ij} the dependent variable, μ the mean, T_i cystic ($i=yes, no$). For basal and peak LH parameters, the same model was used in GLM as described for follicle diameter. P_4 levels at 60 to 66 h after peak LH were tested non-parametrically in the Kruskal Wallis test. For oestrus parameters, data were used from experiment 1 and 3 because of the use of a boar in experiment 2. Onset of oestrus was tested non-parametrically in the Kruskal Wallis test and duration of oestrus in the model as described for follicle diameter at days 1 to 4. Sow body weights and parity were tested in a GLM as described for follicle diameter. Values presented in this study are means \pm se unless stated otherwise.

Results

Follicle development

Follicle development during the first five days after start of treatment is illustrated in Figure 1 for both sows that ovulated and sows that developed cystic ovaries per treatment. At the start of treatment (day 0), follicle diameter of sows that developed cystic ovaries was similar to that of sows that ovulated ($P>0.10$). At days 1 to 4, follicle diameter of sows that developed cystic ovaries was similar to follicle diameter of sows that ovulated ($P>0.10$), but at day 5, follicle diameter was greater in sows that developed cystic ovaries (7.9 ± 0.5 mm vs. 7.1 ± 0.1 mm; $P=0.05$). Follicle diameter at days 6, 14 and 21 after start of treatments are shown in Table 1 for sows that developed cystic ovaries. At day 6 after start of treatments, six out of twelve sows that developed cystic ovaries had a follicle diameter ranging between 7 and 9 mm. In five of the eight sows measured at day 21, follicle diameter was still increasing (18.4 ± 1.7 mm) and in the other three follicle diameter was comparable to follicle size at day 14 (12.6 ± 2.1 mm). In these three animals, follicles were regressing or new ovulation had

occurred at time of slaughter. In sows in which follicle diameter increased, cysts were still present on the ovaries at slaughter.

Oestrus parameters

Of the 12 sows with (partially) cystic ovaries, 11 showed oestrous behaviour (Table 1). For sows of experiment 1 and 3, onset of oestrus was similar for sows that developed cystic ovaries or sows that ovulated (111 ± 13 h vs. 109 ± 4 h; $P>0.10$), but duration of oestrus was longer for sows that developed cystic ovaries (73 ± 11 h vs. 52 ± 3 h; $P<0.05$).

Table 1. Follicle development and oestrus characteristics of sows developing cystic ovaries as compared to sows with ovulation

Sow	Follicle diameter (mm)			Oestrus		Status at slaughter*
	D6	D14**	D21**	Onset	Duration	
				(h)	(h)	
Exp 1						
Sow 1 ¹	9.5	18.1	21.1	123	45	Cysts (n=7), CL (n=21)
Sow 2 ¹	8.6	13.6	18.3	111	63	Cysts (n=30), CL (n=8)
Sow 3	11.5	16.6	19.5	123	21	Cysts, no CL
Sow 4	9.7	10.0	10.0	117	81	Regressing cysts
Sow 5	9.6	11.3	11.1	135	87	Regressing cysts
Ovulation	-	-	-	107 ± 5	57 ± 5	-
Exp 2						
Sow 6	10.7	ND		-	-	Cysts (>25.0 mm)
Sow 7	7.8	ND		282	18	Cysts (>15.0 mm)
Ovulation	-	-		137 ± 6	47 ± 2	-
Exp 3						
Sow 8	7.8	21.1	21.1	117	141	Cysts (n=4), new CL (n=30)
Sow 9 ¹	7.6	15.3	16.8	-	-	Cysts (n=25), CL (n=7)
Sow 10	9.0	15.3	ND	90	60	Cysts with luteal tissue
Ovulation1 [§]	-	-	-	112 ± 6	48 ± 10	-
Sow 11	7.9	18.4		165	87	Cysts (n>20)
Sow 12	7.0	6.0	>12.0	21	75	Cysts (n=19), new CL (n=16)
Ovulation2 [§]	-	-	-	100 ± 7	49 ± 3	-

¹Sows 1,2 and 9 partially ovulated. ND= not determined. * Status at slaughter is time at which animals were slaughtered and their ovaries were examined (range day 20 to day 36). For partially ovulated sows CL were from ovulation during treatment for other sows with CLs, CLs were new, cysts regressed and ovulation occurred. ** Follicle diameter at day 14 ranges from day 11 to day 15 and at day 21 ranges from day 18 to 24. § Ovulation 1 represents the group of ovulating sows with lactational ovulation and start of treatment from day 14 of lactation (IS14) and Ovulation 2 represents the group of sows with ovulation after weaning at day 21 of lactation (C). The number of observations for sows with ovulation is 21 for exp 1, 9 for exp 2, and 39 for exp 3 (Ovulation1 n=22, Ovulation2 n=17).

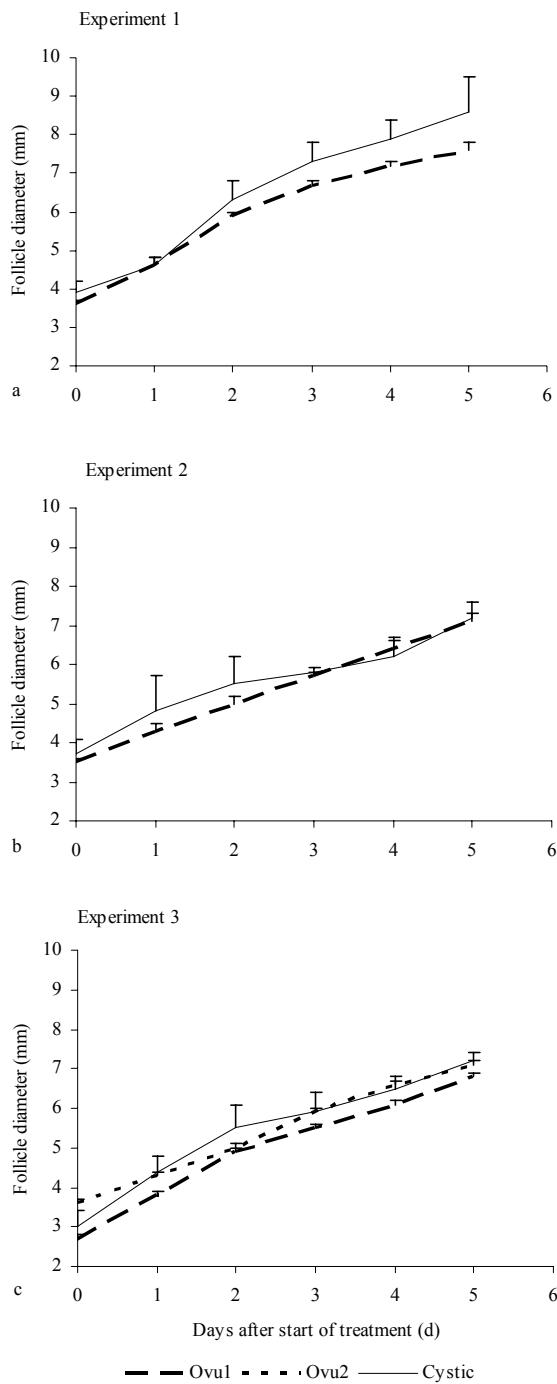


Figure 1. Average follicle development for sows that ovulated (dotted lines) and sows that developed cystic ovaries (black line) per treatment. Start of treatment was at day 14 for sows of experiment 1 and 2 and for one group of sows of experiment 3. In experiment 3 follicle development differed between the two treatments for sows that ovulated and therefore data were not pooled and two lines are presented in Figure 1c. The other group of sows with ovulation of experiment 3 (----) started with their treatment at day 21 of lactation. Ovulation 1 (Ovu1) represent sows that ovulated during lactation with start of treatment at day 14 of lactation and ovulation 2 (Ovu2) represent sows that ovulated after weaning at day 21 of lactation.

Hormone profiles

E_2 , LH and P_4 data for individual sows that developed cystic ovaries and group averages of sows that ovulated are shown in Table 2. Timing of peak E_2 levels relative to start of treatment was later in sows developing cysts than sows with ovulation (121 ± 9 h vs. 94 ± 6 h; $P < 0.05$) but peak levels of E_2 were similar (27.0 ± 2.4 pg/ml vs. 26.0 ± 0.9 pg/ml; $P > 0.10$). Ovulating sows reached basal E_2 levels within 31 h after peak E_2 levels in experiment 1 and within 48 h in experiment 2. Four out of five sows that developed (partially) cystic ovaries did not reach basal E_2 levels within 48h after peak E_2 levels (Table 2).

No differences were found in basal LH levels between sows that developed cystic ovaries and sows that ovulated (1.4 ± 0.3 ng/ml vs. 1.6 ± 0.1 ng/ml; $P > 0.10$) but the increase in LH from basal level to peak LH levels was considerably lower in sows that developed cystic ovaries (0.4 ± 0.1 ng/ml vs. 3.6 ± 0.3 ng/ml; $P < 0.01$). Low peak levels of LH were found in sows that developed cystic ovaries with a highest level of 1.8 ± 0.3 ng/ml compared to 5.1 ± 0.3 ng/ml in sows that ovulated ($P < 0.01$). In Figure 2, the representative hormone profiles of an ovulating sows (2a), a partially ovulating sow (2b) and a sow that developed cystic ovaries (2c) are illustrated and shows the absence of an LH surge in sows that developed cystic ovaries.

P_4 levels in sows with ovulation rose quickly after ovulation to about 3.4 ng/ml at 60 to 66 h after peak LH levels (Figure 2a). Levels of P_4 at 66 h after peak LH levels in sows with cysts were < 1 ng/ml in four out of the five sows that developed cystic ovaries (Table 2 and Figure 2c). Two of the sows with partial ovulation showed increases in P_4 ; for the partially ovulating sow with 21 CLs (Sow 1), levels were comparable to sows that ovulated whilst others remained lower (Table 2). Overall, P_4 levels of sows that developed cystic ovaries were lower at 60 to 66 h after peak LH levels than in sows that ovulated ($P < 0.05$).

Body weight and parity

Body weight at day 1 after farrowing was higher for sows developing cystic ovaries than for sows that ovulated (290 ± 8 kg vs. 272 ± 3 kg; $P = 0.05$), but at day 14 of lactation body weights did not differ (276 ± 8 kg vs. 262 ± 3 kg; $P = 0.11$) and body weight loss during the first two weeks of lactation was also similar (12 ± 4 kg vs. 10 ± 1 kg; $P > 0.10$). Parity of sows developing cystic ovaries was higher than of sows that ovulated (6.7 ± 0.4 vs. 5.1 ± 0.2 ; $P < 0.01$) and an overall correlation was found between body weight at day 1 after farrowing and parity ($r = 0.51$; $P < 0.01$).

Table 2. Hormone levels in of sows developing cystic ovaries as compared to sows with ovulation

	E ₂ [*]		LH		P ₄	
	Peak E ₂ (pg/ml)	peak E ₂ - basal E ₂ (h)	peak E ₂ - peak LH (h)	Basal LH (ng/ml)	peak LH – basal LH (ng/ml)	P ₄ 60-66 h after peak LH (ng/ml)
Exp 1						
Sow 1 ¹	20.2	30	18	2.2	0.5	4.78
Sow 2 ¹	25.6	>48**	12	1.8	0.6	1.19
Sow 3	34.9	>48**	0	0.7	0.3	0.99
Sow 4	28.1	ND**	0	0.5	0.2	0.40
Sow 5	26.0	ND	6	0.3	0.4	0.49
Ovulation	26.0±0.9	31±1.4	14±1.2	1.6±0.1	3.8±0.4	3.41±0.4
Exp 2						
Sow 6	27.4	168	ND	ND	ND	ND
Sow 7	32.2	144	ND	ND	ND	ND
Ovulation	27.8±1.6	51±3.4	ND	ND	ND	ND
Exp 3						
Sow 8	ND	ND	ND	1.4	0.1	4.48
Sow 9 ¹	ND	ND	ND	1.2	1.2	1.72
Ovulation1 [§]	ND	ND	ND	1.7±0.08	2.7±0.3	6.4±0.3
Sow 11	ND	ND	ND	2.4	0.2	ND
Sow 12	ND	ND	ND	2.3	0.3	0.74
Ovulation2 [§]	ND	ND	ND	1.4±0.04	3.7±0.5	7.4±1.7

¹Sows 1, 2 and 10 partially ovulated. *In experiment 2 E2 samples were taken daily. **Basal levels (<7.5 pg/ml) were not reached during the time of sampling. At 48 h after peak E₂ levels, E₂ levels were 8.3 pg/ml, 26.2 pg/ml for sows 2, 3 and 26.1 pg/ml at 24 h post peak E₂ for sow 4 respectively. For sow 5 samples were available until 6h after peak E₂. [§]Ovulation 1 represents the group of ovulating sows with lactational ovulation and start of treatment from day 14 of lactation (IS14) and Ovulation 2 represents the group of sows with ovulation after weaning at day 21 of lactation (C). ND= not determined. The number of observations for sows with ovulation for E₂ is 19 in exp 1 and 9 for exp 2, for LH 17 for exp 1, 14 for exp 3 (ovulation1 n=7, ovulation2 n=7), for P₄ 18 for exp 1 and 11 for exp 3 (ovulation1 n=4, ovulation2 n=7).

Discussion

The aim of this study was to describe follicle development and plasma hormone levels during the follicular phase in which sows developed cystic ovaries and compare these to sows that ovulated following this follicular phase. No information on hormone profiles prior to the development of cystic ovaries is available from other studies examining cystic ovaries in pigs as data comes from sows with experimentally induced cysts (Liptrap 1973; Close and Liptrap, 1975; Liptrap and Doble, 1981; Peter and Liptrap, 1985; Babalola and Shapiro, 1990; Fitko et

al., 1996). In cows a number of studies have examined naturally occurring cysts (Kesler et al., 1979; Vanholder et al., 2005) and van Holder et al. (2005) examined hormone profiles during the follicular phase in which cows developed cystic ovaries. To our knowledge, this has not been studied in pigs. In recent experiments in which lactational oestrus was induced by daily separation of a sow from her litter we repeatedly found sows that developed cystic ovaries. In these sows cysts developed naturally without administration of exogenous hormones. The fact that follicle development and hormone profiles (oestradiol, LH and progesterone) were assessed in these sows, provides unique data about endocrine profiles and development of naturally developing cysts.

Follicle development during the first four days of the follicular phase following weaning or Intermittent Suckling in sows that developed cystic ovaries was similar to that of sows that ovulated within 4 to 8 days. At day 5, follicle diameter was larger in sows that developed cystic ovaries but this difference was mainly caused by one sow. In general, follicle development was not different until time of expected ovulation at day 6 after start of treatment, which is in contrast with Lucy et al. (2001) who state that follicles in sows developing cysts grow rapidly to 8-9 mm, stop growing and eventually fail to ovulate. In cows, follicles seem to develop normally and cysts develop at the time that in other cows ovulation occurs (Vanholder et al., 2005). In summary, follicle development in sows developing cystic ovaries did not deviate during the early follicular phase from sows that ovulated.

Not only the growth of the follicles was similar between sows that either ovulated or developed cystic ovaries, but also (peak) E₂ production of the follicles was similar. The time needed to return to basal levels, however, (measured only in a small number of animals) was extended in sows that developed cystic ovaries as E₂ levels did not return to basal E₂ levels within the expected period. This indicates that sows that developed cystic ovaries experienced an extended E₂ exposure which may be the cause of the longer duration of oestrus of these sows as in a number of studies exposure to (high) E₂ levels has been related to the expression and duration of oestrus (Edwards and Foxcroft, 1983; Lyimo et al., 2000).

In all sows that developed cysts pre-ovulatory LH surges were absent or very small (increase in LH always below 2 ng/ml), whereas sows with ovulation always had an increase in LH of more than 2 ng/ml above basal level. This is concurrent with the general belief that cystic ovaries develop in absence of a clear LH surge (for review Ryan and Raeside, 1991a) and the fact that cysts develop when the LH surge is blocked (Liptrap, 1970; Liptrap, 1973; Peter and Liptrap, 1985). Interestingly, our results show that this may occur even when follicle development (both follicle growth and E₂ production) appears to be normal. So the question is which mechanisms are responsible for the absence of an LH surge and which factors can influence these mechanisms.

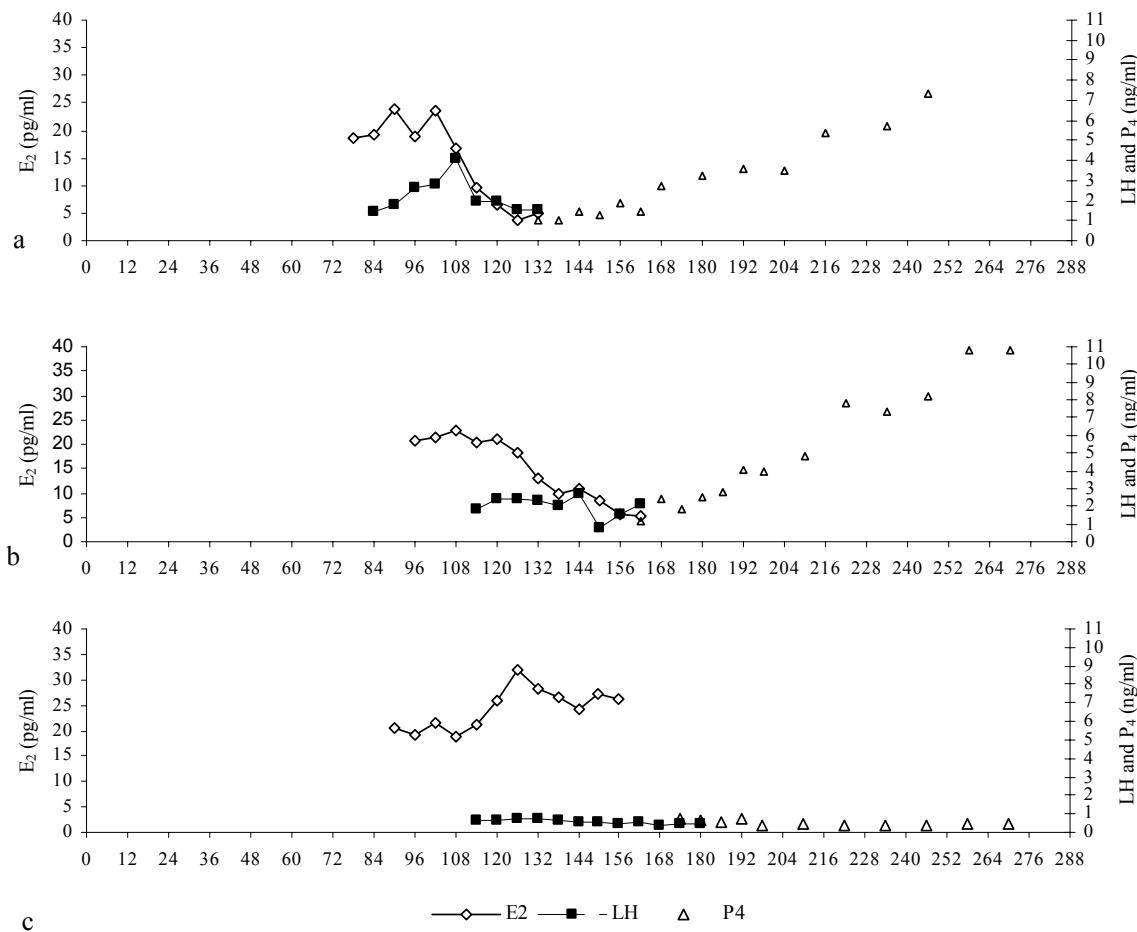


Figure 2. Oestradiol-17 β (E₂), LH and Progesterone (P₄) profiles of representative sow with ovulation (a), a representative sow with partial ovulation (b), and a representative sow that developed cystic ovaries (c).

There are several possible underlying mechanisms which could be responsible for the absence of the pre-ovulatory LH surge. First, available levels of LH in the pituitary have been found to be low early after parturition and as lactation progresses the response of LH increases (e.g. Stevenson et al., 1981; Cox et al., 1988). The lower readily releasable pool of pituitary LH resulted in lower peak levels of the LH surge when comparing lactation periods of 10 (Kirkwood et al., 1984) and 14 days (Willis et al., 2003) vs. 35 days. Indeed, also in our studies lower peak LH levels were observed when lactational oestrus was induced by Intermittent Suckling from day 14 of lactation onwards compared to sows weaned at day 21 of lactation (Chapter V). Varley and Foxcroft (1990) state that cysts develop in sows with limited levels of LH because the down regulation of LH receptors and the subsequent fall in E₂ levels do not occur when LH levels are too low. Perhaps in the sows that developed cysts, LH levels in the pituitary were too limited to respond to E₂ feedback with a pre-ovulatory LH surge. It seems unlikely though that LH levels were limited to the extend that a LH surge could not occur as most of the sows undergoing the same method of separation did ovulate, but with lower LH peak levels.

Second, a more likely mechanism involved in the development of cystic ovaries is a dysfunction of E₂ feedback (Kaneko et al., 2002). Angell et al. (1996) state that a dysfunction in the E₂ feedback mechanism can be a result of insufficient GnRH production, failure of the pituitary to respond to GnRH or a defect at receptor level. In cows with naturally occurring estrogenic cysts, LH was not released after exogenous E₂, but did so after administration of exogenous GnRH (Refsal et al., 1988). This indicates that in animals with cystic ovaries, the pituitary is able to respond to GnRH. Furthermore, mice producing sufficient levels of E₂ in the ovary, but lacking an E₂ receptor in the hypothalamus, are unable to respond to high levels of E₂ with a LH surge and develop large anovulatory follicles (Couse et al., 1999). It is thus possible that a dysfunction of the feedback mechanism of E₂ on the hypothalamus occurs in sows developing cystic ovaries.

The above described mechanisms can play a role in the development of cystic ovaries, but factors are involved influencing the mechanisms resulting in the absence of a LH surge. Two possible factors are related to the metabolic state of the sow and stress.

In humans, the occurrence of cysts has been positively related to obesity and high levels of insulin (for review see Norman et al., 2002). On the other hand, in lactating cows the occurrence of cysts has been related to low insulin levels (Vanholder et al., 2005). Also in lactating sows, insulin levels in sows are low (Prunier et al., 1993) and the majority of the sows developing cysts in the present study did so during lactational oestrus. Insulin levels have been found to stimulate expression of LH receptors on the granulosa cells. It is possible that with the low insulin levels observed during lactation, the responsiveness to LH levels might not be sufficiently stimulated, resulting in follicles not responding to increases in LH (Vanholder et al., 2005). If that were the case, however, the observed LH levels in the present study would have been elevated which was not observed. It seems therefore, unlikely that altered insulin levels induced the development of cysts in our sows through effects on LH

receptor level. Insulin levels could have affected the absence of the LH surge by affecting hypothalamic sensitivity to E₂ or pituitary sensitivity to GnRH (Angell et al., 1996). In their study Angell et al. (1996) used experimentally induced diabetic (low insulin levels), ovariectomised gilts and found that oestradiol benzoate could not induce an LH surge *in vivo* and no increase in LH was observed in response to GnRH administration *in vitro*. These results indicate that in animals a dysfunction in E₂ feedback can be related to low levels of insulin. Thus possibly, the low insulin levels in lactating sows can play a role in the development of cystic ovaries.

Stress may also have been involved in the development of cystic ovaries. Cortisol (Stoebel and Moberg, 1982; Pearce et al., 1988) and ACTH (Liptrap, 1970; Barb et al., 1982; Hennessy and Williamson, 1983) during the follicular phase suppress LH release, the LH surge and can block ovulation. Cortisol blocks the increase in pituitary GnRH receptor concentrations which normally occur under stimulation of E₂ (Daley et al., 2000). No effects of cortisol were found on the ability of the pituitary to respond to GnRH as cortisol treated gilts did respond to GnRH administration by increasing LH levels (Turner et al., 1999a). Thus, cortisol could play a role in the development of cystic ovaries through reducing the pituitary response to GnRH. It seems, however, from studies in (ovariectomised) gilts, that cortisol levels need to be elevated for a number of days before it can suppress LH (Turner et al., 1999a) and inhibit the LH surge (Turner et al., 1999b). Cortisol levels in sows with lactational oestrus induced by Intermittent Suckling (experiment 1), were higher during the first week of separation in sows in which separation occurred at 6 h intervals (Kluivers et al., unpublished results). In this group of sows, the number of sows developing cystic ovaries was higher when compared to separation at 12 h intervals or when weaning occurred at day 21 of lactation (Chapter II). Unfortunately, cortisol levels were not assessed in the other experiments. It therefore remains unclear whether sows developing cystic ovaries had a higher stress response upon separation from their piglets, subsequently suppressing LH release.

In conclusion, in sows that develop cystic ovaries, follicle diameter during the first 4 days of the follicular phase, timing and the level of E₂ profiles and onset of oestrus are similar to sows that ovulated. Characteristic for sows that developed cystic ovaries were the absence of the LH surge, E₂ levels not returning to basal levels within the expected time period, a longer duration of oestrus, and low P₄ levels. Sows that developed cystic ovaries start to deviate in follicle size around the time that ovulation occurs in sows that ovulated. The LH surge and consequently ovulation, do not occur due to a dysfunction in the feedback of E₂ to the hypothalamus possibly related to effects of stress (cortisol) or metabolic state (insulin levels).

VII

General discussion

Introduction

For Intermittent Suckling (IS) to be a successful management system from the sow reproductive point of view there are three important criteria to be met: (1) sows need to respond to IS by showing normal follicle growth and ovulation within limited variation in timing, (2) sows should show oestrous behaviour, (3) sows should become and remain pregnant without compromising subsequent litter size.

In this chapter, the results of our studies regarding the above described criteria in terms of reproductive physiology and their practical implications will be discussed in Parts I and II. In Part III of this chapter, several management factors will be described that can influence the performance of sows within an Intermittent Suckling system. Finally conclusions will be drawn (Part IV) and recommendations for further research will be given (Part V).

I. Follicle growth, oestrous behaviour and ovulation

For IS to be applied in practice, one of the most important criteria is that a large proportion of the sows needs to respond to IS by showing follicle growth, oestrous behaviour and ovulation. It is also important that the timing of oestrus and ovulation occur at a predictable interval from start of IS in order to minimize variation in farrowing dates within groups. This is necessary, because farmers work with week systems in which activities such as insemination, weaning and farrowing occur on particular set days to create a high labour efficiency and a high hygiene status.

Percentages of sows responding to IS

The follicular response of sows to IS can be divided into two main categories, sows showing follicle growth with follicle diameters remaining smaller than pre-ovulatory size (<6mm) and sows developing follicles of pre-ovulatory size (>6mm). Sows with follicle growth larger than 6mm can be subdivided into ovulatory and anovulatory sows. Anovulatory sows either showed regression of pre-ovulatory follicles or continued follicle growth and consequently developed cystic ovaries. The number of sows per category of follicle growth are shown in Table 1.

With an early start of IS at day 14 of lactation, 87% of the sows (69/79) developed pre-ovulatory follicles. Based on the total number of animals, the percentage of sows that ovulated within 8 days after start of treatment was lower (65%; 52/79); P<0.05) when start of IS was early compared to sows weaned at day 21 of lactation (94%; 34/36). With a later start of IS at day 21 of lactation, all sows showed follicle development up to pre-ovulatory size (23/23) and ovulation occurred in 87% (20/23) of the sows, which was comparable to control sows weaned at day 21 of lactation (94%; Table 1). The number of sows with ovulation based

on the number of sows developing pre-ovulatory follicles, was also lower when start of IS was early after farrowing compared to sows weaned at day 21 of lactation (75% vs. 94%, P<0.05; Table 1).

Table 1. Categories of response to IS with regard to follicle development, oestrus and ovulation based on three experiments

	Treatment ¹		
	C	IS14	IS21
Number of sows			
Number of sows with oestrus/ large follicles before start of treatment	17	1	10
Number of animals included at start of treatment	36	79	23
Follicle development < 6 mm			
No pre-ovulatory follicles	0/36	10/79	0/23
Follicle development > 6 mm			
Ovulation within 8 days after start of IS/ weaning	34/36 ^a	52/69 ^b	20/23 ^{b,c}
○ oestrus	34	47	19
No ovulation			
- Regression of follicles	0	7	3
○ oestrus	-	0	3
- Cystic follicles	2	10	0
○ oestrus	2	8	-

¹C: sows weaned at day 21 of lactation; IS14: start of IS at day 14 of lactation; IS21: Start of IS at day 21 of lactation; A χ^2 test was used to test for differences in the number of sows with follicle development <6 mm compared to the number of sows with follicle development >6 mm between treatments. Within the category of sows with follicle development > 6 mm the number of sows that ovulated was compared to the number of sows that did not ovulate. ^{a,b} Different superscripts within one row indicate a difference of P≤ 0.05.

In the category of sows with ovulation, ovulation occurred at day 5 to day 8 after the start of treatment (on average at day 6) and oestrous behaviour was expressed in more than 90% of these sows irrespective of timing of start IS. Unfortunately, oestrous expression was also observed in 59% of the sows that did not ovulate, and developed of cystic ovaries or in which pre-ovulatory follicles regressed.

The number of animals responding to IS with ovulation in the current studies is relatively high when compared to studies performed in the 1970's and 1980's in which percentages of 3% to 50% were found (Crighton, 1970; Henderson and Hughes, 1984; Stevenson and Davis, 1984). These differences in response may be related to the IS regimen. However, it seems more likely that they are related to genetic selection for improved reproductive performance and especially weaning-to-oestrus interval. This selection has

resulted in a reduction of the weaning-to-oestrus interval from 18.7 days (Fahmy et al., 1979) to an average of 5.8 days in 1995 (Vesseur, 1997) indicating that the modern sows used in our studies differ in reproductive performance from the sows used in the older studies.

In summary, start of IS at day 14 of lactation resulted in ovulation in 65% of the sows that had small follicles at the start of IS, and 90% of these sows expressed oestrus. Start of IS at day 21 of lactation resulted in ovulation in 87% of the sows with small follicles at the start of IS, and 95% of these sows expressed oestrus. A number of sows, however, had already large follicles at the start of treatment at day 21, but this was also observed in the control sows. Overall, start of IS at day 21 of lactation was comparable to weaning at day 21 of lactation. Thus, a high number of sows respond to IS with follicle growth (>80%), but the percentage of sows that ovulate is lower when start of IS is relatively early after farrowing.

Reproductive physiology of follicle development, oestrus and ovulation

Not all sows responded to IS with follicle development, oestrus and ovulation. The question is which differences in physiology cause these differences in response of sows described in Table 1.

Sows with large follicles or oestrus and ovulation before start of treatment

A high percentage of sows was excluded from the treatments that started at day 21 of lactation (about 30%), because they already showed considerable follicle growth and/or ovulation before the start of treatment. Spontaneous lactational oestrus seems to occur more often in high parity sows or in sows with a small litter size or low suckling frequency (Gaustad-Aas et al., 2004), and is most likely related to a lower suckling stimulus or lower sensitivity to the inhibition of suckling. In our studies, no difference was found in parity between sows with follicle development before start of treatment and sows ovulating within 8 days after start of treatment (6.0 ± 0.4 vs. 5.3 ± 0.2 ; P=0.10). Litter size, however, was smaller in sows that already had increased follicle growth before start of treatment (8.8 ± 0.2 piglets vs. 10.1 ± 0.1 piglets at day 14 of lactation; P<0.01). A smaller litter size can reduce the release of endogenous opioids which are responsible for the suppression of pulsatile LH secretion during lactation (Barb et al., 1986; Armstrong et al., 1988; De Rensis et al., 1999b) as described in detail in Chapter I. As a result of reduced endogenous opioid release, pulsatile LH secretion can escape from the inhibitory effect and consequently follicles grow out and a sow may show lactation oestrus. Pulsatile LH secretion can also be affected by the metabolic state of the sow as the metabolic state during lactation has been found to be related to pulsatile LH secretion after weaning (Zak et al., 1998). When sows have a high feed intake during lactation and the metabolic state is not too negative, it could be possible that LH pulsatility is increased. In our studies no differences were found in body weight loss or feed intake during the first two weeks of lactation between sows that already had increased follicle development before the start of treatment and sows that ovulated. Body weight at day 13 of lactation, however, was higher in sows with advanced follicle growth or ovulation at start of treatment

when compared to sows that ovulated within 8 days after the start of treatment (284 ± 4 kg vs. 267 ± 3 kg; P<0.05). This difference in body weight does seem to indicate that metabolic state can be involved in advanced follicle growth at the start of treatment.

Generally, increases in LH pulsatility are observed as lactation progresses (Quesnel and Prunier, 1995) and the capability of the pituitary to secrete LH also increases as lactation progresses (Sesti and Britt, 1993a). This could explain why the occurrence of follicle development before start of treatment was observed when the start of treatment was at day 21 of lactation.

In summary, in our sows substantial follicle development or even ovulation before start of treatments occurred in relatively many sows. Data analyses of our sows indicate that this is most likely due to a small litter size, but other factors such as parity, breed and management are also factors of importance in practice. Over the years, an increase in the occurrence of spontaneous lactational oestrus has been observed, for example 7% of the sows used in a study by Soede et al (1995a) had experienced lactational oestrus, which could be related to selection on higher reproductive performance and especially a shorter weaning-to-oestrus interval. As the weaning-to-oestrus interval can differ between breeds (Vesseur et al. 1994), it is likely that the chance of lactational oestrus also differs per breed (see also Part III).

Sows not developing pre-ovulatory follicles

Of the sows included in the treatments, 13% showed some follicle growth, but follicle diameter did not reach pre-ovulatory size (<6mm). In one of our IS studies, LH and FSH pulsatility were measured during the first 12 h of separation. On this first day of separation, no differences were found in FSH and LH parameters between sows that ovulated and sows that did not ovulate, only the frequency of LH pulses tended to be higher in sows that did not ovulate (Langendijk et al., 2007c). These results indicate that during these first 12 h of separation FSH and LH were not limiting. It is, however, unknown what the levels of FSH and LH were after these 12 h. Follicles of these sows did not produce E₂ (Langendijk et al., 2007c) and for a high production of E₂ by the follicles both FSH and LH are necessary (Guthrie et al., 1988). Follicles were found to grow to a diameter of 4 to 6 mm under FSH stimulation only and to 7 to 9mm under LH stimulation only (Guthrie et al., 1988). In these sows follicle diameter did not reach 6mm and both LH and FSH could be limiting for functional follicle development. In sows with similar characteristics, however, (described in the paragraph on page 96), follicles reached diameters greater than 6mm. It seems that in these sows, LH was sufficient. It, however, remains unclear if LH, FSH or both were limiting in this category of sows or whether other factors were involved. This response to IS, was only observed in sows subjected to IS from day 14 of lactation onwards. This seems to indicate that the factor limiting functional development is only limiting at day 14 of lactation. It is therefore interesting to further this category of sows and also LH and FSH levels beyond the first 12 h of separation.

The metabolic state of the sow has also been related to follicle development and ovulation, as a high body weight loss during lactation has been related to a prolonged weaning-to-oestrus interval (for review: Prunier et al., 2003). In our sows, however, body weight loss during the first two weeks of lactation did not differ between sows not developing pre-ovulatory follicles and sows that ovulated within 8 days after the start of treatment ($P>0.10$). Also feed intake during the first two weeks of lactation or body weight at day 1 and day 13 after farrowing did not differ from sows that ovulated within 8 days after the start of treatment ($P>0.10$). Thus, from our results there are no indications that these sows differ metabolically from sows responding to IS with ovulation.

In summary, sows responding to IS with some follicle growth, without reaching pre-ovulatory size was observed only when start of IS was at day 14 of lactation, but it is unclear which factors are limiting follicle growth.

Sows developing pre-ovulatory follicles followed by ovulation

About 93% of the sows responded to the treatments with follicle growth up to pre-ovulatory size ($>6\text{mm}$) within 6 to 7 days after the start of treatment. Within this group, 83% ovulated within 8 days after start of treatment. As described in detail in Chapter III and V, follicle development and the number of ovulating follicles in IS sows were comparable to sows weaned at day 21 of lactation (see also Table 2). Also, the production of E_2 by the growing follicles in IS sows was similar to weaned control sows (Table 2). Follicles of sows subjected to an early start of IS (day 14 of lactation) needed more time to reach peak E_2 levels (Table 2). The increased interval in reaching peak E_2 levels, might explain the later onset of oestrus and consequently the shorter duration of oestrus observed in sows subjected to an early start of IS (Table 2). A late onset of oestrus has been related to a short duration of oestrus (Kemp and Soede, 1995; Steverink et al., 1999) and also in the present study a negative correlation between onset and duration of oestrus ($r=-0.46$; $P<0.01$) was found. Thus, the short duration of oestrus in sows with an early start of IS at day 14 of lactation, can be explained by the late onset of oestrus. Ovulation occurred synchronously on average at day 6 after the start of treatment, but sows subjected to an early start of IS, tended to ovulate 10 h later than weaned control sows.

The LH surge tended to be lower in IS sows (Chapter II). There are several potential factors which could affect peak LH levels in IS sows, either related to lactation and specifically suckling (prolactin, oxytocin and endogenous opioids), or related to the regimen of IS (stress of separation and timing of start IS). Prolactin has been found to affect the pre-ovulatory LH surge, but only resulted in complete inhibition of the LH surge (Pang et al., 1977; Ben-Jonathan et al., 1989). Also endogenous opioids have been found to either completely inhibit the LH surge or had no effect on the LH surge in rats (Pang et al., 1977). It is therefore unlikely that prolactin or endogenous opioids affected peak LH levels. It is unclear whether oxytocin could have affected peak LH levels.

Table 2. Timing of oestrus and ovulation parameters and hormonal events for responder sows ovulating within 8 days after start of treatment

	Treatment ¹		
	C	IS14	IS21
Oestrus and ovulation²			
Onset of oestrus relative to start of treatment (h)	100±4 ^c	113±4 ^d	104±6 ^{c,d}
Ovulation relative to start of treatment (h)	139±4 ^c	149±3 ^d	148±5 ^{c,d}
Duration of oestrus (h)	59±4 ^{a,b}	49±3 ^a	70±5 ^b
Ovulation rate	25±1	27±1	28±2
Hormonal events³			
<i>Timing</i>			
Peak E ₂ levels relative to start of treatment (h)	78±6 ^a	94±5 ^b	ND
Peak LH levels relative to start of treatment (h)	103±4 ^{a,c,d}	119±4 ^{b,d}	98±8 ^{a,b,c}
Interval peak LH to ovulation (h)	39±3	32±1	33±4
<i>Hormone levels</i>			
Peak E ₂ levels (pg/ml)	25±1	26±1	ND
Peak LH levels (ng/ml)	7.2±0.6 ^a	4.9±0.6 ^b	6.3±1.2 ^{a,b}

¹C: sows weaned at day 21 of lactation; IS14: start of IS at day 14 of lactation; IS21: Start of IS at day 21 of lactation.²Number of sows for oestrus is 34, 47, 19 and for ovulation 34, 51, 19 for C, IS14 and IS21 respectively.³Number of sows for E₂ is 16, 19 and 0 and for LH 23, 26 and 8 for C, IS14 and IS21 respectively.^{a,b} Different superscripts within one column indicate a difference of P≤ 0.05; ^{c,d} different superscripts within one column indicate a difference of P≤ 0.10. ND= not determined; lsmeans ± se.

The regimen of IS could affect peak LH levels through stress as cortisol has been found to negatively affect LH release in gilts (Hennessy and Williamson, 1983; Pearce et al., 1988). IS, however, induced repeated acute stress at separation, as shown by acute increases of cortisol (Kluivers et al., unpublished). Acute stress has not been found to affect peak LH levels (Turner et al., 1999a) and it therefore seems unlikely that this reduced peak LH levels in our IS sows. Another factor related to the regimen of IS is the timing of start of IS. Pooled analyses of peak LH levels (Chapter II and Chapter V) show that induction of oestrus early after farrowing resulted in lower peak LH levels (Table 2). Similar results have been found in studies using early weaning (Edwards and Foxcroft, 1983; Kirkwood et al., 1984; Willis et al., 2003). Low peak LH levels early after farrowing are the result of limited LH secretion from the hypothalamus-pituitary axis as the pool of releasable LH increases as lactation progresses (Sesti and Britt, 1993b,c). When IS is initiated a week later, from day 21 of lactation onwards, peak LH levels were comparable to weaned sows (Table 2).

In summary, in ovulating IS sows, follicle development, E₂ production, oestrous behaviour and ovulation rate are similar to weaned sows, but the timing of events is somewhat delayed (about 10 h) and lower peak LH levels were found when start of IS is early after farrowing.

Sows developing pre-ovulatory follicles without subsequent ovulation

Not all sows showing follicle growth up to pre-ovulatory size ovulated. Within this group of anovulatory sows, pre-ovulatory follicles regressed or continued growth and developed into cystic follicles.

Sows subjected to IS from day 14 of lactation onwards and showing regression of pre-ovulatory follicles ($n=7$), did not express oestrous behaviour (Langendijk et al., 2007c, Chapter V) and did not produce E_2 (Langendijk et al., 2007c). These sows show the same characteristics as the sows described earlier, which did not develop pre-ovulatory follicles ($n=10$). Although follicle diameter in these sows did reach pre-ovulatory size, FSH could possibly be the limiting factor in these sows as Guthrie et al. (1988) have shown that follicle can grow up to 7 to 9mm under LH stimulation only, but not under stimulation of only FSH.

In three sows subjected to IS from day 21 of lactation onwards, pre-ovulatory follicles also regressed. These three animals differed from the other sows showing follicular regression as these sows did express oestrous behaviour. It must be noted, however, that the duration of oestrus in these sows was very short (12 h) when compared to sows that ovulated (70 ± 5 h). Nevertheless, as oestrus was observed, it is likely that E_2 was produced in these animals, but unfortunately this was not measured. Follicle development of two of the three sows was comparable to sows that ovulated within 8 days after start of treatment, only in one sow small follicles were observed before regression of follicles at time of expected ovulation. Hormone profiles measured in two of the three sows show that these animals did not have a noticeable pre-ovulatory LH surge and no ovulation as P_4 levels remained low. It remains unclear why in these sows follicles regressed and did not remain present on the ovaries in absence of a LH surge.

In sows developing cystic ovaries, peak E_2 levels were similar to sows with ovulation, but the return of E_2 to basal levels was not within 48 h after peak levels, which was expected based on sows that ovulated. It seems that in sows developing cystic ovaries, there was sufficient FSH and LH during the early follicular phase and the follicle was capable of responding to these hormones as follicles reached pre-ovulatory size and produced E_2 . No ovulation occurred, however, due to a lack of a pre-ovulatory LH surge, most likely caused by a dysfunction in the feedback from E_2 to the hypothalamus (Chapter VI). Why and how this dysfunction occurs is unclear, but it might be related to the early start of IS after farrowing, since the percentage of sows developing cystic follicles was higher when sows were subjected to IS from day 14 of lactation onwards. Furthermore, the development of cystic ovaries may also be related to the metabolic state (Chapter VI), but no differences were found in body weight loss during the first two weeks of lactation, body weight at day 1 or day 13 of lactation or feed intake when compared to sows that ovulated within 8 days after the start of treatment. The only difference found between sows developing cystic ovaries and sows that ovulated was that sows developing cystic ovaries were of higher parity (6.9 ± 0.5 vs. 5.3 ± 0.2 ; $P<0.05$).

In summary, in non-ovulating sows with pre-ovulatory follicle growth regression of pre-ovulatory follicles may be the result of differences in physiology. It remains unclear why regression of pre-ovulatory follicles occurs in sows without oestrous expression. Anovulation in oestrous sows with regression of pre-ovulatory follicles and sows developing cystic ovaries may possibly be related to a dysfunction in E₂ feedback. It remains unclear why in some sows these follicles regress and in other follicles remain on the ovary and develop into cysts.

Consequences for practical implication

Our studies show that a high number of sows respond to IS with follicle growth (>80%). Start of IS during later lactation (day 21) results in a response similar to sows weaned at day 21 of lactation, indicating that when timing of IS is not too early, follicle development, oestrus and ovulation are not compromised by application of IS. With an early start of IS (day 14 of lactation), the number of sows that does not develop pre-ovulatory follicles, shows regression of follicles or develop cystic ovaries is high. The incidence of cystic ovaries seems to be higher in older sows and the incidence of advanced follicle growth or ovulation before start of IS or weaning seems to be higher in sows with a high body weight at farrowing and a small litter size at day 14 of lactation. Unfortunately, these findings can not help in predicting which how sows will respond to IS. Most sows developing cystic ovaries showed oestrous behaviour and would have been inseminated. Consequently, observed pregnancy rates in an IS management system with start of IS at day 14 of lactation, will be lower. Within the category of ovulating sows, not all animals expressed oestrus and, consequently not all animals with ovulation would have been inseminated in practice. In our studies, oestrus detection was carried out in absence of a boar, but it is likely that the presence of a boar during IS, will further stimulate oestrous expression in these sows (Langendijk et al., 2000b). For practical implications, it seems that an early start of IS relative to farrowing is not advisable as at this time the response to IS with follicle growth is variable and ovulation rates are compromised. The response of sows to IS with follicle growth and ovulation may be related to breed and parity of the sows and will be further discussed in Part III of this chapter.

II. Pregnancy

For IS to be applied in practice, it is important that ovulation rates, pregnancy rates and embryo survival rates are high. Furthermore, it is also important that embryo development is not compromised by lactation as this could affect the performance of piglets after birth. In our studies, early embryo survival and embryo development (day 23 and day 30 after ovulation) were examined in IS sows with a different timing of start of IS and duration of IS and will be discussed below.

Pregnancy rates in IS sows

In practice, sows are inseminated based on the expression of oestrous behaviour. In our studies a number of sows showed oestrous behaviour but did not ovulate (see Part I). Therefore, pregnancy rates at day 23 and day 30 can be expressed as a percentage of the number of ovulating or the number of oestrous sows. In Table 3 is shown that pregnancy rates between treatments are similar for both definitions. It must be noted, however, that for detection of differences in pregnancy rates, the number of sows used in our studies is relatively low.

Studies in which sows were weaned relatively early after farrowing show that pregnancy rates are lower than when weaning occurs at a later stage during lactation (Varley and Cole, 1976; Belstra et al., 2002). Furthermore, Gaustad-Aas et al. (2004) showed that pregnancy rates were lower in sows with spontaneous ovulation during lactation as well as ovulation after weaning when ovulation occurred within 3 weeks after farrowing. In our studies, start of IS at day 21 of lactation resulted in a numerically higher percentage of pregnant sows (94%) than when start of IS was at day 14 of lactation (82%; Table 3), supporting the results found in other studies.

Not only timing of start of IS could affect pregnancy rates in IS sows, but also the duration of IS or in other words, continuance of IS during early pregnancy or not (sows weaned at ovulation). When IS continued during early pregnancy, pregnancy rates were found to be lower than when the sow was weaned at ovulation (81% vs. 100%, P=0.05; Table 3). Although pregnancy rates did not seem to be affected by lactation in another study (Gaustad-Aas et al., 2004), within our IS sows, lactation after ovulation seems an important factor of influence.

In conclusion, in our studies with relatively low numbers of animals, continuation of lactation during early pregnancy negatively affected pregnancy rates at day 23 and day 30 after ovulation. Furthermore, although not significantly proven, an early start of IS seems to negatively affect pregnancy rates at day 23 and day 30 after ovulation.

Embryo survival and embryo development

Not only pregnancy rates need to be high, but also the number of embryos and embryo quality should not be compromised.

Timing of start of IS did not affect embryo survival rate at day 30 after ovulation. At day 23 after ovulation, embryo survival rate in IS sows with start of IS at day 14 of lactation was lower than in weaned control sows (see Table 3). In general, the percentage of sows with a high embryo survival (>61%) tended to be lower when start of IS was early (37%) when compared to sows weaned at day 21 of lactation (59%) and sows with IS from day 21 of lactation onwards (65%; P<0.10). Embryo development, placental and uterine parameters at day 30 were not affected by timing of start of IS. At day 23, embryo development tended to be lower in IS sows (Table 3), but this could also be related to the continuance of IS after ovulation, which will be discussed later in this paragraph.

The higher percentage of sows with a relatively low embryo survival rate, and compromised embryo development when IS was initiated from day 14 of lactation onwards can be a result of a suboptimal uterine environment. According to Palmer et al. (1965a), the uterus changes in weight and length till day 28 after parturition and as sows with an early start of IS ovulate around day 21 of lactation, involution of the uterus may not be complete possibly resulting in a suboptimal environment for the embryos.

Uterine development may also be related to P₄ levels which have not only been associated with embryo survival rate (Jindal et al., 1996), but also to uterine blood flow (Ziecik et al., 1986; Ziecik et al., 1992) and secretion of uterine proteins (Vallet et al., 1998). Within sows with start of IS at day 14 of lactation, a positive relation was found between P₄ levels and embryo survival rate which was absent in sows with start of IS at day 21 of lactation (Chapter V). It is might be that with when with an early start the uterine environment is not optimal, high P₄ levels are necessary to increase the quality of the uterine environment necessary for embryos to survive.

Embryo survival rates seemed to be influenced by timing of start of IS, but no effects of continuance of lactation after ovulation were found at day 30 after ovulation (Table 3). Embryo development at day 30 tended to be reduced in sows with continuance of IS during early pregnancy (Table 3). Also at day 23 after ovulation, embryo development was reduced in one IS treatment (IS6; Chapter III). In this group also placental characteristics were negatively affected. Sows in this IS group were subjected to 6 h intervals of suckling and non-suckling. Litters of this group dependent more on milk as the main source of energy (Berkeveld et al., 2007), indicating that milk production of these sows remained high. Continuance of IS during early pregnancy and 12 h of suckling per day also negatively affected uterine development (Chapter V). It is possible that uterine, placental and embryo characteristics were influenced by lactational hormones related to suckling such as oxytocin, prolactin and endogenous opioids. The role of oxytocin in affecting pregnancy parameters is unclear as results in literature are contradictory. In mares, oxytocin did not affect pregnancy rate or embryonic growth (Handler et al., 2006), but in cows oxytocin resulted in a lower embryo survival at day 15 post insemination, possibly through reduction of P₄ or stimulation of PGF2 α (Yildiz and Erisir, 2006). So it might be that oxytocin could negatively affect embryo survival, but it is unclear if oxytocin affected embryo survival in IS sows. Prolactin also negatively affected the number of embryos, embryo and placental weight when administered to rabbit does (Fortun-Lamothe et al., 1999). Prolactin affects embryo and placental development through the quality of uterine secretions, water and ionic transport and endometrial vascularisation (Fortun-Lamothe et al., 1999). Thus, prolactin can not be ruled out as a factor indirectly influencing embryo development in IS sows. There are also indications that exposure of embryos to opioids can affect the development of the nervous system in animals and humans (Robinson, 2000). In humans, however, most studies are conducted using humans addicted to opioids and thus having high levels during the day. In IS sows it is unclear what the levels of endogenous opioids are during the periods of suckling

and non-suckling, but endogenous opioids can not be ruled out as a possible factor. In summary, it is possible that prolactin, oxytocin and endogenous opioids negatively affect embryo, placental and uterine development in sows, but further investigations are necessary to clarify these aspects.

Table 3. Pregnancy rates and embryo survival rates of sows ovulating within 8 days after start of treatment

	Treatment ¹			Weaned at ovulation	IS after ovulation
	C	IS14	IS21		
Pregnancy rate at day 23 to day 30²					
% of ovulating sows	85	82	94	100 ^x	81 ^y
% of oestrous sows	81	73	73	-	-
Embryo survival³					
Day 23	70±5 ^a	54±5 ^b	ND	ND	54±5
Day 30	59±5	52±4	62±5	59±4	53±5
Number of embryos³					
Day 23	19±2	17±2	ND	ND	17±2
Day 30	17±1	13±1	14±1	15±1	12±1
Embryo development³					
Embryo weight (g) at day 23	0.20±0.01 ^c	0.16±0.01 ^d	ND	ND	0.16±0.01
Embryo weight (g) at day 30	1.56±0.06	1.61±0.05	1.54±0.05	1.64±0.05 ^c	1.50±0.05 ^d

¹In this table data of three experiments are pooled; for the parameters weaning at ovulation and IS after ovulation only data from experiment 1 and 3 are included. C: sows weaned at day 21 of lactation; IS14: start of IS at day 14 of lactation; IS21: start of IS at day 21 of lactation; Weaned at ovulation: piglets were weaned at ovulation of the sow and embryos developed in a non-lactating sow; IS after ovulation: IS continued till day 23 (exp 1) or day 20 (exp 3) after ovulation and embryos developed during the first 3 weeks of pregnancy in a lactating sow. ²The number of sows for pregnancy data is 34, 49, 19, 19 and 42 for C, IS14, IS21, weaned at ovulation and IS after ovulation, respectively. ³The number of sows for embryo parameters at day 23 was 16, 16, 0, 0, 16 and at day 30 13, 23, 17, 19, 17 for C, IS14, IS21, weaned at ovulation and IS after ovulation, respectively. ^{x,y} P=0.05 for early pregnancy during IS vs. in weaned IS sows. ; ^{a,b} Different superscripts within one column indicate a difference of P<0.05 between C and IS14 sows. ^{c,d} different superscripts within one row indicate a difference of P<0.10. ND= not determined.

Metabolically related hormones such as insulin and IGF-1 may also have affected embryo survival and development. Willis et al. (2003) found a positive correlation between mean IGF-1 values 10 h after weaning in sows weaned at day 14 of lactation and the number of embryos at day 28 of lactation ($r=0.79$; $P=0.03$). The authors state that this correlation suggests that IGF-1 may be a uterotrophic factor (Willis et al., 2003). Levels of IGF-1 (Van den Brand, 2000) have been found to be low during lactation. The levels of IGF-1 in sows with continuance of IS during early pregnancy have not been measured during pregnancy, but if IGF-1 levels remain low as a result of lactation, it is possible that these low IGF-1 levels may have affected embryo development. Furthermore, maternal IGF-1 levels during early pregnancy have been found to play a role in programming the placenta for growth and functioning during gestation in guinea pigs (Sferruzzi-Perri et al., 2007). Possibly, low levels of IGF-1 during early pregnancy can affect foetal growth during later gestation. Thus, IGF-1 levels may play an important role in embryo and placental development and could have affected these parameters in sows with IS continued after ovulation. Also insulin levels are low during lactation (Prunier et al., 1993) and may affect embryo survival. Ramirez et al. (1997) found effects of exogenous administration of insulin during the weaning-to-oestrus interval on litter size. Insulin increases P₄ levels (Smith et al., 2006) and as P₄ is related to embryo survival (Jindal et al., 1996) insulin could indirectly affect embryo survival through P₄ levels. Possible effects of insulin on P₄ levels are discussed in the following paragraph. Thus, insulin levels could play a role in embryo survival in sows and further investigation is needed to clarify the role of insulin.

The number of viable embryos at day 23 or day 30 did not differ significantly between IS sows and weaned control sows, probably due to the relatively low number of sows used. Already at day 23 after ovulation the numeric difference between weaned control sows and IS sows with start IS at day 14 of lactation was 2 embryos (Table 3), and at day 30 the difference was 4 embryos. In our studies, the number of embryos was not determined after day 30, but the results from day 23 and 30 indicate consequences for litter size. It is difficult to estimate litter size using the number of viable embryos at day 30 as it is unknown what the percentage of foetal mortality will be in sows with a compromised embryo, placental and uterine development at day 30. In order to estimate litter size, the assumption has to be made that foetal mortality will be similar in all treatments despite the effects on embryo development. Assuming that about 13% of the foetuses are lost between day 20 - 30 (survival 62%) and day 85 - 90 (survival 49%) of gestation (Town et al., 2005) resulted in an estimation of 11.7, 13.5, 13.8 piglets born per litter for sows with start IS at day 14 or day 21 of lactation and sows weaned at day 21 of lactation respectively. In this estimation with the assumption that foetal mortality will be similar, an early start of IS litter size will result in a reduction of two piglets per litter. It must be taken into account that foetal mortality can be higher, resulting in a reduction of more than two piglets.

In conclusion, an early start of IS negatively affects embryo survival and continuance of IS after ovulation negatively affects embryo, placental and uterine development. Suckling

related hormones, oxytocin, prolactin and endogenous opioids can not be ruled out as factors of influence in sows with continuance of IS. It is unknown whether the effect on embryo development affect chances of survival during later gestation and development of offspring during gestation and after birth.

Post-ovulatory physiology: progesterone (P_4) levels

Progesterone is an important physiological parameter when exploring causes for possibly lower pregnancy and embryo survival rates in IS systems as it is essential for maintenance of pregnancy and low levels have been associated with embryo mortality in the pig (e.g. Jindal et al., 1996; Van den Brand et al., 2000b). In our studies P_4 levels were lower in sows in which IS continued after ovulation. No association, however, was found between P_4 levels and embryo survival in IS sows at both day 23 or day 30 of pregnancy (Figure 1), which may possibly be explained by lactation and the site of blood sampling.

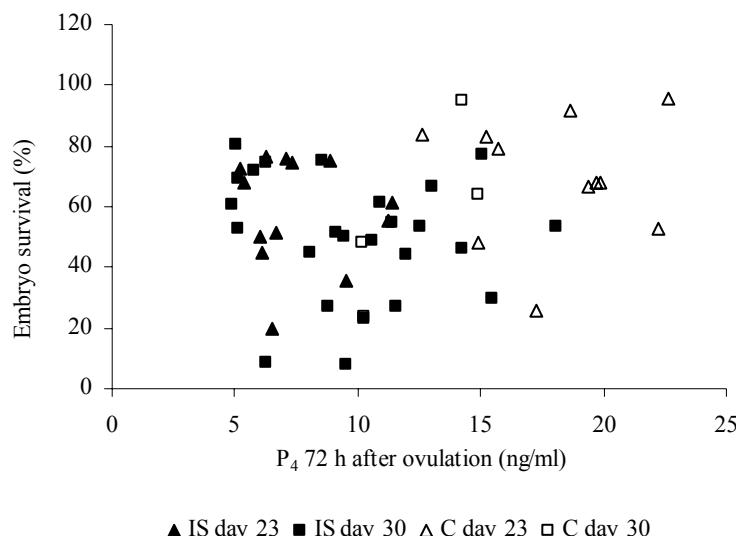


Figure 1. Relation between P_4 levels at 72 h after ovulation and embryo survival for IS sows or weaned control sows (C) at day 23 and day 30 of pregnancy

An important factor which can explain the low P_4 levels, the lack of an association between P_4 levels and embryo survival in our studies compared to other studies is that the fact that our sows were lactating during the rise in progesterone. During lactation, basal liver blood flow and thus metabolic clearance rate are high (Sangsrivavong et al., 2002). This results in a high clearance rate of P_4 from the blood and in our studies blood samples were taken from the jugular vein, after the blood had passed through the liver. Thus, it is possible that the production of P_4 at the ovary is sufficient, but that due to the high metabolic clearance rate lower levels are measured in our samples. This higher lactational metabolic clearance rate might also explain why no effects of feeding level on P_4 were found in IS sows (Chapter IV),

whilst studies using non-lactating animals did find low P₄ levels under high feeding levels (Jindal et al., 1996) and sows (Virolainen et al., 2005a).

For maintenance of pregnancy and development of the uterus it is important that sufficient levels of P₄ reach the uterus. Stefanczyk-Krzymowska et al (1998) have shown that the concentration of P₄ in blood reaching the uterus is elevated through a direct connection between branches of the ovarian and uterine artery. Thus, it is possible that in IS sows production P₄ at the ovary is high and reaches the uterus directly. If this is the case, the lower P₄ levels measured in jugular vein samples do not always reflect what levels reach the uterus and can explain why no relations were found between jugular P₄ and embryo survival in our sows.

P₄ levels at the ovary were not measured and it could be that other lactational related factors (prolactin, oxytocin, endogenous opioids) affected the production and secretion of P₄ at the ovary. High prolactin levels have been found to decrease P₄ levels with 71% (in vitro; Magoffin and Erickson, 1982) and result in low levels of post-ovulatory P₄ in rabbit does (Fortun-Lamothe et al., 1999). Also, high oxytocin levels inhibit release of P₄ from pig luteal cells (Pitzel et al., 1988) and in cows, administration of oxytocin two times daily, resulted in lower levels of P₄ and a slower rise in P₄ than in control animals (Yildiz and Erisir, 2006). If twice daily administration of oxytocin can lower P₄ levels in cows, the expected high levels of oxytocin during 12 h of suckling periods in IS sows could also affect P₄ levels. If the effect of oxytocin is short term, levels of P₄ would be lower after a suckling period than after a period of non-suckling. In our data, however, no differences were found between non-suckling and suckling periods ($P>0.10$; Table 4). It is possible that in sows with suckling periods at 6 h intervals (Experiment 1, Chapter II), levels of prolactin and oxytocin are more elevated as sows experience shorter periods of non-suckling thus a shorter period of time for prolactin levels to reach basal levels. If this is true, it did not affect P₄ levels as no differences were found in P₄ levels between different IS regimens (a 12 h continuous suckling period vs. 6 h interval suckling periods). It must be noted, however, that the lack of a difference in P₄ levels between suckling and non-suckling periods is based on P₄ levels measured in jugular vein blood. At the level of the ovary, where the blood has not passed through the liver, differences in P₄ levels after suckling and non-suckling periods might be detectable.

Next to prolactin and oxytocin, endogenous opioids may also play a role in P₄ release. Administration of an endogenous opioid antagonist (naloxone) increased P₄ secretion from cultured theca cells (in vitro; Kaminski et al., 2001), indicating that high levels of endogenous opioids could reduce P₄ secretion. Thus, not only prolactin and oxytocin, but also endogenous opioids could affect production of P₄ and must not be ruled out.

P₄ production at the ovary might also be affected by hormones (IGF-1, insulin) related to the metabolic state of the sow. IGF-1 can play an important role in the early development of the CL (Miller et al., 2003; Ptak et al., 2003) and stimulates P₄ secretion in pigs (Miller et al., 2003). At day 4 of the oestrous cycle, high concentrations of IGF-1 were found when compared to other days of the cycle (Miller et al., 2003), but during lactation IGF-1 levels are

low (Van den Brand, 2000). Low IGF-1 levels were also found in IS sows (Langendijk et al., 2007b). In sows weaned at day 21 of lactation, however, P₄ levels were high, but IGF-1 levels were still low, which raises the question of the importance of IGF-1 in P₄ secretion. Although, IGF-1 can stimulate P₄ secretion, but its role in P₄ levels in IS sows remains unclear.

Table 4. P₄ levels during suckling and non-suckling periods in sows in which IS continued after ovulation

	P ₄ level at the end of the suckling period (ng/ml)		P ₄ levels at the end of the non-suckling period (ng/ml)	
	N	Means ± se	N	Means ± se
72 h after ovulation	18	7.6±0.5	25	8.7±0.5
96 h after ovulation	19	10.6±0.7	23	10.8±0.6
144 h after ovulation	7	14.4±2.2	18	15.5±1.5

P₄ samples of IS sows in which IS continued after ovulation were subdivided according to the time of sampling (at the end of a suckling or non-suckling period) to test for possible effects of suckling on P₄ levels.

Insulin can also play a role in P₄ secretion as infusion of insulin resulted in an increase in P₄ levels (Smith et al., 2006). Normally during lactation insulin levels are low (Prunier et al., 1993), and in fractioned weaned sows (7 piglets removed at day 21, about 4 suckling piglets left) insulin levels increased to post-weaning levels after the removal of 7 piglets (Eriksson et al., 1987). Furthermore, a short-lasting increase of insulin was observed at each suckling (Eriksson et al., 1987). In our IS sows, insulin levels were not measured, but it seems plausible that insulin levels fluctuate as a result of alternate periods of suckling and non-suckling within an IS regimen. The role of insulin in early pregnant, lactating sows is unknown, but it can play a role in the secretion of P₄.

Insufficient proliferation and luteinisation of granulosa cells in IS sows has been discussed before (Chapter IV and V) as a possible cause of low P₄ levels. It was concluded, however, that this is unlikely as no differences were found in P₄ levels between sows either weaned at day 21 of lactation or Intermittently Suckled from day 21 onwards.

In conclusion, low post-ovulatory P₄ levels in IS sows as measured in our studies are most likely caused by the high metabolic clearance rate induced by lactation. Possibly also other lactational factors, either suckling (prolactin, oxytocin, endogenous opioids) or metabolically (IGF-1, insulin) related, can decrease P₄ secretion at the ovary.

Consequences for practical implication

The studies described in this thesis indicate that continuance of IS after ovulation negatively affects pregnancy rates, embryo development as well as placental and uterine development. Furthermore, an early start of IS (day 14 of lactation) negatively affects embryo survival. Based on these results, it is advised not to start with IS too early after farrowing and not continue with IS during pregnancy. The combination of an early start of IS and continuance after ovulation needs to be avoided. It is, however, necessary to study the effects of continuance of lactation after ovulation on the number and performance of offspring. It is also important to study the effects of a shorter period of continuation of IS after ovulation (for example, until 1 or 2 weeks after ovulation).

III. Management factors

For IS to be applied in practice, it is important that high percentages of sows respond with lactational ovulation and pregnancy. There are several factors which need to be considered in regard to implementation of IS in practice as they can affect the number of sows responding.

Breed

It is known that weaning-to-oestrus interval (Vesseur et al., 1994) and oestrous expression differs between breeds (Buiting and Brascamp, 1979). For IS to be applied in practice it is important to know if different breeds of sows respond differently to IS. In this thesis, Topigs40 sows were used in the first and third experiment and Topigs20 sows for the second experiment. When comparing the two different lines for response to IS with follicle growth as described in Table 5, the percentage of sows responding to IS with follicle growth reaching pre-ovulatory size does not differ between breeds (94% Topigs40 vs. 77% Topigs20). Within the category of sows reaching pre-ovulatory size, however, the percentage of sows with lactational ovulation within 8 days after start of IS, is significantly lower in the Topigs20 sow (53% Topigs20 vs. 84% Topigs40; P<0.05). The percentage of Topigs20 sows with ovulation is lower because, within a large proportion of sows, follicles regress without ovulating (35%). When the Topigs20 sows with regression of pre-ovulatory follicles or no pre-ovulatory follicles were treated with PG600 two weeks after the start of IS, 68% of these sows ovulated (Langendijk et al., 2007c), indicating that with exogenous hormones, the sows are capable of ovulating. As described earlier, the sows with regression of pre-ovulatory follicles did not show an increase in oestradiol levels, possibly related to the fact that the follicles of these sows were not capable of responding to LH pulsatility. In Topigs20 sows, IS was only initiated from day 14 of lactation onwards, which with regard to responsiveness of the follicles to LH, might possibly be too early after farrowing. It is not clear why this is the case in the Topigs20 sow and not in the Topigs40 sow, but might be related to the criteria on which the sows have been genetically selected. Topigs states that the Topigs40 sows is characterized by a short weaning-to-oestrus interval and good expression of oestrus (Topigs,

personal communication) and the Topigs20 (originally a Great York times Dutch Landrace or Finnish Landrace) sow is known for her large litter size and good mothering capabilities (Topigs, personal communication). Furthermore, Topigs40 sows are known for their good feed intake during lactation whilst the Topigs20 is known for having difficulties in taking up sufficient amount of feed during lactation. Indeed, in the present studies feed intake, as a percentage of the amount supplied, was lower in the Topigs20 (88%) than in the Topigs40 sows (95%; $P<0.05$). Although feed intake was lower, no differences were found in body weight loss during the first two weeks of lactation (on average 10 kg for both breeds, $P>0.10$; Table 6). Unfortunately no back-fat data were present for the Topigs20 sows, so no comparisons could be made in back-fat changes between the two breeds. The higher percentage of lactational ovulation in the Topigs40 sow could be related to their high feed intake during lactation, a faster recovery of the hypothalamus-pituitary axis and a qualitative good follicles within the follicle pool present during lactation. In summary, different lines of sows have been selected for different traits and respond different to IS. Possibly, the timing of start of IS (day 14 of lactation) was too early for the Topigs20 sow and when IS is initiated at a later stage during lactation (for example day 21) the sows may respond with higher percentages of sows with lactational ovulation. Thus when applying IS in practice, one should take breed into account in choosing the best strategy.

Parity

In the present IS studies, mostly multiparous sows were used. For IS to be applied in practice, however, it is also important to know how first parity sows would respond to IS. First parity sows have a longer weaning-to-oestrus interval (Walton, 1986; Vesseur et al., 1994) and later ovulation (Walton, 1986) than multiparous sows. Boar exposure during the third week of lactation reduced the weaning-to-oestrus interval in multiparous, but not in primiparous sows, indicating that an increased weaning-to-oestrus interval in primiparous sows can not be reduced by stimulation of a boar (Walton, 1986). An increased weaning-to-oestrus interval is caused by a lack of pulsatile LH secretion both during and after lactation (Zak et al., 1998). Furthermore low feed intake during lactation resulting in a negative energy balance and weight loss (Van den Brand, 2000), can further increase weaning-to-oestrus interval through metabolic effects on pulsatile LH secretion (Zak et al., 1998). It is questionable if primiparous sows that are highly catabolic during lactation will respond to IS. With regard to practical implications of IS, the response of primiparous sows to IS needs to be further investigated.

Table 5. Numbers of sows per category of follicle development response to IS and metabolic parameters per breed

	Topigs40	Topigs20
Number of sows		
Number of sows with oestrus/ large follicles before start of IS	11	0
Number of animals included in the IS treatments	80	22
Follicle development < 6 mm		
	5	5
Follicle development > 6 mm		
Ovulation within 8 days after start of treatment	63/75 ^a	9/17 ^b
○ oestrus	60	6
No ovulation		
- Regression of follicles	4	6
○ oestrus	3	0
- Cystic follicles	8	2
○ oestrus	7	1
Metabolic parameters		
	(n= 146) ¹	(n=24)
Body weight day 1 of lactation (kg)	270±2 ^a	285±5 ^b
Body weight day 13 of lactation (kg)	260±2 ^a	275±5 ^b
Body weight loss during the first two weeks of lactation (kg)	10±1	10±2
Feed intake during the first two weeks of lactation (%)	95±1 ^a	88±2 ^b

¹ The number of animals used for calculation of metabolic parameters for the Topigs40 sows also includes control sows as treatments had not started before day 14 of lactation and no differences were present at days 1 and 13 of lactation between treatments. ^{a,b} Different superscripts within one column indicate a difference of P<0.05; lsmeans ± se

Method of separation

In the experiments described in the present thesis the same method of separation was applied; moving the sows to another unit where contact with their piglets was avoided. When considering practical implications of IS, the currently used method is highly labour intensive. Another possible method of separation is the placement of a partition in the farrowing crate during the hours of separation (physical separation) used by Kuller et al. (2004) and Langendijk et al. (2007a). This method is not as labour intensive, but the sow can still receive auditory and olfactory stimuli from her piglets which could indirectly affect her reproductive performance. In the study of Langendijk et al. (2007a) both methods of separation were examined. Despite the small number of animals (n=6 for each method of separation), pulsatile

LH release in sows in response to IS was more sustained when sows were totally separated from their piglets (Langendijk et al., 2007a). Furthermore, follicle growth and ovulation occurred in 100% of the totally separated sows and in 50% of the physically separated sows. It thus seems that total separation results in a greater reproductive response than physical separation.

Not only the method of separation is important, but also the duration of separation. In older studies different durations of separation were used varying from 22 h to 3 h per day (Grinwich and McKay, 1985). The percentages of sows responding to separation varied greatly and were not very consistent with the duration of separation as for example 6 h of separation resulted in 100% response (Newton et al., 1987b) whilst 12 h of separation resulted in 0% response (Cole et al., 1972). In our first study (Chapter II and III), sows were separated at 12 h intervals (IS12) or 6 h intervals (IS6). The percentages of response did not differ between the groups (100% vs. 92%), but it seemed that when separation occurs at 6 h intervals, there is a higher risk for development of cystic follicles (IS12: 7%; IS6: 33%). Furthermore, in IS6 sows the onset of the LH surge was postponed (Chapter II), embryo development was impaired, body weight loss continued till slaughter (Chapter III) and cortisol levels were high during the first three days and day 7 of separation which was not observed in IS12 sows (Kluivers et al., unpublished). It seems that separation at 6 h intervals can negatively affect reproductive performance due to the higher incidence of cystic follicles, and impaired embryo development. Furthermore, with regard to practical implications, separation at 6 h intervals is highly labour intensive.

In conclusion, the method of separation and the duration of separation can influence the reproductive response of sows to IS. For practical implications it might be useful to study physical separation in more detail and find the optimal duration of separation as it is possible that the optimum can be different from 12 h.

Boar contact

In our studies oestrus detection was carried out by using a back-pressure-test (BPT) in absence of a boar. Studies show that the percentage of animals showing oestrus for a BPT only is lower than when a BPT is combined with boar contact (86% vs. 100%; Langendijk et al., 2000b). In our studies, oestrus detection rates in IS sows were high when even only applying a BPT, but it can be imagined that sows not showing oestrus may benefit from boar stimuli to express lactational oestrus. Furthermore, the duration of oestrus might be prolonged when a boar is present during oestrus detection. This could be beneficial when sows are subjected to IS from day 14 of lactation onwards as in this group 36% of the sows had a duration of oestrus shorter than 36 h compared to 21% and 24% when start of IS or weaning was from day 21 of lactation onwards. Indeed, boar contact increased the duration of oestrus in sows subjected to IS from day 14 of lactation onwards (Topigs20) from 30 h to 63 h (Langendijk et al., 2007c). The boar, however, did not influence pulsatile LH secretion or the number of sows that showed lactational ovulation (Langendijk et al., 2007c). In summary,

boar contact during IS can increase the expression of oestrus in sows and with regard to practical implications it is important that the animals are observed to be in oestrus. Therefore, it might be beneficial to introduce a boar during oestrus detection in IS sows, especially in sows not showing oestrous behaviour clearly.

IV Conclusion

With regard to the first aim of this thesis, '*Is it possible to induce lactational oestrus and ovulation by means of Intermittent Suckling?*', it can be concluded that it is possible to induce lactational oestrus and ovulation in sows by means of Intermittent Suckling. The rate of success is dependent on several factors such as breed of the sow and the timing of start of Intermittent Suckling relative to farrowing. Start of IS too early after farrowing results in a lower percentage of sows with ovulation and a variety of responses with regard to follicle growth: sows not developing pre-ovulatory follicles, sows with regression of pre-ovulatory follicles and sows developing cystic ovaries. It is not always clear why sows respond differently to IS with regard to follicle growth.

With regard to the second aim of this thesis '*What is the quality of lactational oestrus and subsequent pregnancy in sows subjected to Intermittent Suckling?*', it can be concluded that an early start of Intermittent Suckling (day 14 of lactation) negatively affects the quality of lactational oestrus (lower LH levels) and subsequent pregnancy (lower embryo survival). Start of Intermittent Suckling at day 21 of lactation, however, results in a good quality of lactational oestrus (follicle growth, ovulation, oestrus, endocrine status) and pregnancy (pregnancy rate, embryo survival and embryo development), that is comparable to sows weaned after 21 days of continuous lactation. It can also be concluded that a three week continuation of Intermittent Suckling after ovulation negatively affects the quality of pregnancy (lower progesterone levels and less developed embryos). Ending lactation at ovulation results in high post-ovulatory progesterone levels and embryo survival and embryo development up to day 30, comparable to sows weaned after a 21 day continuous lactation. In conclusion, the results of the experiments in the present thesis indicate that the quality of lactational oestrus and subsequent pregnancy is comparable to weaned sows when Intermittent Suckling does not start too early after farrowing and Intermittent Suckling is not continued during early pregnancy.

With regard to the practical aim '*Does Intermittent Suckling work in practice?*', it is difficult to draw conclusions, as more factors are important for application of Intermittent Suckling to in practice than have been examined in this thesis. This thesis, however, does give insight into the physiology of lactational oestrus and showed that lactational oestrus was induced in more than 80% of the Topigs40 sows within 1 week after start of IS and more than 70% of the Intermittently Suckled sows were pregnant at day 23 to day 30, but this may be dependent on

breed and other related management factors. With regard to lower percentages of ovulating sows it is advisable not to start with Intermittent Suckling too early after farrowing. The optimal timing, however, may depend on the breed of sow. With regard to progesterone levels during pregnancy and embryo development it is advisable not to continue Intermittent Suckling during early pregnancy, which means for most sows that the IS period would be limited to only one week. The optimal starting time of Intermittent Suckling and the optimal duration of Intermittent Suckling need further study and has to be combined with the optimal Intermittent Suckling strategy for piglets.

Concerning piglet performance within an IS regimen, studies have shown that Intermittent Suckling stimulated creep feed intake, but decreased body weight at weaning, because creep feed intake did not compensate for the reduced milk intake (Kuller et al., 2004; Kuller et al., 2007; Berkeveld et al., 2007). Nevertheless, the post-weaning growth check was reduced in Intermittently Suckled piglets which was one of the major aims for the use of Intermittent Suckling (Kuller et al., 2004; Kuller et al., 2007; Berkeveld et al., 2007). Piglet performance within an Intermittent Suckling regimen appears to be affected by timing of start of Intermittent Suckling and also the duration of Intermittent Suckling. One week of Intermittent Suckling already stimulated creep feed intake (Gerritsen et al., unpublished), but feed intake increased more when duration of IS was 10 days (Kuller et al., 2004). Thus, the question is if one week of Intermittent Suckling is long enough to significantly reduce the post-weaning growth check. If a longer period of Intermittent Suckling is needed for the piglets, this interferes with the advise of one week of Intermittent Suckling for optimal sow reproduction to avoid continuance of Intermittent Suckling during early pregnancy.

In conclusion, Intermittent Suckling may work in practice, but is currently not ready for practical use. It is necessary to examine sow performance of other breeds in more detail, find the optimal timing and duration of Intermittent Suckling for the sow and the piglet and combine these into one protocol. Furthermore, the method of separation needs investigation to reduce the amount of labour related to Intermittent Suckling.

V Recommendations for future research

This thesis has given insight into the endocrinology of lactational oestrus, but also gave into the possibilities of using induction of lactational oestrus by Intermittent Suckling in practice. Nevertheless, there are some remaining questions interesting to study in future research.

Firstly, with regard to follicle development results from this thesis show that sows respond differently to Intermittent Suckling, but it remains unclear what causes these differences in response. There are indications that limited levels of FSH or LH may be involved in the response and it is therefore interesting to study FSH in these different responses in follicle growth. Other possible factors such as IGF-1 should also be considered.

Secondly, the pre-ovulatory LH surge necessary for ovulation was found to be lower when induction of oestrus was early after farrowing. Despite this lower LH surge, ovulation

occurred in a high number of animals. A few animals partially ovulated without showing an increase of 2 ng/ml from basal levels. This raises the question how high should the increase in LH be for ovulation to occur? It would be interesting to study at what levels of LH ovulation is triggered and why some animals can partially ovulate without an apparent LH surge.

Thirdly, our studies have shown that it is possible to ovulate during lactation, but when lactation continues after ovulation P₄ levels are lower than in non-lactating animals. There are indications that the low P₄ levels are related to the metabolic state of the sow (insulin, IGF-1), suckling (prolactin, oxytocin, endogenous opioids) or lactation itself (liver blood flow). It is unknown what the levels of insulin, IGF-1, prolactin, oxytocin and endogenous opioids are in Intermittently Suckled sows during suckling and non-suckling periods and how these change during the course of lactation under an IS regimen. Furthermore, it is also unclear whether the low levels of P₄ measured in systemic blood during high lactational clearance rates reflect the P₄ levels at the ovary and uterus. If this there is a difference in systemic vs. ovary and uterus P₄ levels, feeding level for example on P₄ in systemic blood may have consequences at ovary level and subsequently uterine development and embryo survival and embryo development, whilst no differences were measured in systemic blood. Therefore, it would be interesting to study P₄ levels at ovary and uterine level.

Fourthly, lactation during early pregnancy was found to negatively affect embryo development. During pregnancy, imprinting of genes is necessary for the development of the embryo. Changes in the environment can affect the expression of genes and thus consequently affect placental development and embryo development. Normally in sows, pregnancy during lactation does not occur and this different environment could have effects on imprinting and subsequently litter size and performance of the subsequent litter. Thus, lactation during early pregnancy is an interesting model for studying imprinting and epigenetics, but it is of great importance to know if there are effects on litter performance when implementing IS with extended lactation in practice.

Fifth, when evaluating the implementation of Intermittent Suckling on practice, the conclusion was drawn that implementation of IS in practice is currently not possible. As described earlier in this chapter, factors such as breed, parity, method of separation and boar contact may affect the number of sows responding to Intermittent Suckling with follicle growth and ovulation. Therefore further studies taking these factors into account are necessary.

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Summary

Introduction

Weaning of piglets at the relatively young age of 3 to 4 weeks can compromise piglet health and welfare related with a reduced post-weaning feed intake, reduced post-weaning growth and the occurrence of diarrhoea. Extending lactation length and stimulation of creep feed intake during lactation will possibly improve piglet welfare around weaning. Extending lactation length, however, is economically undesirable as sows are normally anoestrus during lactation. Consequently, an extension of lactation length will reduce the number of litters per sow per year. Intermittent Suckling (IS), a management system in which sow and piglets are separated for a certain period of the day during lactation has been proposed as a method to extend lactation length, stimulate piglet creep feed intake and overcome the anoestrus block of the sow. This thesis focuses on the reproductive performance of sows in an IS system. For IS to be a successful management system from the sow reproductive point of view, several criteria must be met: sows need to respond to IS with follicle growth and ovulation with limited variation in timing, sows should show oestrous behaviour, and sows should become pregnant without compromising subsequent litter size. Consequently, the aims of this thesis were to: (1) to study if by application of IS lactational oestrus and ovulation can be induced in a high proportion of sows and (2) to examine the quality of this lactational oestrus by studying hormone levels and pregnancy parameters of the subsequent pregnancy.

Regimen of Intermittent Suckling

To study the response of sows to IS concerning follicle development, oestrus and ovulation (Chapter II), but also consequences for early pregnancy in sows with lactational oestrus (Chapter III) experiment 1 was carried out. Within this experiment two regimens of IS were compared with a control treatment in which multiparous sows (Topigs40) were weaned at day 21 of lactation (C; n=23). Both IS regimens were initiated at day 14 of lactation and separation occurred either for 12 h per day either continuously (IS12; n=14) or at 6 h intervals (IS6; n=13). Ovulation occurred within 7 days after start of IS/weaning in 100% of the C sows, in 93% of the IS12 sows and 83% of the IS6 sows. Analysis of hormone profiles indicated that peak oestradiol levels tended to be higher in IS sows, the pre-ovulatory LH surge tended to be lower in IS12 vs. C sows (5.1 ± 1.7 ng/ml vs. 8.4 ± 5.0 ng/ml) and post-ovulatory progesterone levels were significantly lower in IS sows.

All sows showing pre-ovulatory follicle growth were inseminated at each day of oestrus and slaughtered at day 23 after ovulation. Pregnancy rates did not significantly differ between treatments (75% IS12, 78% IS6 vs. 94% C). Also embryo survival rates were similar (57% IS12, 51% IS6 vs. 70% C). Embryo development, placental development and uterine development were negatively affected by the regimen of IS; several parameters related with development were less in IS6 sows compared to C sows, with IS12 sows being intermediate.

This study showed that by means of IS, lactational oestrus could be induced in more than 80% of the sows. The pre-ovulatory LH surge, progesterone levels, the number of

ovulating sows, and embryo development were negatively affected by IS or the regimen of IS. Possible causes for the negative effects of IS may be related with the early start of IS at day 14 of lactation, stress, factors related to lactation such as feeding level or hormones (endogenous opioids). The differences between IS regimens remain unclear, but may be related with stress and lactational factors such as a high milk production.

Feeding level and post-ovulatory progesterone levels

In several studies progesterone levels have been positively related to embryo survival during early pregnancy. Furthermore, low levels of progesterone have been related with high feeding levels. In IS sows of experiment 1 (Chapter II), fed at a high lactational feeding level, progesterone levels were low. Thus, the question was raised if the high feeding levels of the IS sows (6-7 kg/day compared to 2.5 kg/day in weaned control caused the low progesterone levels (Chapter IV). Topigs20 sows (n=21) were subjected to IS from day 14 of lactation onwards and separation occurred for 12 h per day until day 6 after ovulation. Ovulation occurred in a low number of animals (n=9) and the remaining sows were treated with PG600 at day 28 of lactation (n=12). At ovulation, sows were allocated to a High (on average 6.5 kg) or a Low (High minus 2.5 kg) feeding level until weaning at 6 days after ovulation. P₄ levels did not differ between sows fed a High or Low feeding level. Progesterone levels were comparable to the levels of IS sows in the first experiment. It is unclear why progesterone levels were low, but may be a result of a high metabolic clearance rate, or low progesterone production due to insufficient luteinisation or suppression of progesterone secretion through the metabolic state of the sow (insulin) or suckling related hormones.

Timing of start of IS and continuation of IS after ovulation

A third experiment was set up to examine factors related to the IS regimen that could have resulted in both the low LH and progesterone levels found in the first experiment, namely that fact that IS started on day 14 of lactation and that IS continued during early pregnancy. The first aim of this study was to examine the effects of timing of start IS on LH, progesterone levels and subsequent pregnancy (Chapter V). Sows (Topigs40; n=95) were either subjected to IS from day 14 (IS14) or day 21 (IS21) of lactation onwards and in control sows (C) weaning occurred at day 21 of lactation. Ovulation occurred in 85%, 87% and 89% of the IS14, IS21 and C sows respectively. An early start of IS (IS14) resulted in a numerically, but not significantly lower LH surge (4.3 ± 0.3 ng/ml IS14 vs. 5.0 ± 0.6 ng/ml IS21 vs. 5.2 ± 0.4 ng/ml C). Post-ovulatory progesterone levels at day 7 and embryo survival at day 30 tended to be lower in IS14 than in IS21 sows.

A second aim of this experiment was to study the effects of continuance of IS after ovulation on P₄ levels and subsequent pregnancy. At ovulation IS sows (IS14 and IS21) were allocated to either ending IS at ovulation (W_{ov}) or continuance of IS up to 20 days after ovulation (W_{ov+20}). Continuation of IS after ovulation resulted in lower progesterone levels at

day 7 and day 12 after ovulation (day 12 W_{ov+20} : 28 ± 2 ng/ml vs. W_{ov} : 46 ± 2 ng/ml), and tends to negatively affect embryo development.

In summary, an early start of IS tends to result in a lower embryo survival rate, possibly caused by a suboptimal uterine environment due to incomplete involution. Continuation of IS after ovulation negatively affects progesterone levels and tends to have negative effects on embryo development. It is unclear what causes the low progesterone levels in lactating sows, but factors related to the metabolic state of the sows (insulin) suppressing progesterone production or the high lactational metabolic clearance rate may play a role. The low progesterone levels can have a negative effect on uterine development resulting in a suboptimal environment for the embryos which will have consequences for their development.

Naturally developing cystic ovaries

The ovarian response of sows to IS as observed during the three experiments was diverse. The majority of the sows ovulated, but other responses included: no induction of pre-ovulatory follicle growth and pre-ovulatory follicle growth but regression of follicles. The physiological background of these responses are discussed in Chapter VII. Another observed response to IS was the development of cystic ovaries. As during the experiments follicle size was assessed daily and for many sows hormone levels (oestradiol, LH and progesterone) were assessed, a unique opportunity arose to increase our understanding of endocrinology and development of naturally occurring cysts. Data from sows that developed cysts were compared with their normally ovulating counterparts in order to examine when and how sows that develop cysts start to deviate from ovulating sows (Chapter VI). In total 12 sows developed cystic ovaries. Characteristic for sows that developed cystic ovaries was the absence of the LH surge, oestradiol levels that did not return to basal levels within the expected time period, a longer duration of oestrus and low progesterone levels. Differences in follicle growth in sows that developed cystic ovaries were observed at the time that normally ovulation occurs. Possibly a dysfunction in the feedback of oestradiol to the hypothalamus is related to the absence of the LH surge, which might be caused by stress or the metabolic state of the sow.

Sow reproductive performance in an Intermittent Suckling regimen

The results presented in this thesis show that by means of IS it is possible to induce lactational oestrus and ovulation. The rate of success, however, is dependent on several factors such as breed of the sows and timing of start of IS relative to farrowing. Furthermore, the quality of lactational oestrus and subsequent pregnancy can be comparable to that of weaned sows when IS does not start too early after farrowing and Intermittent Suckling is not continued during early pregnancy. The variable response to IS in follicle growth and the absence of ovulation in a number of sows could be related to the early start of IS relative to parturition, stress or metabolic factors (insulin, IGF-1). Consequences of these findings for the implementation of

IS in practice are discussed in detail in the General discussion (Chapter VII). In this chapter also recommendations for further research are described; for example to study the effects of IS on litter size as within the experiments described in this thesis, embryo survival was only examined during early pregnancy (day 23 and day 30) and it is currently unknown if IS affects survival during later stages of pregnancy.

Samenvatting

Inleiding

Het spenen van biggen op een relatief jonge leeftijd van 3 tot 4 weken, kan de gezondheid en het welzijn van de biggen negatief beïnvloeden door een verlaagde voeropname en groei na spenen en het voorkomen van diarree. Het verlengen van de lactatie en het stimuleren van de voeropname tijdens de lactatie, kan mogelijk het welzijn van biggen rondom spenen verhogen. Echter, het verlengen van de lactatie is vanuit economisch oogpunt onwenselijk aangezien zeugen tijdens de lactatie anoestrus zijn. Als gevolg hiervan zou een verlengde lactatie resulteren in een afname van het aantal worpen per zeug per jaar.

Intermittent Suckling (IS), oftewel tijdelijk spenen, is een management systeem waarin zeug en biggen dagelijks tijdens de lactatie voor een bepaalde periode van elkaar worden gescheiden. De verwachting is dat bij toepassing van dit systeem de biggen gestimuleerd worden om voer op te nemen tijdens de lactatie wat mogelijk het welzijn rondom spenen kan verhogen. Daarnaast wordt verwacht dat bij toepassing van IS bij een deel van de zeugen een bronst kan worden geïnduceerd tijdens de lactatie. Indien dit mogelijk is zou ook de lactatie kunnen worden verlengd.

In dit proefschrift wordt de nadruk gelegd op de reproductie van de zeug binnen een IS systeem. Er zijn verschillende criteria waaraan het IS systeem moet voldoen voordat een dergelijk systeem succesvol in de praktijk kan worden toegepast, namelijk: zeugen moeten op IS reageren met follikelontwikkeling en ovulatie binnen een gelimiteerde periode, zeugen moeten bronstgedrag laten zien, en zeugen moeten drachtig worden zonder negatieve effecten op de toomgrootte. Om dit te onderzoeken, worden in dit proefschrift de volgende vraagstellingen beantwoord: (1) Kan bij toepassing van IS een lacatiebronst en ovulatie opgewekt worden in een hoog percentage van de zeugen? (2) Wat is de kwaliteit van deze lacatiebronst met betrekking tot hormoonniveaus en embryonale overleving en ontwikkeling?

Het regime van tijdelijk spenen (IS)

Om de respons van zeugen op IS te bestuderen met betrekking tot follikelontwikkeling, bronst en ovulatie (Hoofdstuk II) maar ook om de consequenties van IS op de vroege dracht in zeugen met een lacatiebronst (Hoofdstuk III) te bestuderen werd een experiment uitgevoerd. Binnen dit experiment werden twee verschillende regimes van IS vergeleken met een controle groep waarin zeugen (Topigs40) werden gespeend op dag 21 van de lactatie (C; n=23). In beide IS regimes werd IS gestart op dag 14 van de lactatie en werden zeugen en biggen van elkaar gescheiden gedurende 12 uur per dag. De scheiding was of wel gedurende 12 uur aaneengesloten (IS12; n=14) of gedurende twee periodes van 6 uur (IS6; n=13). Binnen 7 dagen na start IS/spenen ovuleerde 100% van de C zeugen, 93% van de IS12 zeugen en 83% van de IS6 zeugen. Analyses van de hormoonprofielen gaven aan dat er een tendens was naar hogere piekniveaus van oestradiol in IS zeugen, een tendens in een lagere pre-ovulatoire LH piek in IS12 zeugen en significant lagere post-ovulatoire progesteron IS zeugen.

Gedurende elke dag van de bronst werden alle zeugen met pre-ovulatoire follikels geïnsemineerd en op dag 23 na ovulatie geslacht. Er werden geen significante verschillen gevonden tussen behandelingen in drachtheidpercentages (75% IS12, 78% IS6 and 94% C;). Het percentage embryonale overleving was eveneens niet verschillend tussen behandelingen (57% IS12, 51% IS6 vs. 70% C). Embryonale-, placenta- en baarmoederontwikkeling werden negatief beïnvloed door het IS regime, waarbij de ontwikkeling het sterkst negatief beïnvloed werd door het IS6 regime.

Dit experiment toonde aan dat bij toepassing van IS in meer dan 80% van de IS zeugen een lactatiebronst geïnduceerd werd. Desalniettemin werden de pre-ovulatoire LH piek, progesteron niveaus, het aantal zeugen met ovulatie en embryonale ontwikkeling negatief beïnvloed door IS of het regime van IS. Mogelijke oorzaken voor de negatieve effecten van IS zijn het de vroege start van IS op dag 14 van de lactatie, separatie stress, of factoren gerelateerd aan lactatie zoals voerniveau, hormonen (endogene opioïden). De oorzaak voor verschillen tussen de twee IS regimes is onduidelijk maar mogelijk kunnen stress of factoren gerelateerd aan lactatie zoals een hoge melkproductie hierin een rol spelen.

Voerniveau en post-ovulatoire progesteron niveaus

In verschillende studies is een positieve relatie aangetoond tussen progesteron tijdens de vroege dracht embryonale overleving. Lage progesteron niveaus, gemeten bij IS zeugen uit experiment 1 (Hoofdstuk II) kunnen mogelijk een negatief effect hebben op embryonale overleving. Een mogelijke oorzaak voor de lage gemeten progesteron niveaus is het hoge voerniveau waarop de IS zeugen werden gevoerd (6-7 kg/dag vs. 2.5 kg/dag in controle zeugen). Hoge voerniveaus resulteren namelijk in een versnelde omzetting van progesteron in de lever waardoor er dus lage progesteron niveaus gemeten worden. De vraag was of, in de IS zeugen, de hoge voerniveaus de lage progesteron niveaus veroorzaakten (Hoofdstuk IV). Zeugen (Topigs20; n=21) werden dagelijks onderworpen aan IS vanaf dag 14 van de lactatie gedurende een aaneengesloten periode van 12 uur per dag tot 6 dagen na ovulatie. Het aantal zeugen dat ovuleerde in response op IS was laag (n=9) en de overige zeugen werden met PG600 behandeld op dag 28 van de lactatie om ovulatie te induceren. Op het moment van ovulatie werden zeugen ingedeeld in twee groepen; een Hoog voerniveau (gemiddeld 6.5 kg/dag) of een Laag voerniveau (Hoog minus 2.5 kg/dag) voor 6 dagen. Er werd geen verschil gevonden in progesteron niveaus tussen zeugen op het Hoge of Lage voerniveau. Deze progesteron niveaus waren vergelijkbaar met de progesteron niveaus gemeten bij de IS zeugen uit experiment 1 (Hoofdstuk II). Het is onduidelijk waardoor de lage progesteron niveaus in IS zeugen veroorzaakt worden. Mogelijke oorzaken zijn een verhoogde lever activiteit als gevolg van lactatie resulterend in een verhoogde omzetting van progesteron of een verlaagde progesteron productie als gevolg van een onvoldoende luteinisatie, onderdrukking door de afgifte van zoog-gerelateerde hormonen of de conditie van de zeg.

Moment van start en voortzetting van tijdelijk spenen (IS) tijdens de vroege dracht

Een derde experiment werd uitgevoerd (Hoofdstuk V) om te bestuderen of de vroege start van IS (dag 14 van de lactatie) of het feit dat IS werd voortgezet tijdens de vroege dracht leidden tot de lage pre-ovulatoire LH piek en lage progesteron niveaus die werden gevonden in experiment 1. Zeugen (Topigs40; n=95) werden verdeeld over twee IS behandelingen met start IS op dag 14 (IS14) of dag 21 (IS21) van de lactatie en controle zeugen (C) werden gespeend op dag 21 van de lactatie. Binnen 8 dagen na start IS/spenen ovuleerde 85%, 87% en 89% van de respectievelijk IS14, IS21 en C zeugen. Een numeriek maar niet significant lagere LH piek werd gevonden bij een vroege start van IS (4.3 ± 0.3 ng/ml IS14 vs. 5.0 ± 0.6 ng/ml IS21 vs. 5.2 ± 0.4 ng/ml C). Er was een tendens tot lagere progesteron niveaus op dag 7 na ovulatie en tot een lagere embryonale overleving bij start IS op dag 14 vs. dag 21.

Het tweede doel was om de effecten van voortzetting van IS tijdens de vroege dracht op progesteron niveaus en embryonale overleving en ontwikkeling te bestuderen. Zeugen werden op ovulatie (IS14 en IS21) ingedeeld in twee groepen: onmiddellijk spenen (W_{ov}) of voortzetting van IS tot dag 20 na ovulatie (W_{ov+20}). De zeugen werden op dag 30 van de dracht geslacht. Voortzetting van IS tijdens de vroege dracht resulteerde in lage progesteron niveaus op dag 7 en dag 12 na ovulatie (dag 12: $W_{ov+20}: 28 \pm 2$ ng/ml vs. $W_{ov}: 46 \pm 2$ ng/ml), en een tendens tot een slechtere embryonale ontwikkeling.

Samenvattend kan gesteld worden dat een vroege start van IS leidt tot een tendens naar lagere embryonale overleving, wat mogelijk veroorzaakt wordt door een suboptimaal baarmoedermilieu doordat de baarmoeder nog niet volledig hersteld is van de vorige dracht. Voortzetting van IS tijdens de vroege dracht heeft een negatief effect op progesteron niveaus en een tendens tot een slechtere embryonale overleving. Het is onduidelijk wat de oorzaak is van de lagere progesteron niveaus in lacterende zeugen, maar mogelijke oorzaken zijn hormonen gerelateerd aan de metabole status van de zeug (insuline) die de productie van progesteron onderdrukken of de verhoogde omzetting van progesteron door de lever. De lagere progesteron niveaus kunnen een negatief effect hebben op de ontwikkeling van de baarmoeder waardoor embryo's zich in een suboptimale omgeving bevinden en als gevolg hiervan slechter ontwikkelen.

Spontaan ontwikkelende cysteuze ovaria

Toepassing van IS tijdens drie experimenten resulteerde in een gevarieerde respons ten aanzien van follikelgroei: (1) beperkte follikelgroei, (2) follikelgroei tot pre-ovulatoire grootte gevolgd door ovulatie, (3) follikelgroei tot pre-ovulatoire grootte maar vervolgens regressie van follikels en (4) follikelgroei tot pre-ovulatoire grootte, niet gevolgd door ovulatie maar door doorgroei van follikels leidend tot cysteuze ovaria. De fysiologische achtergrond van de eerste 3 responsen wordt bediscussieerd in Hoofdstuk VII. Het voorkomen van de vierde respons, cysteuze ovaria, gaf de unieke kans om inzicht te krijgen in de endocrinologie en ontwikkeling van spontaan ontwikkelde cysten, omdat tijdens de experimenten dagelijks

follikelontwikkeling werd gescoord en hormoonprofielen (oestradiol, LH, progesteron) werden bepaald. Data van zeugen die cysteuze ovaria ontwikkelden zijn vergeleken met geovuleerde zeugen om te bestuderen wanneer en waarom zeugen die cysteuze ovaria ontwikkelden afweken van geovuleerde zeugen (Hoofdstuk VI). In totaal ontwikkelden 12 zeugen cysteuze ovaria. Karakteristiek voor zeugen die cysteuze ovaria ontwikkelden was het uitblijven van de pre-ovulatoire LH piek, en vervolgens oestradiol niveaus die niet terugkeerden naar basaal niveau binnen de verwachte periode, een langere bronstduur, en lage progesteron niveaus. De follikelgrootte week af van de geovuleerde zeugen vanaf het moment dat ovulatie werd verwacht. Het uitblijven van de pre-ovulatoire LH piek kan mogelijk een gevolg zijn van een disfunctie in de terugkoppeling van oestradiol op de hypothalamus. Stress en de metabole status van de zeug spelen hierbij mogelijk een rol.

Reproductie van de zeug tijdens tijdelijk spenen (IS)

De resultaten van dit proefschrift geven aan dat het mogelijk is om lactatiebronsten te induceren door toepassing van tijdelijk spenen (IS). Het succespercentage is afhankelijk van verschillende factoren waaronder het ras van de zeug en het moment van start van IS ten opzichte van werpen. Kwalitatief is een lactatiebronst opgewekt tijdens IS en de aansluitende vroege dracht vergelijkbaar met gespeende zeugen zolang niet te vroeg na werpen wordt gestart met IS en niet wordt voortgezet tijdens de vroege dracht. De gevarieerde respons in follikelgroeい en het uitblijven van ovulatie bij een aantal zeugen zijn mogelijk gerelateerd aan de vroege start van IS t.o.v. werpen, stress of metabole factoren (insuline, IGF-1). Consequenties van deze bevindingen voor de toepassing van tijdelijk spenen in de praktijk worden bediscussieerd in Hoofdstuk VII. Ook worden in dit hoofdstuk vragen voor verder onderzoek beschreven zoals bijvoorbeeld de vraag of IS effect heeft op de uiteindelijke toomgrootte aangezien in de experimenten beschreven in dit proefschrift embryonale overleving alleen is bekeken tijdens de vroege dracht (dag 23 en dag 30 van de dracht) en het onbekend is wat de overleving is tijdens latere stadia van de dracht.

Curriculum vitae

Curriculum vitae

Rosemarijn Gerritsen werd op 15 juli 1980 geboren in Valkenswaard en groeide op in Huizen. In 1998 behaalde zij het International Baccalaureate-diploma (tweetalig VWO) aan International School Hilversum ‘Alberdingk Thijm’ te Hilversum. Datzelfde jaar begon zij met de opleiding Zoötechniek aan Wageningen Universiteit en Researchcentrum. In november 2003 werd de MSc Animal Science afgerond met afstudeervakken bij Gezondheidsleer & Reproductie, Fokkerij & Genetica, een stage in Canada bij Prof. dr. G.R. Foxcroft aan the University of Alberta, en een student-assistentschap bij Gezondheidsleer en Reproductie. Vanaf december 2003 was zij werkzaam als assistent in opleiding (AIO) bij de huidige leerstoelgroep Adaptatiefysiologie van Wageningen Universiteit en Researchcentrum (WUR) waarvan dit proefschrift het resultaat is.

Rosemarijn Gerritsen was born on July 15th 1980 in Valkenswaard and was raised in Huizen. In 1998, she graduated from high school International School Hilversum ‘Alberdingk Thijm’ in Hilversum with an International Baccalaureate certificate. During the same year she started with the BSc Animal Science at Wageningen University and Researchcentre. In November of 2003 she completed her MSc with theses on Health & Reproduction and Animal Breeding & Genetics, an internship in Canada at the research group of Prof. dr. G.R. Foxcroft at the University of Alberta, and a job as technical-assistant at the Health & Reproduction group. From December 2003 onwards she was employed at the Adaptation Physiology group of Wageningen University and Researchcentre as a PhD student of which this thesis is the result.

Publications

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Conference papers/abstracts

- Gerritsen R, Langendijk P, Soede NM, Hazeleger W, Dieleman SJ, Taverne MAM, Kemp B. Intermittent Suckling: a system that benefits piglets and sows? 7th International Conference on Pig Reproduction practitioners day, Kerkrade, The Netherlands , 16 June 2005- Porcine reproduction; from research to practice, 51-57.
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Training and Supervision Plan		Graduate School WIAS
Name PhD student	Rosemarijn Gerritsen	 The Graduate School WAGENINGEN INSTITUTE of ANIMAL SCIENCES
Group	Adaptation Physiology	
Daily supervisors	Dr. ir. N.M. Soede, Dr. ir. P. Langendijk	
Supervisor	Prof. dr. ir. B. Kemp	
The Basic Package (3 ECTS)		Year
WIAS Introduction Course		2005
Course on philosophy of science and/or ethics		2005
International conferences (4 ECTS)		
International conference on porcine reproduction and ICPR+, Kerkrade, The Netherlands		2005
European society for domestic animal reproduction (ESDAR), Murcia, Spain		2005
European society for domestic animal reproduction (ESDAR), Portoroz, Slovenia		2006
European society for domestic animal reproduction (ESDAR), Celle, Germany		2007
International Pig Conference: Paradigms in Pig Science, Loughborough, UK		2007
Seminars and workshops (2 ECTS)		
Themadag Platform Voortplanting: Male fertility, Wageningen, The Netherlands		2004
WIAS Science day, Wageningen, The Netherlands		2005-2007
Seminar Reproduction in Dairy Cattle, from research to practice, Wageningen, The Netherlands		2005
Symposium Early development, Utrecht, The Netherlands		2006
Satellite symposium: embryo-maternal communication, Celle, Germany		2007
Presentations (9 ECTS)		
Scientific Meeting, Utrecht, The Netherlands, oral presentation		2004
ICPR practitioners day, Kerkrade, The Netherlands, oral presentation		2005
ESDAR, Murcia, Spain, 2 poster presentations and 1 oral presentation		2005
ESDAR, Portoroz, Slovenia, poster presentation		2006
WIAS Science day, Wageningen, The Netherlands, oral presentation		2006
Groot Groep dagen (veterinair congres), oral presentation		2006
Paradigms in Pig Science, Loughborough, UK, poster presentation		2007
WIAS Science day, Wageningen, poster presentation		2007
In-Depth Studies (9 ECTS)		
PHLO- vruchtbaarheid varken		2005
Course Reproduction and Epigenetics, Uppsala, Sweden		2005
Science meets society: Robustness in the context of animal production		2007
Understanding genotype by environment interactions		2007
WIAS advanced statistics course: design of animal experiments		2007
Discussion group PhD students Wageningen and Utrecht		2004-2006
Statutory Courses (4 ECTS)		
Use of Laboratory Animals		2004
Professional Skills Support Courses (3 ECTS)		
Course Techniques for Scientific Writing		2004
Course Supervising MSc thesis work		2005
Intervisie en supervisie MSc en BSc studenten		2005
PhD Competence assessment		2006
NWO talentendag		2006
Didactic Skills Training: teaching and supervising (23 ECTS)		
Lecture course SIC Pig (YLS-30703)		2006-2007
Lecture course Health, Welfare and Management (ADP-30306)		2007
Practical Reproduction and Fertility practical (ADP-21803)		2004-2006
Practical Biology of Animal Reproduction practical (YLS-11306)		2004-2007
Practical Use of laboratory animals (YLS-32306)		2005-2007
10 MSc students (7 major, 3 minor) and 1 BSc student		2004-2007
Education and Training Total		57 ECTS

Colophon

The research described in this thesis was part of a project of the Adaptation and Resistance research programme entitled ‘Extending lactation length: improving piglet health and welfare without compromising sow reproductive performance’. This project was a cooperation between Wageningen University and Researchcentre, Animal Sciences Group Lelystad and the Veterinary Faculty of Utrecht University.

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