Reprod Dom Anim **43**, 59–65 (2008); doi: 10.1111/j.1439-0531.2007.00855.x
ISSN 0936-6768

Early Embryo Survival and Development in Sows with Lactational Ovulation

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Contents

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 hours 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 5 days after the start of treatment. Sows were inseminated each day of oestrus and slaughtered at D23 after ovulation. Intermittent suckling 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 IS, 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 (IS) regimens 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 with that of conventionally weaned sows (C) in the first week after weaning (oestrus: 96% IS vs 100% C; Gerritsen et al. in press). It is not clear if these sows ovulating during IS have a normal fertility. First, Gerritsen et al. in press found that the LH surge and post-ovulatory plasma progesterone (P_4) levels are significantly lower in IS sows. As high levels of P_4 have been found to positively affect embryo survival in pigs (Jindal et al. 1996) the low P_4 levels in IS sows might have consequences for uterine development and embryo development and survival. Secondly, 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 IS 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: (i) Control (C); continuous lactation with weaning at day 21 of lactation; (ii) Intermittent suckling12 (IS12); (iii) Intermittent suckling6 (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 to 20:00 hours and for the IS6 treatment from 8:00 to 14:00 hours and from 20:00 to 02:00 hours. 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. Intermittent suckling 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 hours) a commercial feed [12.8 MJ/kg metabolizable energy (ME), 145 g/kg crude protein (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 hours). Intermittent suckling 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 20:00 hours 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 (Gerritsen et al. in press). 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 by Gerritsen et al. in press.

Three batches of in total 50 multiparous TOPIGS 40 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 Gerritsen et al. in press). Detailed information on oestrus behaviour, hormone profiles, follicle development and ovulation has been published in a different paper (Gerritsen et al. in press).

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 $\geqslant$7 mm for 2 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 three 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, Münster, 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 Fig. 1. Variation in embryo development was measured by calculating the coefficient of variance for embryo weight and head size per litter.

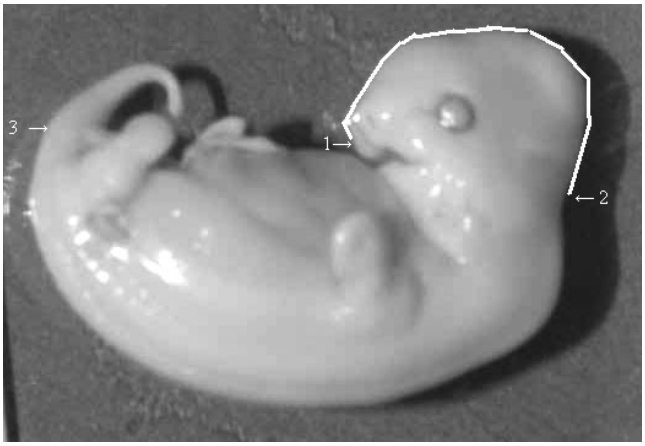

Fig. 1. Embryo measurement. Head size was measured as the circumference from mouth (1) to neck (2) and trunk size was measured as the circumference from neck (2) to tailbone (3)

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, Mannheim, 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 non-viable 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 in 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 analysed using SAS (SAS Institute, Cary, NC, USA). Differences between treatments in initial and day 23 pregnancy rates were analysed using Fischer's exact test. Embryo and placental parameters were averaged per sow and then analysed. All parameters were analysed 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

analysed 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 analysed in a covariate model with ovulation rate as covariate. The number of viable, non-viable embryos, the number of placentas, placental attachment sites and uterine parameters were analysed 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 analysed in a covariate model with weight or back-fat at farrowing as covariate. Sow protein and fat loss during lactation were analysed 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 IS 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 of 10 IS12 sows and 2 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 non-viable embryos at day 23, however, tended to be affected by the regime of IS as IS6 sows had 3.4 non-viable embryos on average and IS12 sows only had 1.4 non-viable 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 P_4 levels at 75 h after ovulation and embryo survival ($p > 0.10$).

Table 1. Pregnancy rates per treatment

	C	IS12	IS6
Initially pregnant sows[a]	16/17	11/13	9/9
Day 23 pregnant sows	16/17	9/12[b]	7/9

[a]Initially pregnant sows were defined as sows with placental attachment sites at D23 and for IS12 one sow culled before day 23, with embryos.
[b]One sow was culled at day 8 of pregnancy, therefore number of sows pregnant at day 23 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).

Early embryo development

At day 23 after ovulation, development of C embryos was enhanced when compared with that of IS6 sows

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

	C		IS12		IS6	
Parameter	Mean	SE	Mean	SE	Mean	SE
Slaughter day after ovulation (day)	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
Non-viable	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 $p < 0.10$.
CL, corpora lutea.

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

	C (n = 12)		IS12 (n = 9)		IS6 (n = 7)	
Parameter	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 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 $p = 0.10$.
CV, coefficient of variance.

(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 P_4 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 ($p > 0.10$).

Glucose levels in sow plasma and allantoic fluid

The regimens of IS 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 in 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 P_4 levels at 75 h after ovulation or embryo survival or embryo development at day 23 of gestation ($p > 0.10$).

Sow body weight and back-fat thickness

The change in sow body weight during lactation (21 days in C sows and approximately 41 days in IS sows) is illustrated in Fig. 2. Weight change was similar among the three treatments during the first 3 weeks of lactation, i.e. including the first week with IS ($p > 0.10$). 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 3 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

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

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

C: n = 16; IS12: n = 9; IS6: n = 7.
[a]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.
[b]The sow plasma glucose concentration/allantoic plasma concentration was higher for C (2.9) compared with IS sows (2.4); $p = 0.05$.

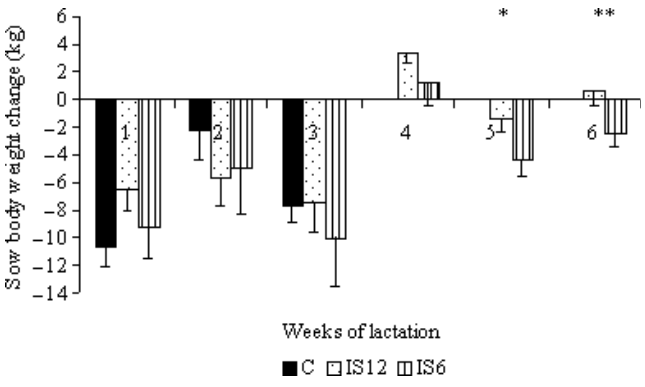

Fig. 2. Sow body weight change ± SE during weeks of lactation for C, IS12 and IS6 sows. The number of observations was 17 for C, 13 for IS12 and 9 for IS6. Week 1 is day 0 – day 6, week 2 is day 6 – day 13, week 3 is day 13 – day 20, week 4 is day 20 – day 27, week 5 is day 27 – day 34 and week 6 is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

($p > 0.10$). 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 3 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 ($p < 0.05$).

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 were occurred during lactation. Other studies in which IS 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 (Thompson et al. 1981; Britt and Levis 1982) 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 with our study.

Although pregnancy and embryo survival rates were not significantly lower in sows undergoing IS, the rates were substantially lower when compared with 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 postpartum (approximately 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 postpartum results in compromised farrowing rates (8–14 days 50%; 15–21 days 59.5%) when compared with sows served during lactation at 22–28 days postpartum (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 postpartum 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 postpartum and lactating sows served 8–14 days postpartum 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 (Gerritsen et al. in press). The amplitude of the pre-ovulatory LH surge has been found to affect the levels of P_4 after ovulation (Einarsson and Rojkittikhun 1993) and relations have been found between P_4 levels and embryo parameters (Jindal et al. 1996; Van den Brand et al. 2000). Therefore, it is possible that low LH surges found in early weaned and IS sows indirectly affected embryo survival. In the present study, P_4 levels in IS sows were lower than in conventionally weaned sows, but no relation was found between embryo survival and development and P_4 levels at 75 h after ovulation. Therefore it seems unlikely that low levels of P_4 caused the smaller embryos in the IS6 group directly. However, the low plasma P_4 levels may have an indirect negative effect on embryo survival. P_4 levels are thought to positively influence the number of LH/human chorionic gonadotropin (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 P_4 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 approximately 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 P_4 levels and could be a reflection of a less favourable environment for embryo development. Plasma P_4 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. in press). 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 repeated adrenocorticotropic hormone (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 IS, 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 P_4 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 postpartum and/or the fact that the sows are lactating during the follicular phase and early pregnancy.

Acknowledgements

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

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Submitted: 22.12.2006

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