

# Use of Natural Stimuli to Induce Ovulation and Maximise Fertility in Lactating Sows

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## Executive Summary

The overall aim of the experiments undertaken as part of this project was to optimize lactation oestrus expression and reproductive output of sows induced to ovulate using natural stimuli. These natural stimuli took the form of boars, manipulations of suckling load and loose, multisuckle pens. To achieve this, four experiments were conducted, and a brief summary of the aims, methods and key results from each experiment will be included in this executive summary.

The first experiment was conducted on a commercial facility (n = 603 sows), and was designed to determine the effects of sow parity, suckled litter size and split weaning on day 18 of lactation on the incidence of lactation oestrus and measures of subsequent reproductive performance. Overall, the data from this experiment demonstrated that while daily, full boar exposure in a DMA area is an effective stimulant of lactation oestrus, primiparous sows have a reduced capacity to ovulate during lactation in response to boar stimulation, compared to older sows. It is also evident from these data that reducing suckled litter sizes to 7 piglets in late lactation enhanced the lactation oestrus response by both multi- and primiparous sows. Overall, Mating sows in lactation was associated with a reduction in subsequent litter size by about 0.7 piglets, compared to mating post-weaning. Furthermore, farrowing rate, although acceptable, was generally lower for sows that were mated during lactation.

The second experiment compared the effect of three durations of sow and piglet separation (0, 7 or 16 hours; n = 16 sows/treatment) on days 17, 18 and 19 of lactation on piglet weight gain, the timing of oestrus and subsequent reproduction. All sows received 10 minutes of fenceline boar exposure whilst remaining in their farrowing crate. Respectively, 16, 7 and 0 hours of sows-piglet separation resulted in 81%, 50% and 19% of sows expressing oestrus during lactation. However, 16 hours of sow-piglet separation resulted in lower farrowing rates for those sows mated during lactation and reduced piglet growth compared to the 0 and 7 hours of separation. Based on the increased farrowing rate and lack of a negative effect on piglet growth, 7 hours of sow-piglet separation appears to be the most commercially attractive option for oestrus stimulation during lactation. Interestingly, when the number of piglets produced per 100 sows farrowing prior to the oestrus stimulation protocol was calculated, the 7 hour sow-piglet separation protocol increased the number of piglets produced by 185 and 548 compared to the conventional management protocol and 16 hour sow-piglet separation protocol, respectively.

The third experiment determined the impact of split-weaning (reducing suckled litter size to 7 piglets) on day 18 post-parturition or when lactation oestrus occurred, on oocyte developmental competence *in vitro* and embryo survival *in vivo*. The data from this study demonstrated that split weaning on day 18 (prior to lactation ovulation) improves embryo development and survival in sows mated during lactation. Interestingly, split weaning at first detection of oestrus was also associated with improved pregnancy rates and increased embryo weights.

In the fourth study, it was demonstrated that transferring 3 sows and their litters to a loose housing system for the last 10 days of a 28 day lactation did not markedly increase the incidence of lactation oestrus. However, housing a boar next to the group housed sows for 20 minutes, and allowing nose-to-nose contact through the fence resulted in 81% of group-housed sows ovulating during lactation. However, within the system used in this study, group housing sows and litters for the last 10 days of lactation did reduce piglet liveweight gain and tended to increase piglet mortality during this period, when compared to continual housing sows in the traditional farrowing crates.

When taken together, the current data clearly demonstrated that sow-piglet separation, full physical boar exposure in a detection mating area and fenceline boar exposure to group housed sows, resulted in a high proportion of sows expressing oestrus and ovulating during lactation and conceiving when mated during lactation. It is also clear that a permanent reduction in suckled litter size in late lactation increases the capacity of sows to ovulate during lactation, and positively effects both embryo survival and development. It is also evident that while three days of seven hours of sow-piglet separation resulted in only 50% of sows ovulating during lactation, it was shown to provide significant improvements to total reproductive output (litter size and piglets born/100 sows) when the performance of the sows mated post-weaning was also considered. Similarly, a permanent reduction in suckled litter size prior to complete weaning improved the reproductive output of sows mated post-weaning. Based on the current data it is suggested that strategies which reliably stimulate lactation oestrus may also improve the performance of those sows which do not ovulate until after weaning. It is likely, that oestrus stimulation strategies imposed during late lactation reflect positive effects on the metabolic status of the sow as well as an increase in positive inputs (boar pheromones) into the hypothalamic-pituitary axis, which improve ovarian follicle growth, and possibly oocyte quality, prior to weaning.

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# 1. Introduction

The ability to stimulate lactating sows to ovulate and conceive within 35 days after farrowing would enable piglet weaning age to be increased and still result in approximately 2.4 litters per sow per year. However, during lactation, sows are usually anoestrus, with the later stages of follicle growth and ovulation prevented by the suckling induced inhibition of Luteinising Hormone (LH) release. It is also common for sows to mobilise body reserves in order to fulfil their nutrient requirements for maintenance and milk yield, with the resultant catabolic state further reducing endocrine and metabolic support for follicle growth and development. It is, therefore, reasonable to suggest that increasing ovarian maturity, by improving gonadotrophin and metabolic support for growth of the follicle-oocyte complex, will enable ovulation to occur during lactation. In general terms, gonadotrophin and metabolic support for ovarian follicle growth can be improved by exposing the sow to factors which stimulate LH release (eg boar exposure), by reducing the metabolic demand on the sow (eg modification of suckled litter size) or by decreasing suckling frequency (eg enforcing or encouraging sow-piglet separation).

In a recently completed study investigating the effects of suckled litter size on oestrus expression in lactating sows (Terry et al., 2013), we clearly demonstrated that boar exposure was an effective stimulant of oestrus and ovulation in lactating sows. Specifically, regardless of litter size suckled, fenceline boar exposure commencing on day 18 of lactation resulted in an interval to oestrus of  $4.6 \pm 0.26$  days and 78% of sows expressing oestrus by day 25 of lactation (Terry et al., 2013). Although the proportion of sows expressing oestrus was lower when suckled litter size was maintained at 10 compared to 7, 5 or 3 piglets (0.56 versus 0.90), when sows suckling 10 or 11 piglets received full, physical boar exposure from day 14 of lactation onwards 100% of sows ovulated within 6 days. Together, our data indicate that boar exposure can stimulate an ovulatory response similar in timing to that which occurs following complete weaning, but that full physical boar exposure is required to optimise the response, especially in the absence of any alteration in suckling load.

We have also recently demonstrated that a suckled litter size of 10 piglets as opposed to 7 piglets prior to and after mating reduced subsequent litter size in sows mated on day 23 of lactation and weaned on day 30 (Terry et al., 2013). However, it remains to be established whether this reflects a reduction in ovulation rate, oocyte quality or embryo survival. The PhD studies (unpublished) of Shona Grant indicate that reducing suckled litter size to 5 piglets for the 7 days prior to weaning significantly improves ovarian follicle growth. Studies in gilts and weaned sows demonstrate that reducing nutrient intake not only decreases the number of follicles selected for ovulation, but also impairs the capacity of the shed oocytes to develop into viable embryos and survive to parturition (Zak et al., 1997; van Wettere et al., 2011). Further, post-mating nutritional intake has been shown to alter embryo development and survival, as well as pregnancy maintenance (reviewed by van Wettere and Hughes 2007). Alterations in maternal metabolic status impact follicle function either directly, via the effect of metabolic hormones and metabolites on somatic cell and oocyte function, or indirectly, via altered secretion of gonadotrophins. Based on this, it is logical to assume that the endocrine and metabolic environment to which ovarian follicles are exposed during lactation will be far from optimal, resulting in reduced or impaired development of the somatic cell-oocyte unit. Interestingly, continuation of lactation, in conjunction with 12 hourly periods of enforced sow-piglet separation (intermittent suckling), has been associated with reduced progesterone production and embryo weight compared to sows induced to ovulate during lactation but weaned immediately post-mating (Gerritsen et al., 2009). In contrast, we have recently demonstrated good litter sizes (total born of 13.5) in sows mated before day 23 of lactation and continuing to suckle 7 piglets until day 30 post-parturition. Our data suggest,

therefore, that maintenance of lactation during the first 7 to 10 days of pregnancy exerts little effect on pregnancy maintenance and litter size when suckled litter size is relatively low.

A reduction in suckling frequency can also induce an ovulatory response. This can be achieved through enforced separation of piglets and sows for a period of time each day (6 - 12 hours), often referred to as intermittent suckling (Langendijk et al., 2007). However, the percentage of sows ovulating in response to intermittent suckling varies enormously between genotypes, leading Gerritsen et al. (2008) to conclude that intermittent suckling is not a practical option. Equally, the additional labour component associated with physically separating sows and piglets makes the commercial viability of intermittent suckling questionable. An alternative option is to modify lactation housing conditions to enable, and indeed encourage, the sow to regulate suckling frequency and duration herself. In standard farrowing crates and pens, frequency of suckling bouts peak around day 9-13 after birth, and gradually decreases thereafter (Puppe and Tuchscherer, 2000; Valros et al., 2002). However, regardless of stage of lactation, the time spent suckling is reduced in farrowing pens compared to farrowing crates (Stolba et al., 1990). Interestingly, time spent suckling was even further decreased in complex group housing situations and was associated with lactation ovulation in 100% of sows (Stolba et al., 1990). Moving sows and litters from a crate to a group housing situation on day 20 of lactation resulted in a significant decrease in the frequency of nursing events, as well as lactation ovulation in 66% of sows (Bryant and Rowlinson, 1984). The data of Rowlinson and Bryant (1981) provides further support for a stimulatory effect of group housing on ovarian activity, with sows displaying a mean interval to oestrus of 11, 9.2, 12.2 and 7.4 days when grouping occurred on days 10, 15, 20 and 25 of lactation, respectively. In a subsequent study, Rowlinson and Bryant (1982) demonstrated an additive effect of boar exposure on oestrus expression in group housed, lactating sows. Interestingly, the occurrence of lactation ovulation in group housed sows appears to be associated with less time spent suckling (Hulten et al., 2006). It is, therefore, proposed that group housing of sows, probably due to reduced suckling intensity and frequency, in combination with daily boar contact will be an effective method of stimulating oestrus in lactating sows. Conversely, given the incidence of spontaneous, asynchronous oestrus expression in group housed sows receiving no additional stimulation (Hulten et al. 2006), ease of reproductive management will be greatly increased if the onset of lactation oestrus can be controlled and synchronised.

Accepting that group housing in conjunction with boar exposure appears likely to stimulate oestrus expression in a high proportion of lactating sows, it is vital that the design of group housing systems meets the requirements of the sow and litter as well as those of commercial production systems. In particular, group housing during lactation must meet the welfare and behavioural requirements of the sow and litter, maximise piglet performance and survival pre- and post- weaning, and optimise sow reproduction and longevity. Two systems currently exist which appear to fulfil these requirements. One is the use of individual farrowing crates or pens for the first seven to fourteen days post-parturition followed by movement to simple group lactation pens ('follow-on system').

In conclusion, it is evident that boar exposure represents a reliable and powerful stimulus of oestrus expression in lactating sows. Importantly, it may not be necessary to alter suckling intensity in order to obtain a rapid and synchronous response. Although suckling load does appear to affect subsequent litter size, it is unclear whether this is due to a negative effect on the quality of the oocytes shed (i.e. impaired endocrine and nutritional support pre-mating) or luteal function and uterine environment (i.e. impaired endocrine and nutritional support post-mating). Further, group housing of sows also appears to promote lactation ovulation, most likely due to a reduction in suckling intensity and a

consequential improvement in sow metabolic status. However, the effects of group housing during lactation on incidences of lactation ovulation have received little attention in the past 30 years, and the optimal system (or systems) to promote lactation ovulation whilst maximising sow and litter performance and welfare has yet to be established.

## **2. Methodology**

A total of four experiments were conducted as part of this project. The methods for each project are presented separately, along with the specific aims and hypotheses to be tested. All studies were conducted in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (NHMRC, 2004). All experimental procedures were conducted with approval from the Animal Ethics Committee of The University of Adelaide.

### ***Experiment One: Lactation oestrus induction in multi- and primi- parous sows in an Australian commercial pork production system***

#### ***Background and Aims to this study***

The current study tested two hypotheses; first, that the use of full daily boar exposure coupled with split weaning, in a commercial setting, will increase the incidence of lactation oestrus; second, that the incidence of lactation oestrus will be lower for first parity sows compared to multiparous sows.

#### ***Materials and Methods***

This study was conducted in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (NHMRC, 2004). All experimental procedures were conducted at a large commercial breeding unit in South Australia, with approval from the Animal Ethics Committee of The University of Adelaide, South Australia.

#### ***Animals, housing and diet***

The experiment consisted of 13 replicates conducted between June 2012 and October 2012. A total of 603 Large White x Duroc x Landrace primiparous sows ( $n = 303$ ) and multiparous sows ( $n = 300$ ; parity 2 to 3;  $2.5 \pm 0.03$ ) were individually housed in conventional farrowing crates from 1 week before expected farrowing until weaning. Piglets were weaned on day  $30.7 \pm 0.05$  post-parturition (day 0 = first 24 hours post-parturition). On the day of farrowing (day 0) sows were fed 1 kg of a lactation diet (14.0 MJ/kg DE, 17.1% protein, and 0.872% available lysine). On day 1 post-partum this was increased to 2.5 kg of the same lactation diet; by day 5 of lactation sows were receiving 4 kg, and thereafter sows were fed ad libitum to a maximum of 9 kg through to weaning. On day 1 of lactation, litters were standardised to 11 piglets per sow.

#### ***Treatments***

Before shed entry multiparous (MP;  $n = 300$ ) and primiparous (PP;  $n = 303$ ) sows were allocated randomly based on parity to either the control (a conventional lactation with no boar exposure; No BE,  $n = 149$ ) or to a boar exposure (BE,  $n = 454$ ) in lactation treatment.

### ***Conventional lactation treatment (No BE).***

Sows not receiving BE (No BE; n = 149) were housed separately from sows receiving boar exposure during lactation to ensure they were not exposed to any boar pheromones that may be present on sows after full physical boar exposure. Sow live weight and P2 backfat were measured at the start of lactation ( $1.8 \pm 0.1$  days post-farrowing; range: 1 - 3) and again on the day of weaning ( $29.9 \pm 0.9$  days post-farrowing; range: 28 - 32). A pre-prandial blood sample was taken by jugular venipuncture into a 9 ml Heparin-Lithium coated collection tube (Vacurette, Greiner Labortechnik, Austria) two days post-weaning between 0700 and 0800 h to measure progesterone concentration to be able to determine if ovulation had occurred during lactation. Blood samples were maintained on ice and were centrifuged at  $1500 \times g$  for 15 minutes and plasma was then stored at  $-20^{\circ}\text{C}$ , until assayed for progesterone.

Blood samples from the No BE sows were analysed for progesterone (P4) concentration in 50  $\mu\text{l}$  of plasma, in duplicate using a coated tube radio immunoassay, according to the manufacturer's instructions (M118; Beckman Coulter, Brea, CA, USA). The lowest detectable concentration was 1 ng per ml. The intra assay coefficient of variation was less than 10%. The inter assay coefficient of variation was less than 15%. Sows with a P4 concentration  $\geq 4$  ng/ml on day 2 post-weaning were defined as expressing a spontaneous lactation oestrus.

### ***Boar exposure treatments***

Sow live weight and P2 backfat were measured at the start of lactation ( $2.0 \pm 0.04$  days post-farrowing; range 1 - 4), on day 17 of lactation and again on the day of weaning ( $30.8 \pm 0.05$  days post-farrowing; range 29 - 34). On day 17 of lactation, sows were stratified within parity group, according to their average live weight (LW) loss ( $5.7 \pm 0.58$  kg) between days 1 and 17 of lactation and suckled litter size and allocated to one of the two BE treatments: BE with an unchanged litter size (BE, n = 302) or BE and the litter permanently reduced (split weaned) to 7 piglets (BESPW7, n = 152). Increased numbers of sows were allocated to the BE treatments to ensure that there were adequate numbers of sows with a litter size of 9 or more piglets at weaning, in each parity group, to allow comparison to the split weaned group (BESPW7). All sows started full physical boar exposure on day 18 of lactation and were artificially inseminated at the first detection of a standing oestrus.

BE (unchanged litter size). Sows not receiving a change in litter size at day 18 (BE; n = 302; MP n = 151; PP n = 151) had their litter weighed on day 17 of lactation and no piglets were removed from the litter (average number of piglets suckling,  $9.5 \pm 0.01$ ).

BESPW7 (litter size split weaned to 7 piglets). Only sows with 9 or more piglets suckling (average  $10.2 \pm 0.06$ ; range 9-11) on day 17 were eligible for allocation to the BESPW7 (n = 152; n MP = 75; n PP = 77) treatment. On day 17 of lactation litters were weighed and the heaviest piglets were identified for split weaning and were then removed from the lactation crate between 0700 and 0800 h on day 18 of lactation.

### ***Boar exposure and oestrus detection***

Beginning on day 18 of lactation sows were taken daily from the farrowing crate to a detection mating area and, in groups of 2 - 3 sows, given full physical boar exposure with a mature boar for 15 minutes. The detection mating area was separate from the lactation shed and involved a maximum of a 5 minute walk for the sows. Within the first three minutes of entering the pen, sows were checked for oestrus using the back pressure test, with the display of a standing reflex defined as the first sign of oestrus. Sows were

artificially inseminated (AI) at the first detection of oestrus and again every 24 hours until the end of behavioural oestrus or when the sow had received a total of three inseminations, whichever came first. Cervical inseminations were performed using a sponge-tipped disposable catheter with each insemination containing an 80 ml dose of fresh, extended semen ( $3 \times 10^9$  spermatozoa per inseminate; < 4 days old). Semen was purchased from a commercial artificial insemination collection centre. The interval from the start of treatment (day 18) to the first expression of a standing oestrus during lactation was recorded. The duration of oestrus expression (days) was also recorded.

### ***Post-weaning housing of all sows, and BE and artificial insemination of sows not mated during lactation***

At weaning, sows mated in lactation were removed from their lactation crates and placed into group housing pens for the remainder of gestation. Multiparous sows were housed in separate group pens to primiparous sows.

At weaning, sows which did not exhibit a lactation oestrus, including sows from the No BE treatment, were removed from their lactation crates and placed into group pens, with primiparous and multiparous sows separated. From weaning they received daily nose to nose BE with a mature boar in a detection mating area. When sows first exhibited an oestrus post-weaning they were artificially inseminated in an “AI station” by first detecting oestrus by fence line contact with a mature boar. Sows were inseminated at the first detection of oestrus and again every 24 hours until the end of behavioural oestrus or when the sow had received a total of two inseminations, whichever came first. Cervical inseminations were performed using a sponge-tipped disposable catheter with each insemination containing an 80 ml dose of fresh, extended semen ( $3 \times 10^9$  spermatozoa per inseminate; < 4 days old). Semen was purchased from a commercial artificial insemination collection centre. Sows were mixed back into group housing accommodation two hours after their final insemination. Within the group pens, sows were given 2 m<sup>2</sup> of floor space at all times.

### ***Pregnancy status and farrowing rates***

Pregnancy status was determined in all sows by transabdominal ultrasound at approximately  $28 \pm 4$  days post AI. The number of piglets born alive, stillborns, and mummies were recorded from the subsequent litter of the sows that were inseminated during lactation.

### ***Statistical Analysis***

Data are expressed as mean  $\pm$  standard error of the mean (SEM). In all analyses the sow was the experimental unit. A general analysis of variance (ANOVA) with experimental replicate built-in with sow parity and liveweight on day 1 of lactation as a co-variate was used to determine the treatment effects on sow body condition. Data for effects of parity on sow body condition were analysed using an ANOVA with experimental replicate built-in and with liveweight on day 1 of lactation as a co-variate. The cumulative proportion of sows expressing oestrus and farrowing rate were analysed as chi-squared. The effects of treatment and parity on average piglet weight, average piglet weight gain and average daily piglet growth rate were analysed by averaging the litter weight data with the sow as the experimental unit, using an unbalanced ANOVA and with experimental replicate built-in to the model and with piglet weight on day 4, sow liveweight loss from day 1-weaning and lactation length used as co-variates. Subsequent reproduction including total born, born alive, stillborns and mummies were analysed using an ANOVA with experimental

replicate built in and sow parity and sow live weight loss between first day of lactation and weaning used as a covariate.

All analyses, except for the Chi-squared analyses, were performed using Genstat, 10th Edition (Rothamsted Experimental Station, Harpenden). Probability values stated as being  $P < 0.05$  were described as significant.

### ***Experiment Two: Effect of interrupted suckling on sow reproductive performance and piglet growth***

#### ***Background and Aims to this study***

This experiment was designed to determine the incidence of lactation oestrus in response to commercially practical interrupted suckling and boar exposure regimes. The effect of these regimes on piglet growth was also determined, as was the impact on sow and piglet circulating cortisol levels.

The aims of this experiment were to: to determine the effect on sow lactation oestrus expression and reproductive performance as well as piglet performance of three days of interrupted suckling for 0, 7 or 16 hours in conjunction with sow's receiving fenceline, physical contact with a boar whilst remaining in their farrowing crate. The durations of the interrupted suckling regimes were designed to coincide with the start and finish of a standard working day.

#### ***Material and methods***

Experimental work was conducted during winter / spring on a 7,500 sow commercial facility situated approximately 70 km north of Adelaide, South Australia.

#### ***Experimental Design***

A total of 48, Large White cross Landrace multiparous sows were used in two experimental blocks. Following parturition, sows were allocated to experience either zero (0IS), seven (7IS) or sixteen (16IS) hours of piglet separation (or interrupted suckling; IS) from day 17.0  $\pm$  0.18 days post-partum for three days. During separation, piglets remained in the farrowing crate of their dam, with separation achieved through the use of a board placed between the sow and creep area. Separation commenced at 8 am and finished at 3 pm in the 7IS treatment group, and commenced at 4 pm and ended at 8 am in the 16IS treatment group. Sows in all three treatment groups received ten minutes of physical nose-to-nose contact with a mature boar through the open door of their farrowing crate from start of IS until weaning on day 27.0  $\pm$  0.18 post-partum. During the night, a boar was housed in a farrowing crate in the farrowing shed. Following weaning, sows were moved to group accommodation, with sows which failed to exhibit oestrus during lactation receiving full, physical contact with a mature boar in a detection mating area until expression of oestrus.

Sows were checked for oestrus using the back pressure test, with the display of a standing reflex defined as the first sign of oestrus. Sows were artificially inseminated at the first detection of oestrus and again every 24 h until the end of behavioral oestrus or when the sow had received a total of 3 inseminations, whichever came first. Cervical inseminations were performed using a sponge-tipped disposable catheter with each insemination containing an 80-mL dose of fresh, extended semen ( $3 \times 10^9$  spermatozoa per inseminate; <4 d old). Semen was purchased from a commercial artificial insemination collection

center. The interval from the start of treatment (Day 17) to the first expression of a standing oestrus during lactation (lactation oestrus) or post-weaning (post-weaning oestrus) was recorded. The duration of oestrus expression (days) was also recorded.

### ***Experimental Measures***

Two days after the start of treatment, blood samples were collected from sows one hour after the start and one hour after the cessation of IS, or the equivalent times for OIS animals. A blood sample was collected from two piglets per litter two days prior to the start of treatment and on day three of treatment. Samples were collected from OIS piglets at 8 am and 5 pm, equivalent to one hour into the IS treatment for the 7 IS and 16IS treatment groups. Samples were collected from 7IS piglets at 8 am and from 16IS piglets at 5 pm. Samples were maintained on ice and centrifuged at 1,500 for 15 minutes, plasma was then stored at -20°C until assayed for cortisol. Piglets were weighed individually the day prior to the start of treatment and at weaning.

The timing of oestrus, number of artificial inseminations, pregnancy rates, farrowing rates and subsequent litter size (total born, born alive, still born and mummies) were recorded for all sows and treatments.

### ***Cortisol Analysis***

Plasma (50 µl) was assayed for cortisol by coated tube radioimmunoassay (IM1841; Immunotech, Prague, Czech Republic) according to the manufacturer's instructions. The intra assay CV was 7.1%. The inter assay CV were 8.7% at 34.1 nM and 8.5% at 143.8 nM. The minimum detectable level (lowest standard) was 20 nM. Values lower than the detection limit (10.5% of the samples) were assigned a value of 20 nM for statistical purposes. Cross reactions with other steroids according to the kit manufacturer were, 11-Desoxycortisol, 18%; Corticosterone, 8.4%; 21-desoxycortisol, 7.5%; Desoxycorticosterone, 7.3%; progesterone, 1.8%.

### ***Statistical Analysis***

Data are expressed as mean ± SEM. In all analyses the sow or litter were the experimental unit. A general ANOVA was used to determine treatment effects on all variables. Litter size suckled and litter weight at start of treatment were included in the model as co-variates when determining treatment effects on litter performance. The cumulative proportion of sows expressing oestrus and farrowing rate were analysed by chi-squared. All analyses, except for the chi-squared analyses, were performed using Genstat (10th Edition, Rothamsted Experimental Station, Harpenden, UK). Probability values stated as being  $P < 0.05$  were described as significant,  $P < 0.1$  were accepted as a tendency.

### ***Experiment Three: Effect of split-weaning prior to lactation oestrus or post lactation oestrus on oocyte quality and embryo survival***

All animal procedures were conducted at the University of Adelaide's piggery at Roseworthy, South Australia with approval from the Animal Ethics Committee of The University of Adelaide.

### ***Experimental animals, housing and management***

This experiment was conducted over 5 replicates from April to December 2012. Ninety-eight Large White x Landrace multiparous sows ranging in parity from 2 to 6 were selected and allocated to treatment based on parity (average parity  $3.3 \pm 0.2$ ; Mean  $\pm$  SEM) to one of five treatment groups;

- 1) litter size maintained at 11 piglets for the duration of lactation and sows slaughtered 21 days post parturition (control),  $n = 20$ ;
- 2) litter size maintained at 11 piglets for the duration of lactation and sows slaughtered 30 days post lactation oestrus and insemination (control mated),  $n = 22$ ;
- 3) litter size maintained at 11 piglets until day 18 at which point the litter was reduced to 7 piglets, and sows were slaughtered 21 days post parturition (split-wean 7 (SW 7)),  $n = 19$ ;
- 4) litter size maintained at 11 piglets until day 18 then reduced to 7 piglets, and sows were slaughtered 30 days post lactation oestrus and insemination (split-wean 7 mated (SW 7 mated)),  $n = 21$ ;
- 5) litter size maintained at 11 until first expression of oestrus at which point litter size was reduced to 7 piglets, and sows were then slaughtered 30 days post lactation oestrus and insemination (oestrus split-wean 7 mated (OES SW 7 mated)),  $n = 16$ .

Within 24 hrs of farrowing, litter sizes were standardised to 11 piglets per sow. Sows were housed in conventional farrowing crates for the duration of lactation and moved to individual sow stalls once weaned. During lactation, sows were fed a commercial lactation diet supplying 14.6 MJ DE/kg, 18.7% CP, 1.0% total lysine. The diet was fed at increasing amounts from 1 kg on the day of farrowing to a maximum of 8 kg/day over three meals by day 7 of lactation. At weaning sows were fed 2.5 kg/day of a commercial dry sow diet supplying 13.0 MJ DE/kg, 13.8% CP, 0.7% total lysine.

Control and SW 7 sows were weaned from their litters on day 21 post-parturition which coincided with slaughter. All sows in the mating groups were weaned from their litter at a minimum of 30 days post parturition and at least 10 days post lactation oestrus and mating (average weaning age  $32.7 \pm 0.2$  days; Range 30 - 37 days). Sows were allowed 35 days to express a lactational oestrus upon which they were weaned from their litter.

### ***Sow measures***

Sows were weighed and P2 backfat measured on days 1 and 18 post parturition as well as at oestrus, weaning and slaughter. P2 backfat was measured over the last rib, 65 mm from the vertebrae, using a 5 MHz linear probe (Aquila Vet, Pie Medical Equipment).

### ***Oestrus stimulation and oestrus detection***

From day 18 post-parturition, all sows received 15 min of daily direct physical contact with a mature boar in a detection mating area (DMA). Boar contact commenced at 08:00 hrs, with oestrus defined as the exhibition of a standing reflex in response to the manual application of pressure to the sow's back (the "back pressure" test). Daily boar contact continued until oestrus was no longer detected and the duration of oestrus was then recorded. Sows were artificially inseminated at first detection of oestrus and repeated daily until oestrus was no longer detected, or until the sow had received four inseminations, whichever occurred first. Terminal mix semen was obtained from a local supplier (SABOR Pty. Ltd; Clare, SA, Australia).

### ***Split-weaning and piglet measures***

All piglets were weighed as a whole litter on days 3 and 7 post parturition. On day 18, piglets from both SW 7 treatments were weighed individually and the heaviest 4 piglets were weaned leaving a litter size of 7 while the piglets from both control treatments and OES SW mated were weighed as a whole litter on day 18. At first detection of a lactation oestrus, piglets from OES SW 7 mated sows were weighed individually and the heaviest 4 piglets were weaned. Finally, whole litter weights were recorded at weaning for all treatments.

### ***Transrectal ultrasound***

Transrectal ultrasound with a 7.5 MHz linear-array transducer (Esaote, Genova, Italy) was performed to assess ovarian follicular growth on days 14 and 18 post parturition and every second day from day 18 until first expression of oestrus. Once oestrus was detected, transrectal ultrasound was performed at 08:00 and 18:00 hrs for the duration of oestrus to determine timing of ovulation. Both the left and the right ovary were scanned and recorded images were later viewed using the scanner program MyLabOne version 5.10 (MyLabOne, Esaote, Genova, Italy). The diameters of all visible antral follicles were measured. Ultrasound was performed in the farrowing crate and no restraint of the sow was required.

### ***Plasma collection and analysis***

A single blood sample was collected on days 3 and 18 post parturition as well as at first detection of oestrus, then 3, 10 and 15 days post oestrus. For sows that did not express a lactational oestrus, a blood sample was taken at weaning to determine if a silent ovulation had occurred. A single blood sample was also taken at slaughter. Sows were restrained using a snare and a pre-prandial blood sample was taken by jugular venipuncture into a 9 mL Heparin-Lithium coated collection tube (Vacurette®, Griener Labortechnik, Austria). Blood samples were maintained on ice and processed within 1 hr of collection. Blood samples were centrifuged for 15 min at 3000 rpm and plasma was stored at -20°C.

All hormone analyses were performed by the Adelaide Research Assay Facility, University of Adelaide. Plasma samples were analysed for progesterone (P4), luteinising hormone (LH) and insulin depending on the time of sample. Table 1 shows which samples were analysed for which hormone.

**Table 1 Analysis of hormones in sow plasma collected at different time points.**

<b>Time of blood sample</b>	<b>Hormones analysed</b>
Day 3 post partum	Insulin
Day 18 post partum	Insulin, P4
Lactation oestrus	LH, P4
Days 3, 10 and 15 post lactation oestrus	P4
Weaning with no lactation oestrus	P4
Day 21 post partum slaughter	LH
Day 30 post lactation oestrus slaughter	P4

### ***Plasma hormone analysis***

Blood plasma progesterone was determined in duplicate using a coated tube radioimmunoassay (IM1188; Beckman Coulter, Brea, CA, USA). The minimum detectable limit of the assay was 0.12 ng/ml. The intra-assay coefficient of variation of the assays was 7.4%. The inter-assay coefficient of variation of the assays was 3.2%.

Blood plasma insulin was determined in duplicate using a double antibody radioimmunoassay (PI-12K; Millipore, St. Charles, MO, USA). The minimum detectable limit of the assay was 3.125 uU/ml. The intra-assay coefficient of variation of the assays was 6.1%. The inter-assay coefficient of variation of the assays was 4.4%.

Blood plasma LH was assayed in duplicate by double antibody radioimmunoassay using reagents obtained from Dr A. F. Parlow (National Hormone & Peptide Program, Harbor-UCLA Medical Center). The LH preparation AFP-11043B was used for iodination and preparation of standards. LH iodination was performed by Prosearch Australia (Malvern, VIC., Australia). The antiserum, (AFP-15103194) was used at a final dilution of 1:300,000. The standard curve ranged from 0.4 ng/ml to 25 ng/ml. Briefly, 100 µL sample (either neat or diluted 10 fold with PBS) or standard was incubated with 400 µL antibody for 24 hrs, followed by the addition of 100 µL iodinated LH (20,000 cpm per tube) and further incubation overnight. To all tubes except the totals and non-specific binding tubes, ice cold Sac-Cel (IDS, Cat # AASAC1; Goat anti-rabbit; 50 µL) was added, and the tubes were vortexed and left at 4°C for 20 mins. One millilitre of ice cold water was added and the tubes were immediately centrifuged at 4000 rpm for 20 mins. The supernatant was aspirated, the radioactivity determined in a gamma counter and levels of LH were calculated by interpolation from the standard curve. The minimum detectable limit of the assay was 0.4 ng/ml. The intra-assay coefficient of variation of the assay was less than 15%.

### ***Pre-mating slaughter (control and SW 7 sows)***

On day 21 post parturition, sows were transported to a local abattoir and their reproductive tracts recovered within 15 mins of slaughter. Both ovaries were removed and placed into individual tubes containing Phosphate Buffered Saline (PBS) warmed to 30°C and transported to the laboratory.

### ***Oocyte recovery***

Upon arrival at the laboratory, the reproductive tract and ovaries were weighed. Ovaries were maintained at 38°C during processing at the laboratory. On each ovary, the diameter of all visible surface follicles was measured using electronic vernier callipers (Absolute Digimatic Caliper, Mitutoyo, Japan) and the follicular contents were collected by aspiration into sterile 10 mL tubes using an 18-gauge needle and vacuum pump (Cook Australia, Queensland, Australia) at a pressure of 25 mm Hg. The time from ovary collection to aspiration was 2-3 hrs. Cumulus oocyte complexes (COCs) were recovered from the follicular fluid using a dissecting microscope and placed into *in vitro* maturation (IVM) medium. Remaining follicular fluid was centrifuged at 3000 rpm for 5 mins and stored at -80°C for further analysis.

### ***In vitro production of embryos***

All chemicals were obtained from Sigma Chemical Co. (St, Louis, MO, USA) unless otherwise stated. In vitro oocyte maturation was conducted as described in Weaver et al., (2014). Following 40 - 44 hours of in vitro maturation, COC's transferred to a 0.1%

hyaluronidase solution for 30 seconds to remove excess cumulus cells leaving the corona radiata cells intact. The oocytes were washed three times in fertilisation medium and transferred to a culture well containing 500 µl of the IVF medium under 300 µL of mineral oil. Terminal mix semen (SABOR Pty. Ltd., Clare, South Australia), was used for *in vitro* fertilisation. Spermatozoa from the samples with the highest viability were added to the oocytes to give a final concentration of  $0.5 \times 10^6$  spermatozoa /mL in each well. Spermatozoa were co-incubated with oocytes in a humidified atmosphere of 5% CO<sub>2</sub> in air at 38.6°C for 5 hrs. Following incubation, spermatozoa and remaining cumulus cells were removed from the surface of the zona pellucida using a fine bore glass pipette. Presumptive zygotes were incubated, with cleavage rate recorded 48 hrs post fertilisation and embryo development assessed and blastocyst total cell counts were recorded on day 6 post fertilisation.

Day 6 and 7 embryos were placed on a microscope slide in a drop of glycerol containing 1mg/mL Hoeschst 33342 and covered with a coverslip. Blastocyst cell nuclei were counted using a fluorescent microscope (Olympus Optical Co. Ltd., Tokyo, Japan) to provide a total cell count.

#### ***Follicular fluid hormone analysis***

Follicular fluid progesterone was determined in duplicate using a coated tube radioimmunoassay (IM1188; Beckman Coulter, Brea, CA, USA) after diluting samples either 10 fold or 100 fold with PBS, according to the manufacturer's instructions. The minimum detectable limit of the assay was 0.12 ng/mL. The intra-assay coefficient of variation of the assay was less than 7.4%.

Follicular fluid oestradiol was determined using a double antibody radioimmunoassay (DSL4800; Beckman Coulter, Brea, CA, USA) after diluting samples 100 fold with PBS, according to the manufacturer's instructions. The minimum detectable limit of the assay was 4 pg/ml. The intra-assay coefficient of variation of the assay was 20%.

Follicular fluid LH was assayed in duplicate using the same method described above for plasma LH analysis. The minimal detectable limit and intra-assay coefficient of variation was the same as plasma analysis.

#### ***Day 30 post-mating slaughter (control mated, SW 7 mated and OES SW 7 mated sows)***

On day 28 post first artificial insemination, sows were ultrasound scanned trans-abdominally to determine pregnancy status and non-pregnant sows were returned to the breeding herd. Pregnant sows were slaughtered at a commercial abattoir on day 30 ± 0.1 of pregnancy and their reproductive tracts were recovered within 15 mins of slaughter.

Upon arrival at the laboratory, the entire reproductive tract was weighed and the left and right horns of the uterus were identified. The following measurements were taken: individual ovarian weight, number of corpora lutea (CL) on each ovary, individual CL weight, chorioallantoic fluid volume, wet placental weight, number of viable and non-viable fetuses, fetal weight, fetal crown-rump length and weight of the empty uterine horns. Whole viable embryos and a section of highly vascularised tissue from each placenta were placed in separate cryogenic tubes and snap-frozen in liquid nitrogen for further gene expression analysis. Samples were stored at -80°C until analysis.

### ***Statistical analysis***

A chi square test was used to determine any treatment effects on the proportion of sows expressing oestrus and the proportion of sows becoming pregnant. The percentage of sows that expressed lactation oestrus and time to lactation oestrus were combined for Control mated and OES SW 7 mated sows as they were essentially the same treatment up until oestrus expression. Values in the text are expressed as Mean  $\pm$  SEM. A general linear model, with replicate and parity built in, and sow bodyweight change from day 1 to 18 as a covariate, was used to study the effects of split weaning on all variables measured (SPSS Statistics Version 21, Chicago, IL, USA). Differences between treatments were examined using least significant difference, with differences considered significant when  $P < 0.05$ .

### ***Experiment Four: Effect of group housing during lactation and fenceline boar contact on lactation oestrus expression***

This study was conducted at the Roseworthy piggery using 108 multiparous sows (parity  $2.8 \pm 0.07$ ) were used in this trial. Two weeks after farrowing, sows were allocated to one of the following four treatments (with allocation based on farrowing date, parity and suckled litter size):

- Treatment One (**CrateNoBE**; n = 18 sows): sows housed in farrowing crates throughout lactation, weaned as per normal piggery practice and mated at their first post-weaning oestrus. Sows taken daily to a group pen for 20 minutes
- Treatment Two (**CrateBE**; n = 18): sows housed in farrowing crates through lactation, and commencing daily, full physical boar contact (in DMA) for 15 minutes from day 18 of lactation.
- Treatment Three (**MultisuckleNoBE**; n = 36 sows): sows moved to multisuckle pens in weaner rooms (3 per pen) on day 18 of lactation.
- Treatment Four (**MultisuckleBE**; n = 36 sows): sows moved to multisuckle pens on day 18 of lactation, and received daily, 20 minutes of fenceline contact with a mature boar in their pen.

All sows were weaned on day 28 of lactation. Sows receiving boar exposure during lactation, or housed in multisuckle pens, were artificially inseminated at first detection of oestrus (either in lactation or post-weaning). From all sows the following measures were collected: weight on day 18 and day 28 of lactation, timing of oestrus, pregnancy status and subsequent total born. In addition, piglets were weighed individually on day 18 and 28 of lactation.

### ***Statistical Analysis***

Data are expressed as mean  $\pm$  SEM. In all analyses the sow or litter were the experimental unit. A general ANOVA was used to determine treatment effects on all variables. Litter size suckled and litter weight at start of treatment were included in the model as covariates when determining treatment effects on litter performance. The cumulative proportion of sows expressing oestrus and farrowing rate were analysed by chi-squared. All analyses, except for the chi-squared analyses, were performed using Genstat (10th Edition, Rothamsted Experimental Station, Harpenden, UK).

### 3. Outcomes

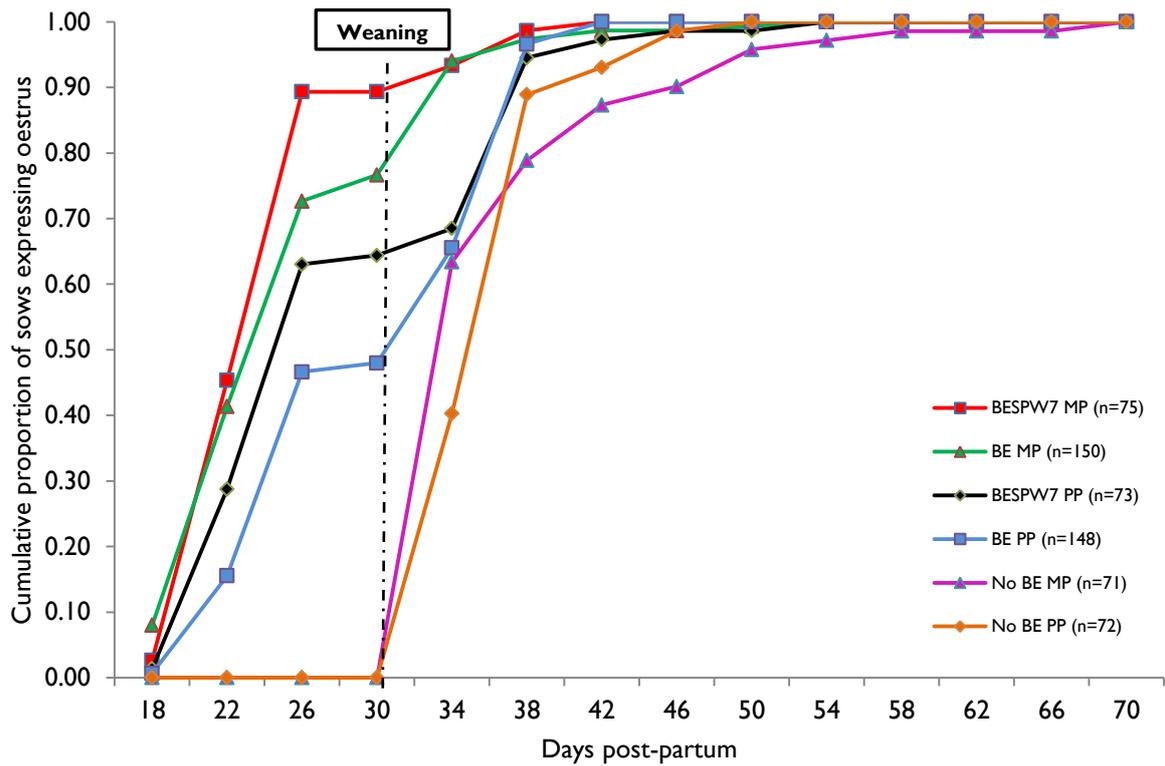
#### *Experiment One: Lactation oestrus induction in multi- and primi- parous sows in an Australian commercial pork production system*

##### *Sow body condition throughout lactation*

Sow live weight (LW) on day 1 of lactation did not differ between treatments (Table 2). At weaning, the No BE sows had lower LW and a greater percentage of LW loss over the course of lactation than both the BE treatments. From day 1 and throughout lactation PP sows consistently had a lower LW than MP sows. P2 backfat measurements at day 1 and weaning were similar across all treatments. P2 backfat was consistently lower in PP compared to MP sows throughout lactation.

##### *Expression of oestrus*

The distribution of oestrus expression from farrowing to oestrus is shown in Figure 1 for all PP and MP sows from all treatments. There was an effect of parity on the cumulative proportion of MP and PP sows expressing oestrus by day 26 post-partum in the BESPW7 (89% vs 61% respectively;  $P < 0.01$ ) and BE (76% vs 47% respectively;  $P < 0.01$ ) with a higher proportion of MP sows from both treatments expressing oestrus by this stage (Figure 1). Between 0 and 7 days from the onset of stimulation, both BE treatments exhibited a higher proportion of oestrus compared to the No BE sows demonstrating that an oestrus during lactation is as synchronous as mating post-weaning (Table 3). Providing both MP and PP sows with BE significantly increased the incidence of lactation oestrus, with a further increase observed when litter size was reduced to seven piglets (Table 3). A total of 24% of MP sows and 8% of PP sows in the non-BE treated sows ovulated spontaneously during lactation based on the progesterone levels post-weaning. In all treatments the incidence of lactation oestrus was higher for MP sows than PP sows (Table 3). When treatments were pooled together MP sows exhibited a 29% higher incidence of lactation oestrus than PP sows (81% compared to 52%, respectively;  $P < 0.05$ ). Parity or treatment did not affect the incidence of post-weaning oestrus expression or the weaning to oestrus interval ( $P > 0.05$ ).



**Figure 1** Cumulative proportion of primiparous and multiparous sows expressing oestrus relative to the days post-partum beginning on day 18 and finishing on day 70 post-partum

BE to lactation oestrus interval was similar within treatment; however, when treatment groups were combined, PP sows took slightly longer than MP sows to exhibit a lactation oestrus (Table 4). The oestrus to weaning interval was similar between treatments and parity (Table 4). When treatment groups were combined, MP sows had a longer duration of lactation oestrus than PP sows.

Table 2 Sow live weight (LW), live weight loss, and P2 backfat at day 1 and 17 post-farrow and weaning.

Item	Treatment			Parity	
	No BE	BE	BESPW7	Primiparous	Multiparous
n	149	303	153	303	302
<b>LW, kg</b>					
Day 1, pp	232.9 ± 2.0	237.6 ± 1.4	237.0 ± 1.9	202.4 ± 1.4 <sup>c</sup>	270.3 ± 1.4 <sup>d</sup>
Day 17, pp	na <sup>1</sup>	231.9 ± 1.4	230.4 ± 1.9	195.6 ± 1.6 <sup>c</sup>	267.3 ± 1.6 <sup>d</sup>
Weaning, pp	214.5 ± 1.9 <sup>a</sup>	224.7 ± 1.4 <sup>b</sup>	225.9 ± 1.9 <sup>b</sup>	189.2 ± 1.4 <sup>c</sup>	256.3 ± 1.4 <sup>d</sup>
<b>LW loss, kg</b>					
Days 1-17	na <sup>1</sup>	5.7 ± 0.6	6.6 ± 0.8	7.3 ± 0.6 <sup>c</sup>	4.7 ± 0.6 <sup>d</sup>
Days 17-wean	na <sup>1</sup>	7.2 ± 0.5	4.0 ± 0.7	4.3 ± 0.6	8.0 ± 0.6
Days 1-wn	18.0 ± 1.1 <sup>a</sup>	13.0 ± 0.7 <sup>b</sup>	10.8 ± 1.0 <sup>b</sup>	13.2 ± 0.7	14.0 ± 0.7
<b>% LW loss, kg</b>					
Days 1-17	na <sup>1</sup>	2.5 ± 0.2	2.8 ± 0.3	3.6 ± 0.3 <sup>c</sup>	1.6 ± 0.3 <sup>d</sup>
Days 17-wean	na <sup>1</sup>	3.0 ± 0.2 <sup>a</sup>	1.6 ± 0.3 <sup>b</sup>	2.1 ± 0.3 <sup>c</sup>	2.9 ± 0.3 <sup>d</sup>
Days 1-wean	7.7 ± 0.5 <sup>a</sup>	5.4 ± 0.3 <sup>b</sup>	4.5 ± 0.4 <sup>b</sup>	6.5 ± 0.3 <sup>c</sup>	5.0 ± 0.3 <sup>d</sup>
<b>P2, mm</b>					
Day 1, pp	16.0 ± 0.3	15.6 ± 0.2	15.1 ± 0.3	14.8 ± 0.2 <sup>c</sup>	16.3 ± 0.2 <sup>d</sup>
Day 17, pp	na <sup>1</sup>	14.6 ± 0.2	14.4 ± 0.3	13.5 ± 0.2 <sup>c</sup>	15.5 ± 0.2 <sup>d</sup>
Weaning, pp	13.9 ± 0.2	13.8 ± 0.2	14.1 ± 0.2	12.7 ± 0.2 <sup>c</sup>	15.1 ± 0.2 <sup>d</sup>
<b>P2 loss, mm</b>					
Days 1-17	na <sup>1</sup>	0.8 ± 0.2	0.5 ± 0.2	1.0 ± 0.2 <sup>c</sup>	0.4 ± 0.2 <sup>d</sup>
Days 17-wean	na <sup>1</sup>	0.9 ± 0.1 <sup>a</sup>	0.3 ± 0.2 <sup>b</sup>	0.9 ± 0.1 <sup>c</sup>	0.5 ± 0.1 <sup>d</sup>
Days 1-wean	1.9 ± 0.3 <sup>a</sup>	1.7 ± 0.2 <sup>a</sup>	0.9 ± 0.2 <sup>b</sup>	2.0 ± 0.2 <sup>c</sup>	1.1 ± 0.2 <sup>d</sup>

<sup>ab</sup> For treatment effects, means within a row without a common superscript differ (P<0.05); <sup>cd</sup> For parity effects, means within a row without a common superscript differ (P<0.05); <sup>1</sup>Sows in the No BE treatment weren't weighed on day 17

### ***Subsequent reproductive performance***

The subsequent number of piglets born alive was highest for sows in the No BE and the BESPW7 treatment (Table 5). Parity did not affect the total numbers of piglets born, the number born alive or mummified; however, stillborn numbers were increased for MP sows. Mating sows pre-weaning resulted in a lower total born and born alive, compared to sows mated post-weaning (P<0.05). There was no interaction between treatment and the timing of mating for sows and gilts. Compared to their counterparts mated during lactation, farrowing rates were higher for BE MP sows and all BESPW7 sows mated post-weaning (Table 6). There was no effect of treatment or parity on the pooled farrow rate.

**Table 3 Lactation (Lact) and post-weaning (Pw) oestrus expression and the weaning to oestrus interval (WOI)**

Parity	No BE		BE		BESPW7		No BE	BE	BESPW7
	MP	PP	MP	PP	MP	PP	Both	Both	Both
<i>n</i>	74	75	151	151	75	77	149	302	152
Lact Oestrus <sup>1</sup> , %	24 <sup>a</sup>	8 <sup>b</sup>	76 <sup>c</sup>	47 <sup>d</sup>	89 <sup>e</sup>	61 <sup>f</sup>	16 <sup>g</sup>	62 <sup>h</sup>	75 <sup>i</sup>
Lact Oestrus <sup>1</sup> , Day 0 - 7 <sup>2</sup> , %	76 <sup>a</sup>	88 <sup>ab</sup>	91 <sup>bc</sup>	92 <sup>bc</sup>	99 <sup>c</sup>	98 <sup>c</sup>	82 <sup>g</sup>	91 <sup>h</sup>	98 <sup>i</sup>
Pw Oestrus <sup>3</sup> , %	72/74	73/75	35/36	78/80	8/8	27/30	145/149	113/116	35/38
WOI <sup>4</sup> , d	5.2±1.1	4.9±1.1	4.4±1.1	4.5±1.0	5.4±1.2	4.5±1.1	5.0±1.0	4.4±1.0	4.8±1.1

<sup>abcdet</sup> Within a row means without a common superscript differ ( $P < 0.05$ ); <sup>gh</sup> Within a row means for the parity combined data without a common superscript differ ( $P < 0.05$ ); <sup>1</sup> Lactation oestrus percentage of sows in the No BE treatment is based on P4 analysis  $\geq 4$  ng/ml on day 2 post-weaning; <sup>2</sup> Lactation oestrus expression within 4 days and 7 days from the onset of stimulation, where stimulation begun on day 18 of lactation BE and BESPW7 treatments and from weaning for the No BE treatment; <sup>3</sup> % anoestrus sows at weaning expressing oestrus post-weaning; <sup>4</sup> Weaning to oestrus interval of sows anoestrus during lactation.

**Table 4 Boar exposure (BE) to lactation oestrus interval and the duration of lactation oestrus**

	Treatment		Parity	
	BE	BESPW7	PP	MP
<i>n</i>	302	152	228	226
BE to lactation oestrus interval <sup>1</sup> , d	4.6 ± 0.1	4.5 ± 0.2	4.9 ± 0.2 <sup>a</sup>	4.4 ± 0.1 <sup>b</sup>
Oestrus to weaning interval <sup>1</sup> , d	8.4 ± 0.2	8.3 ± 0.2	8.2 ± 0.2	8.4 ± 0.2
Duration of lactation oestrus <sup>1</sup> , d	2.8 ± 0.1	2.9 ± 0.1	2.6 ± 0.1 <sup>a</sup>	3.1 ± 0.1 <sup>b</sup>

<sup>ab</sup> Within a row means without a common superscript differ ( $P < 0.05$ ); <sup>1</sup> Only including sows which had a lactation oestrus.

**Table 5 Effect of treatment, parity and the timing of artificial insemination (AI) on the total born (TB), born alive (BA), still born (SB) and mummified fetus (Mum) at the subsequent litter**

	n	Parity	TB	BA	SB	Mum
<b>Treatment</b>						
No Boar Exp	115	All	12.0 ± 0.3	11.2 ± 0.3 <sup>a</sup>	0.9 ± 0.1	0.1 ± 0.03
Boar exposure	223	All	11.3 ± 0.2	10.4 ± 0.2 <sup>b</sup>	0.9 ± 0.1	0.1 ± 0.04
Boar exposure + SPW	114	All	11.9 ± 0.3	11.0 ± 0.3 <sup>ab</sup>	0.9 ± 0.1	0.2 ± 0.05
<b>Parity</b>						
Primiparous	223	N/A	11.4 ± 0.2	10.8 ± 0.2	0.7 ± 0.04 <sup>a</sup>	0.1 ± 0.1
Multiparous	219	N/A	11.9 ± 0.2	10.8 ± 0.2	1.1 ± 0.04 <sup>b</sup>	0.2 ± 0.1
<b>Timing of AI</b>						
Pre-weaning	216	All	11.3 ± 0.2 <sup>a</sup>	10.4 ± 0.2 <sup>a</sup>	0.9 ± 0.1	0.2 ± 0.04
Post-weaning	236	All	12.0 ± 0.2 <sup>b</sup>	11.1 ± 0.2 <sup>b</sup>	0.9 ± 0.1	0.1 ± 0.04
<b>Treatment x Timing of AI x parity</b>						
No Boar Exp						
Post-weaning	57	PP	11.6 ± 0.4	10.9 ± 0.4	0.7 ± 0.2	0.1 ± 0.1
Post-weaning	58	MP	12.7 ± 0.4	11.5 ± 0.4	1.2 ± 0.2	0.1 ± 0.1
Boar exposure						
Pre-weaning	54	PP	11.1 ± 0.4	10.3 ± 0.4	0.8 ± 0.2	0.1 ± 0.1
Pre-weaning	80	MP	10.8 ± 0.3	9.6 ± 0.3	1.2 ± 0.1	0.3 ± 0.1
Post-weaning	61	PP	11.6 ± 0.4	11.1 ± 0.4	0.5 ± 0.2	0.1 ± 0.1
Post-weaning	28	MP	12.0 ± 0.6	10.9 ± 0.6	1.1 ± 0.3	0.03 ± 0.1
Boar exposure + SPW						
Pre-weaning	36	PP	11.7 ± 0.5	11.2 ± 0.5	0.5 ± 0.2	0.1 ± 0.1
Pre-weaning	46	MP	11.8 ± 0.5	10.9 ± 0.4	1.0 ± 0.2	0.3 ± 0.1
Post-weaning	25	PP	11.0 ± 0.6	10.1 ± 0.6	0.9 ± 0.3	0.1 ± 0.1
Post-weaning	7	MP	14.1 ± 1.2	13.0 ± 1.2	1.1 ± 0.5	0.1 ± 0.2

<sup>ab</sup>Within a column and main effect, means without a common superscript differ (P<0.05); TB, total born; BA, born alive; SB, still born, Mum, mummified fetus

**Table 6 Subsequent farrowing rate for sows artificially inseminated (AI) in lactation or post-weaning**

Treatment	No BE			BE			BESPW7		
	MP	PP	Both	MP	PP	Both	MP	PP	Both
<i>n</i>	74	75	149	151	151	302	75	77	152
Farrow rate, AI in lactation, %	na <sup>1</sup>	na <sup>1</sup>	na <sup>1</sup>	73*	82	77	73*	77*	75*
Farrow rate, AI post-weaning, %	83	83	83	83*	81	82	88*	96*	94*
Pooled farrow rate, % <sup>2</sup>	83	83	83	76	82	79	75	84	79

\* Within a column and between farrow rate in AI in lactation and AI post-weaning, means within a treatment and parity group without a common superscript differ (P<0.05); <sup>1</sup>Sows in the No BE treatment weren't artificially inseminated whilst lactating; <sup>2</sup>Pooled farrow rate of sows mated in lactation and post-weaning

**Progesterone concentrations of sows in the conventional lactation treatment (no boar exposure)**

A higher proportion of MP sows were suspected of ovulating during lactation than PP sows. The average P4 concentration was highest for all sows, MP and PP, which were suspected of ovulating during lactation (Table 7). The weaning to oestrus interval was highest for all sows which were suspected of ovulating during lactation (Table 7). The litter size weaned did not differ between sows suspected or not suspected of ovulating during lactation.

**Table 7 Average progesterone concentration and weaning to oestrus interval of primiparous (PP) and multiparous (MP) sows suspected of ovulating during lactation within the no boar exposure treatment.**

Parity	PP		MP		Pooled for parity	
	No	Yes	No	Yes	No	Yes
Suspected lactation ovulation (No or Yes) <sup>1</sup>						
<i>n</i> (%) sows	69 (92%)	6 (8%)	55 (76%)	17 (24%)	124 (84%)	23 (16%)
Average P4 concentration, ng/ml	0.4±0.3 <sup>a</sup>	11.7±0.9 <sup>b</sup>	0.6±0.3 <sup>a</sup>	10.8±0.6 <sup>b</sup>	0.5±0.2 <sup>c</sup>	11.0±0.5 <sup>d</sup>
Weaning to oestrus interval, d	5.0±0.5 <sup>a</sup>	11.2±1.5 <sup>b</sup>	4.5±0.5 <sup>a</sup>	14.3±0.9 <sup>b</sup>	4.8±0.3 <sup>c</sup>	13.5±0.8 <sup>d</sup>
Litter size weaned	9.3±0.2	9.3±0.6	9.3±0.2	9.4±0.4	9.3±0.1	9.3±0.3

<sup>ab</sup>Within a row and main effect, means without a common superscript differ ( $P < 0.05$ ); <sup>cd</sup>Within a row and main effect of parity, means without a common superscript differ ( $P < 0.05$ ); <sup>1</sup>Suspected of ovulating during lactation based on progesterone concentration greater than or equal to 4 ng/ml, 2 days post-weaning

**Piglet growth**

Piglet weight on day four was slightly higher for sows in the BESPW7 treatment; however, there was no difference in weight at day 17. Consistently throughout lactation piglets from MP sows had higher average weights compared to those from PP sows (Table 8). Growth rate from day 4 to day 17 of lactation was greater for piglets from MP sows than from PP sows; however, no differences in piglet growth rates from 4-17 days were observed between treatments. Piglet weight gain from day 17 to weaning was greatest following a reduction in litter size in the BESPW7 treatment and for MP sows. Sows not receiving BE had lower average piglet weights on the day of weaning compared to both treatments receiving BE.

**Table 8 Average piglet weight and daily piglet growth rate throughout lactation**

	Treatment			Parity	
	No BE	BE	BESPW7	PP	MP
Average n piglets, day 4	10.2 ± 0.1 <sup>a</sup>	9.9 ± 0.1 <sup>b</sup>	10.5 ± 0.1 <sup>a</sup>	10.3 ± 0.1 <sup>c</sup>	10.0 ± 0.1 <sup>d</sup>
Average n piglets, day 17	na <sup>1</sup>	9.5 ± 0.1	10.2 ± 0.1	10.0 ± 0.1	9.5 ± 0.1
Average n piglets weaned	9.3 ± 0.1 <sup>a</sup>	9.3 ± 0.1 <sup>a</sup>	6.9 ± 0.1 <sup>b</sup>	8.8 ± 0.1	8.6 ± 0.1
Average piglet weight, kg					
Day 4	2.1 ± 0.02 <sup>a</sup>	2.1 ± 0.02 <sup>a</sup>	2.2 ± 0.02 <sup>b</sup>	2.0 ± 0.02 <sup>c</sup>	2.3 ± 0.02 <sup>d</sup>
Day 17	na <sup>1</sup>	5.5 ± 0.03	5.5 ± 0.04	5.1 ± 0.04 <sup>c</sup>	5.9 ± 0.04 <sup>d</sup>
Day of weaning	8.2 ± 0.08 <sup>a</sup>	8.6 ± 0.06 <sup>b</sup>	8.6 ± 0.08 <sup>b</sup>	7.9 ± 0.06 <sup>c</sup>	9.1 ± 0.06 <sup>d</sup>
Average piglet weight gain, kg					
Day 4 - 17	na <sup>1</sup>	3.3 ± 0.03	3.3 ± 0.04	3.0 ± 0.03 <sup>c</sup>	3.6 ± 0.03 <sup>d</sup>
Day 17 - weaning	na <sup>1</sup>	3.1 ± 0.05 <sup>a</sup>	3.5 ± 0.06 <sup>b</sup>	3.0 ± 0.05 <sup>c</sup>	3.5 ± 0.05 <sup>d</sup>

<sup>ab</sup> For treatment effects, means within a row without a common superscript differ ( $P < 0.05$ ); <sup>cd</sup> For parity effects, means within a row without a common superscript differ ( $P < 0.05$ ); <sup>1</sup>Piglets in the No BE treatment weren't weighed on day 17 of lactation

### ***Experiment Two: Effect of interrupted suckling on sow reproductive performance and piglet growth***

#### ***Sow reproductive performance***

The proportion of sows coming into oestrus during lactation was significantly higher for the 16IS compared to 0IS treatment group (0.81 versus 0.19; Table 9). The incidence of lactation oestrus expression tended ( $P < 0.1$ ) to be different between all three treatments (Table 9). The proportion of sows coming into oestrus post-weaning was significantly lower for the 16IS compared to 0IS treatment group (0.19 versus 0.81; Table 9). By day 5 post-weaning, all sows had expressed oestrus and been artificially inseminated. The timing of lactation oestrus relative to the start of treatment and farrowing was similar for all treatment groups (Table 9). On average, sows mated during lactation, continued to lactate for  $5 \pm 0.21$  days before weaning occurred. The interval from parturition to first detection of oestrus, regardless of pre- or post-weaning, was significantly shorter for sows in the 16IS compared to 0IS treatment groups (Table 9). The cumulative proportion of sows expressing oestrus following the commencement of treatment is described in Figure 1. The incidence of lactation oestrus was higher ( $P < 0.05$ ) on day 6 post-treatment start in the 16IS compared to 7IS and 0IS treatments.

Regardless of the timing of mating, a significantly lower proportion of sows in the 16IS compared to the 7IS and 0IS treatments farrowed a subsequent litter (Table 10). Regardless of treatment, farrowing rates were significantly lower for sows mated in lactation compared to post-weaning. The farrowing rate of sows mated during lactation was 100% for the 7IS and 0IS treatments, and 54% for the 16IS treatment group. The interval between successive farrowings was eight days less for sows mated during as opposed to after lactation ( $P < 0.05$ ; Table 10). The number of piglets farrowed was unaffected by the timing of oestrus relative to weaning; however, sows mated during as opposed to after lactation produced significantly fewer mummified foetuses at the subsequent farrowing (Table 10). Interestingly, the total number of piglets born at the subsequent farrowing per 100 sows entering the interrupted suckling regimes was numerically higher for the 7IS (1310 piglets) compared to the 0IS (1125 piglets) and 16IS (762 piglets).

**Table 9 Effect of 0, 7 or 16 hours of sow and piglet separation from day 17 to 20 of lactation on the timing of oestrus**

	Duration of sow - piglet separation			Pooled SEM
	0 hours	7 hours	16 hours	
Timing of lactation oestrus, days				
Relative to farrowing	23.0	21.9	22.2	0.31
Relative to start of stimulation	4.9	5.1	5.0	0.19
Timing of oestrus, days <sup>1</sup>				
Relative to farrowing	29.6 <sup>d</sup>	26.7 <sup>de</sup>	23.8 <sup>e</sup>	0.68
Relative to start of stimulation	12.7 <sup>d</sup>	9.6 <sup>de</sup>	6.7 <sup>e</sup>	0.69
Weaning to oestrus, days <sup>2</sup>	4.3	4.3	4.7	0.14
Proportion sows expressing:				
Lactation oestrus	0.19 <sup>ad</sup>	0.50 <sup>bde</sup>	0.81 <sup>ce</sup>	
Post-weaning oestrus	0.81 <sup>ad</sup>	0.50 <sup>bde</sup>	0.19 <sup>ce</sup>	
Oestrus	1.00	1.00	1.00	

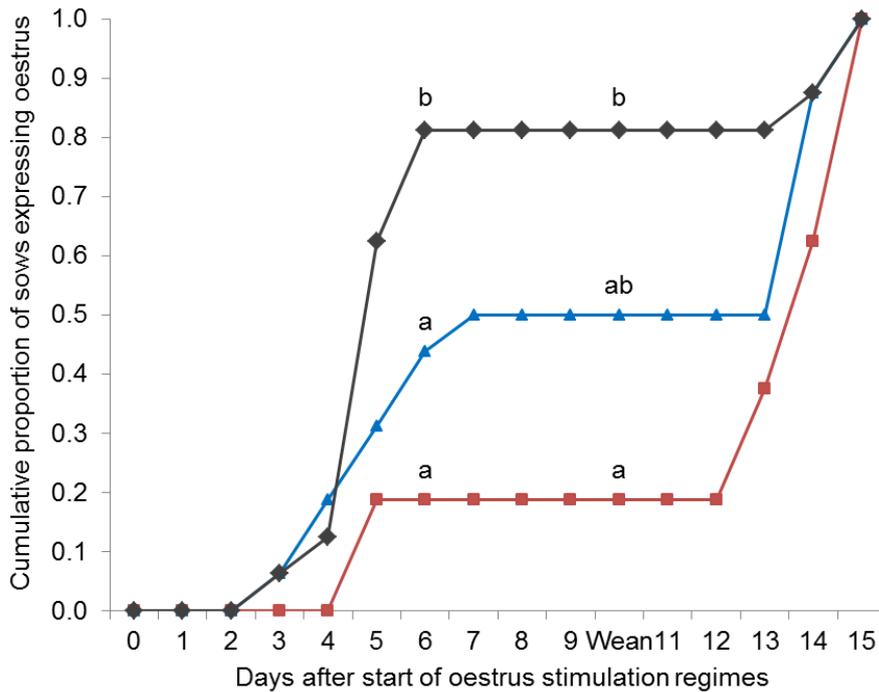
Values within a row with different superscripts differ; <sup>abc</sup> P<0.1; <sup>d,e</sup> P < 0.01

<sup>1</sup>Includes both lactation and post-weaning oestrus; <sup>2</sup>Sows which didn't express a lactation oestrus

**Table 10 Effect of 0, 7 or 16 hours of sow and piglet separation from day 17 to 20 of lactation and timing of oestrus / insemination (lactation versus post-weaning) on subsequent reproductive performance**

	Subsequent reproductive performance						Pigs / 100 sows
	Farr %	Int F - F	TB	BA	BD	Mm	
Sow-piglet separation							
0 hours	0.93 <sup>a</sup>	145.4	12.09	11.20	0.85	0.04	1125.3
7 hours	1.00 <sup>a</sup>	143.6	13.12	11.41	1.23	0.47	1310.0
16 hours	0.63 <sup>b</sup>	141.7	12.12	10.48	1.35	0.29	762.3
Timing of oestrus							
In lactation	0.75 <sup>a</sup>	139.3 <sup>a</sup>	12.31	10.98	1.03	0.10 <sup>a</sup>	922.5
Post-weaning	0.96 <sup>b</sup>	147.3 <sup>b</sup>	12.60	11.18	1.23	0.40 <sup>b</sup>	1209.6
Pooled SEM		0.687	0.457	0.374	0.218	0.079	

Farr % = Proportion of inseminated pigs farrowing a litter, adjusted for losses due to non-reproductive reasons; Int F-F = interval between successive farrowings; Pigs / 100 sows = total number of piglets born per 100 sows per system; TB = Total piglets born at the subsequent farrowing; BA = number of piglets born alive at the subsequent farrowing; BD = number of piglets born dead at the subsequent farrowing; Mm = number of mummified foetuses produced at the subsequent farrowing. <sup>ab</sup> within a column different superscripts indicate significance at P<0.05.



**Figure 2** Effect of 0 (■), 7 (▲) or 16 (◆) hours of sow and piglet separation from day 17 to 20 of lactation on the cumulative proportion of sows expressing oestrus. <sup>a,b</sup> Values within a day with different superscripts differ significantly;  $P < 0.05$

### **Piglet and litter characteristics**

Suckled litter size on day 17 of lactation and at weaning was similar for all treatment groups (Table 11). On day 17 of lactation, total litter weight and individual piglet weight were similar for all treatment groups. There was a tendency ( $P = 0.08$ ) for sows in the 0IS treatment to wean heavier piglets and litters compared with those in the 16IS treatment group. Piglet and litter weight gain from day 17 of lactation to weaning was significantly lower for the 16IS compared with 0IS treatment groups, but similar for the 0IS and 7IS treatments (Table 11).

**Table 11 Effect of 0, 7 or 16 hours of sow and piglet separation from day 17 to 20 of lactation on litter characteristics and growth between day 17 and 27 (weaning)**

	Duration of sow - piglet separation			Pooled SEM
	0 hours	7 hours	16 hours	
Suckled litter size				
Day 17 post-partum	9.44	9.81	10.06	0.328
At weaning	9.06	9.63	9.94	0.364
Total litter weight (kg)				
Day 17 post-partum	52.82	52.80	52.60	1.451
At weaning	77.44*	75.48*	68.61*	2.790
Gain	24.00 <sup>a</sup>	22.87 <sup>a</sup>	16.43 <sup>b</sup>	1.553
Individual piglet weight (kg)				
Day 17 post-partum	5.39	5.42	5.38	0.195
At weaning	8.07*	7.92*	7.14*	0.293
Gain	2.66 <sup>a</sup>	2.50 <sup>a</sup>	1.77 <sup>b</sup>	0.141
Piglet age at weaning (days)	26.9	27.1	27.1	0.324

<sup>a,b</sup> Values within a row with different superscripts differ significantly; P < 0.01

\*Values within a row differ; P < 0.08

### ***Sow and piglet cortisol plasma levels***

Piglet cortisol levels were unaffected by treatment (Table 12). Sow cortisol levels during separation were significantly higher in the 7IS compared to 0IS treatment groups (Table 12). The difference in cortisol levels between the period of IS and after IS was significantly higher for the 7IS compared to 0IS treatments (Table 12).

**Table 12 Effect of 0, 7 or 16 hours of sow and piglet separation from day 17 to 20 of lactation on the maternal and piglet cortisol levels during and after separation on day 2 of the treatment**

	Duration of sow - piglet separation			Pooled SEM
	0 hours	7 hours	16 hours	
Mean piglet cortisol (ng/ml)				
Two days prior to treatment start <sup>1</sup>	24.38	24.22	23.60	1.503
On the third day of treatment <sup>2</sup>	24.83	25.18	28.52	2.169
Difference <sup>3</sup>	0.45	0.95	4.92	2.783
Mean sow cortisol (ng/ml)				
During separation <sup>4</sup>	19.87 <sup>a</sup>	27.18 <sup>b</sup>	24.58 <sup>ab</sup>	1.584
After separation <sup>5</sup>	18.73	14.99	18.49	1.521
Difference <sup>6</sup>	-3.13 <sup>c</sup>	-12.18 <sup>d</sup>	-6.08 <sup>cd</sup>	2.107

<sup>a,b</sup> Values within a row with different superscripts differ significantly; P < 0.05

<sup>c,d</sup> Values within a row differ; P < 0.06

<sup>1</sup> Samples collected at time of day equivalent to 1 hour into separation for 7 and 16 hour treatments and 8 am for 0 hour treatments

<sup>2</sup> Samples collected 1 hour into separation period, and 8 am for 0 hour treatments

<sup>3</sup> Difference between cortisol levels prior to start of treatment and the third day of treatment

<sup>4</sup> Samples collected one hour after start of separation

<sup>5</sup> Samples collected one hour after end of separation

<sup>6</sup> Difference between levels during and after separation

## Experiment Three: Effect of split-weaning prior to lactation oestrus or post lactation oestrus on oocyte quality and embryo survival - Materials and Methods

To simplify data analysis and presentation, the data from this experiment has been presented in two parts. The first relates to the embryo survival treatment. The second relates to the oocyte quality treatments.

### ***Part One: Effect of split weaning on embryo survival***

#### ***Sow body composition***

Sow liveweight was similar for all treatments on days one and 18 post parturition as well as at weaning and day 30 of gestation. However, at oestrus, control mated sows tended to be heavier than SW 7 mated sows (Table 13). Control mated sows lost less weight from day 18 post parturition to oestrus than both SW 7 mated and OES SW 7 mated sows, however, there were more control mated sows on heat on day 18 which resulted in a zero weight change; while from oestrus to weaning, both SW 7 mated sows and OES SW 7 mated sows gained more weight than control mated sows (Table 13). Furthermore, control mated sows lost more weight from day one to weaning and day 18 to weaning than both SW 7 mated and OES SW 7 mated sows (Table 13). There was no effect of treatment on P2 backfat at any time point during the experiment.

#### ***Litter characteristics***

Three days post parturition, total litter weight, average piglet weight and litter size did not differ between treatments (Table 14). However, on day 18 post parturition, prior to split weaning, litter weight and litter size was higher in SW 7 mated sows compared to OES SW 7 mated sows (Table 14). After split weaning on day 18, litter weight and size as well as individual piglet weight were all lower in SW 7 mated sows compared to control mated and OES SW 7 mated sows (Table 14). Subsequently at weaning, control mated sows had a larger litter size and heavier litter weight than both SW 7 mated and OES SW 7 mated sows, however, there was no difference in individual piglet weight (Table 14).

**Table 13 Sow body composition on days one and 18 post parturition as well as at oestrus, weaning and on day 30 of gestation.**

	Treatment		
	control mated	SW 7 mated	OES SW 7 mated
<i>n</i>	22	21	16
<b>Sow liveweight, kg</b>			
Day 1 post-parturition	296.6 ± 5.7	283.5 ± 6.4	295.6 ± 6.4
Day 18 post-parturition	280.7 ± 6.7	270.1 ± 7.4	283.9 ± 7.4
Day of oestrus	285.1 ± 6.1 <sup>d</sup>	269.2 ± 6.2 <sup>e</sup>	277.9 ± 6.2 <sup>de</sup>
Day of weaning	273.6 ± 7.1	270.0 ± 7.9	282.7 ± 7.9
Day 30 of gestation	275.9 ± 6.2	270.0 ± 6.3	273.6 ± 6.2
<b>Sow liveweight change, kg</b>			
Days 1-18	-16.0 ± 2.5	-13.4 ± 2.8	-11.8 ± 2.7
Days 18-oestrus	-2.3 ± 1.1 <sup>x</sup>	-6.8 ± 1.0 <sup>y</sup>	-6.0 ± 1.0 <sup>y</sup>
Oestrus to weaning	-0.9 ± 1.9 <sup>a</sup>	7.7 ± 1.8 <sup>b</sup>	4.8 ± 1.7 <sup>b</sup>
Weaning to day 30 of gestation	-7.3 ± 2.4	-6.8 ± 2.4	-3.8 ± 2.4
Day 1 to weaning	-23.0 ± 3.0 <sup>a</sup>	-13.5 ± 3.3 <sup>b</sup>	-13.0 ± 3.3 <sup>b</sup>
Day 18 to weaning	-7.1 ± 1.9 <sup>a</sup>	-0.1 ± 2.1 <sup>b</sup>	-1.2 ± 2.1 <sup>b</sup>
<b>Sow backfat, mm</b>			
Day 1 post-parturition	26.35 ± 1.16	25.42 ± 1.30	26.03 ± 1.29
Day 18 post-parturition	24.62 ± 1.06	24.78 ± 1.17	24.22 ± 1.17
Day of oestrus	24.76 ± 1.34	23.77 ± 1.37	23.08 ± 1.27
Day of weaning	22.57 ± 1.10	23.13 ± 1.22	22.92 ± 1.22
Day 30 of gestation	22.76 ± 0.95	24.04 ± 0.89	22.63 ± 0.88
<b>Sow backfat change, mm</b>			
Days 1-18	-1.73 ± 0.60	-0.56 ± 0.67	-1.82 ± 0.67
Days 18-oestrus	-0.53 ± 0.66	-1.17 ± 0.62	-1.14 ± 0.57
Oestrus to weaning	-1.29 ± 0.89	-0.08 ± 0.84	-0.16 ± 0.77
Weaning to day 30 of gestation	-0.97 ± 0.63	-1.19 ± 0.57	-0.91 ± 0.58
Day 1 to weaning	-3.78 ± 0.85	-2.19 ± 0.95	-3.12 ± 0.95
Day 18 to weaning	-2.05 ± 0.77	-1.65 ± 0.86	-1.30 ± 0.86

<sup>abc</sup> Within a row, means without a common superscript differ (P < 0.05)

<sup>xyz</sup> Within a row, means without a common superscript differ (P < 0.01)

<sup>def</sup> Within a row, means without a common superscript tended to differ (P < 0.1)

**Table 14 Effect of split weaning on day 18 post parturition or at lactation oestrus on litter size, total litter weight and individual piglet weight.**

	Treatment		
	Control mated	SW 7 mated	OES SW 7 mated
<i>n</i>	22	21	16
<b>Day 3</b>			
Litter weight (kg)	23.9 ± 1.0	21.6 ± 1.1	23.6 ± 1.0
Litter size	10.9 ± 0.05	10.9 ± 0.05	10.9 ± 0.05
Individual piglet weight (kg)	2.2 ± 0.1	2.0 ± 0.1	2.2 ± 0.1
<b>Day 18</b>			
Litter weight (kg)	65.1 ± 2.1 <sup>ab</sup>	61.3 ± 2.4 <sup>a</sup>	67.9 ± 2.1 <sup>b</sup>
Litter size	10.7 ± 0.1 <sup>ab</sup>	10.4 ± 0.1 <sup>a</sup>	10.8 ± 0.1 <sup>b</sup>
Individual piglet weight (kg)	6.1 ± 0.2	5.9 ± 0.2	6.3 ± 0.2
Number piglets weaned (kg)	0.0 ± 0.0 <sup>x</sup>	3.4 ± 0.1 <sup>y</sup>	0.0 ± 0.0 <sup>x</sup>
Total litter weight weaned (kg)	-	23.0 ± 1.3	-
Individual piglet weight weaned (kg)	-	6.4 ± 0.2	-
Number piglets retained	10.8 ± 0.0 <sup>x</sup>	7.0 ± 0.0 <sup>y</sup>	10.8 ± 0.0 <sup>x</sup>
Total litter weight retained (kg)	66.5 ± 1.6 <sup>x</sup>	38.3 ± 1.8 <sup>y</sup>	68.1 ± 1.6 <sup>x</sup>
Individual piglet weight retained (kg)	6.1 ± 0.2 <sup>x</sup>	5.4 ± 0.2 <sup>y</sup>	6.3 ± 0.2 <sup>x</sup>
<b>Oestrus</b>			
Number piglets weaned	0.0 ± 0.0 <sup>x</sup>	0.0 ± 0.0 <sup>x</sup>	3.7 ± 0.1 <sup>y</sup>
Total litter weight weaned (kg)	-	-	32.0 ± 0.7
Individual piglet weight weaned (kg)	-	-	8.6 ± 0.1
Number piglets retained	-	-	7.0 ± 0.0
Total litter weight retained (kg)	-	-	48.4 ± 0.5
Individual piglet weight retained (kg)	-	-	6.9 ± 0.1
<b>Weaning</b>			
Number piglets weaned	10.8 ± 0.1 <sup>x</sup>	7.0 ± 0.1 <sup>y</sup>	7.0 ± 0.1 <sup>y</sup>
Total litter weight weaned (kg)	104.6 ± 3.3 <sup>x</sup>	75.7 ± 3.7 <sup>y</sup>	70.7 ± 3.3 <sup>y</sup>
Individual piglet weight weaned (kg)	9.5 ± 0.4	10.7 ± 0.5	10.1 ± 0.4

<sup>abc</sup> Within a row, means without a common superscript differ (P < 0.05)

<sup>xyz</sup> Within a row, means without a common superscript differ (P < 0.01)

<sup>def</sup> Within a row, means without a common superscript tended to differ (P < 0.1)

### ***Lactation oestrus expression***

Control mated and OES SW 7 mated sows have been combined (*n* = 38) for analysis of incidence of oestrus expression and timing of oestrus as the two treatments were identical prior to first detection of oestrus. Seven sows (11.9 % of all sows) exhibited lactation oestrus on day 18 post parturition when boar contact commenced. These sows were allocated to either control mated (*n* = 5) or OES SW 7 mated (*n* = 2) treatments.

The cumulative proportion of sows expressing a lactation oestrus is shown in Figure 3. There was no effect of treatment on the proportion of sows that expressed lactation

oestrus or the time taken to express oestrus (Table 15;  $P > 0.05$ ). There was also no difference between treatments on the duration of lactation oestrus (control mated,  $3.1 \pm 0.3$  days; SW 7 mated,  $2.9 \pm 0.2$  days; OES SW 7 mated,  $3.4 \pm 0.2$  days;  $P > 0.05$ ) (excluding sows on heat on day 18).

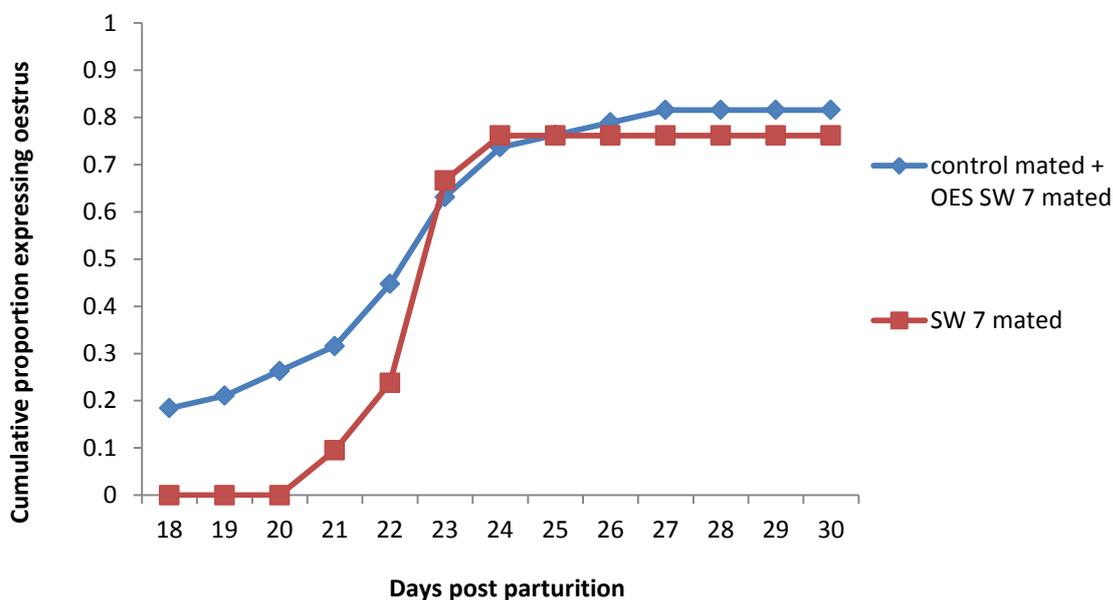


Figure 3 Cumulative proportion of sows that expressed lactation oestrus.

Table 15 The proportion of sows that expressed lactation oestrus and the time taken to express lactation oestrus.

	<i>n</i>	Proportion expressing lactation oestrus	Days to oestrus <sup>2</sup>
<b>Including sows on heat on day 18</b>			
Control mated and OES SW 7 mated combined <sup>1</sup>	38	0.82	$21.2 \pm 0.5$
SW 7 mated	21	0.76	$22.4 \pm 0.5$
<b>Excluding sows on heat on day 18</b>			
Control mated and OES SW 7 mated combined <sup>1</sup>	31	0.77	$22.7 \pm 0.4$
SW 7 mated	21	0.76	$22.4 \pm 0.5$

<sup>1</sup> Until oestrus expression, control mated and OES SW 7 mated treatments were identical

<sup>2</sup> Number of days from farrowing to oestrus

### Ovarian follicle growth

Ovarian follicle growth over the period of lactation is shown in Figure 4. There was no effect of treatment on the average diameter of the three largest follicles at any time

point during lactation ( $P > 0.05$ ). At the first detection of oestrus, the average diameter of the three largest follicles did not differ between control mated ( $4.8 \pm 0.21$  mm), SW 7 mated ( $4.43 \pm 0.20$  mm) or OES SW 7 mated ( $3.99 \pm 0.17$ ) sows ( $P > 0.05$ ). Similarly, diameter did not differ between control mated, SW 7 mated or OES SW 7 mated sows at the ovarian scan prior to ovulation ( $6.4 \pm 0.45$ ,  $5.2 \pm 0.39$  and  $6.4 \pm 0.44$  mm respectively;  $P > 0.05$ ). The timing of ovulation was also similar for control mated, SW 7 mated and OES SW 7 mated sows ( $2.1 \pm 0.18$ ,  $2.5 \pm 0.18$  and  $2.3 \pm 0.18$  days post behavioural oestrus detection respectively;  $P > 0.05$ ), however, when the timing of ovulation was expressed relative to oestrus length, ovulation tended to occur sooner in OES SW 7 mated sows compared to SW 7 mated sows ( $71 \pm 4.9$  % versus  $87 \pm 4.7$  % of the way through oestrus, respectively  $P > 0.1$ ). Control mated sows ovulated  $76 \pm 5.0$  % of the way through oestrus.

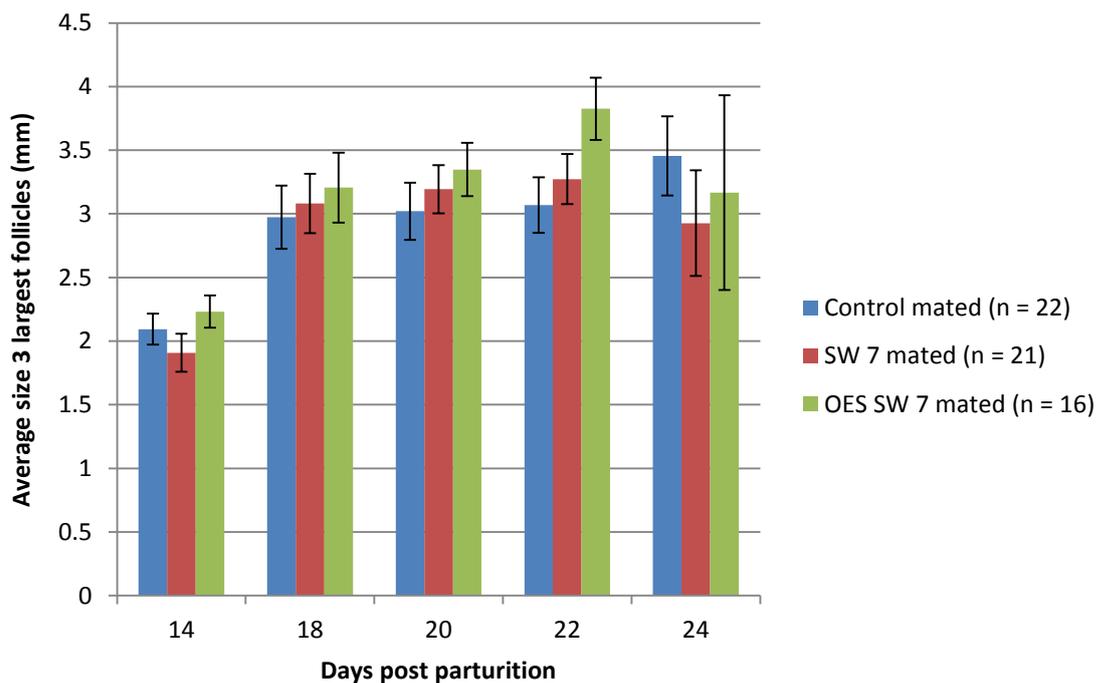


Figure 4 Average size of the largest 3 ovarian follicles on days 14, 18, 20, 22 and 24 post parturition measured by transrectal ultrasound. Ovaries were scanned until expression of oestrus.

### Day 30 gestation reproductive parameters

At 30 days post lactation oestrus detection, pregnancy rate was higher in SW 7 and OES SW 7 mated sows compared to control mated sows (Table 16). Furthermore, when sows already in oestrus on day 18 post parturition were excluded from the analysis, pregnancy rate remained lower in control sows (60%) compared to SW 7 mated (94%) and OES SW 7 mated (93%) sows ( $P < 0.05$ ). Sows that were in oestrus on day 18 and subsequently mated ( $n = 7$ ), had lower pregnancy rates (29%) than sows that were mated after day 18 ( $n = 40$ ) (88%;  $P < 0.05$ ). However, there was no difference in embryo survival between the two sows that were in oestrus on day 18 and confirmed pregnant, compared to those mated after day 18 ( $37.2 \pm 12.9\%$  ( $n = 2$ ) versus  $61.8 \pm 3.9\%$  ( $n = 40$ );  $P = 0.218$ ). The two sows that were on heat on day 18, mated and were pregnant at day 30 of gestation were both control mated sows and both exhibited oestrus for three days. All other sows that were on heat on day 18 exhibited oestrus for 2.5 days or less ( $2.0 \pm 0.27$  days).

There was no difference in day of slaughter for all three treatments (control mated, 30.0 ± 0.22; SW 7 mated, 30.2 ± 0.21, and OES SW 7 mated, 30.2 ± 0.21;  $P > 0.05$ ). While ovulation rate was similar for all three treatments, the number of viable embryos was higher in SW 7 mated sows compared to control mated sows and OES SW 7 mated sows (Table 16). As a result, embryo survival was greater in SW 7 mated sows compared to both control mated and OES SW 7 mated sows (Table 16).

**Table 16** The effect of treatment on pregnancy rate, ovulation rate, embryo survival and embryo characteristics at day 30 of gestation.

	Treatment		
	Control mated	SW 7 mated	OES SW 7 mated
Pregnancy rate <sup>1</sup>	60% <sup>a</sup>	94% <sup>b</sup>	81% <sup>b</sup>
<i>n</i> <sup>2</sup>	9	15	13
# corpora lutea	23.5 ± 1.5	22.2 ± 1.2	23.3 ± 1.1
# viable embryos	12.6 ± 1.1 <sup>a</sup>	16.0 ± 1.0 <sup>b</sup>	11.2 ± 0.9 <sup>a</sup>
Embryo survival (%)	56.4 ± 5.0 <sup>axy</sup>	73.7 ± 4.2 <sup>bx</sup>	49.5 ± 3.9 <sup>ay</sup>
Embryo weight (g)	1.39 ± 0.06 <sup>a</sup>	1.64 ± 0.05 <sup>b</sup>	1.60 ± 0.05 <sup>b</sup>
Embryo crown-rump length (mm)	23.1 ± 3.0	22.9 ± 2.5	24.4 ± 2.3

<sup>abc</sup> Within a row, means without a common superscript differ ( $P < 0.05$ )

<sup>xyz</sup> Within a row, means without a common superscript differ ( $P < 0.01$ )

<sup>def</sup> Within a row, means without a common superscript tended to differ ( $P < 0.1$ )

<sup>1</sup> Of sows inseminated in lactation

<sup>2</sup> Number of sows inseminated in lactation and pregnant 30 days post artificial insemination

Embryo weight was higher in both SW 7 mated sows and OES SW 7 mated sows compared to control mated sows (Table 16) and ranged from 0.96 to 2.30 g. There was no effect of treatment on ovarian, corpora lutea or placenta weight, however, full uterine weight was higher in SW 7 mated sows compared to control mated and OES SW 7 mated sows (Table 17). Irrespective of treatment, full uterine weight was positively correlated to embryo number ( $P < 0.001$ ;  $R^2 = 0.65$ ). Furthermore, while placental weight was positively correlated with allantochorionic fluid volume ( $P < 0.05$ ;  $R^2 = 0.17$ ), there was no relationship between placenta weight and embryo weight ( $P > 0.05$ ).

There was no relationship between day of slaughter and embryo number, embryo survival, placental weight or allantochorionic fluid volume, however, as day of slaughter increased, embryo weight tended to increase by 0.096 g ( $P < 0.1$ ;  $R^2 = 0.10$ ). A greater percentage of SW 7 mated sows had multiple embryos (two or three) within the same placenta (47%,  $n = 7$ ) compared to control mated (11%,  $n = 1$ ) and OES SW 7 mated (8%,  $n = 1$ ) sows at day 30 of gestation ( $P < 0.05$ ). However, multiple embryos only accounted for six percent of total embryos. Overall, individual embryos were heavier than embryos that shared a placenta (1.65 ± 0.02 g versus 1.45 ± 0.05 g;  $P < 0.001$ ).

**Table 17 The effect of treatment on ovarian, corpora lutea, uterine and placenta weights at day 30 of gestation.**

	Treatment		
	Control mated	SW 7 mated	OES SW 7 mated
<i>n</i>	9	15	13
Mean ovarian weight (g)	10.70 ± 0.56	10.87 ± 0.48	11.29 ± 0.44
Total corpora luteal weight (g)	9.65 ± 0.67	9.90 ± 0.56	9.62 ± 0.55
Individual corpora luteal weight (g)	0.42 ± 0.02	0.44 ± 0.02	0.41 ± 0.02
Full uterine weight (kg)	4.41 ± 0.27 <sup>axy</sup>	5.43 ± 0.23 <sup>bx</sup>	4.28 ± 0.22 <sup>ay</sup>
Empty uterine weight (kg)	1.81 ± 0.14	1.98 ± 0.12	1.84 ± 0.12
Mean individual placenta weight (g)	22.72 ± 1.39	23.73 ± 1.17	24.11 ± 1.14
Placental efficiency (embryo weight / placenta weight)	0.068 ± 0.003	0.071 ± 0.003	0.070 ± 0.003
Mean individual allantochorionic fluid volume (mL)	181.3 ± 10.2	180.0 ± 8.8	188.8 ± 8.7

<sup>abc</sup> Within a row, means without a common superscript differ ( $P < 0.05$ )

<sup>xyz</sup> Within a row, means without a common superscript differ ( $P < 0.01$ )

<sup>def</sup> Within a row, means without a common superscript tended to differ ( $P < 0.1$ )

### ***Sow percent body weight loss effect on reproductive parameters***

Irrespective of treatment, sows that lost more than five percent of their day one post parturition body weight at lactation oestrus expression had reduced embryo survival but higher pregnancy rates (Table 18). Furthermore, sows that lost more than seven percent of their body weight tended to have reduced embryo survival (Table 18). Additionally, sows that lost more than five percent body weight from day one to 18 post parturition ( $n = 21$ ) also had reduced embryo survival compared to sows that lost less than five percent body weight ( $n = 16$ ) ( $58.0 \pm 3.6$  versus  $62.6 \pm 3.7\%$  respectively;  $P < 0.05$ ).

From lactation oestrus to weaning, sows were grouped into either lost weight ( $n = 11$ ) or gained weight ( $n = 26$ ). There was no effect of weight gain or loss on ovulation rate, embryo number or embryo survival ( $P > 0.05$ ). Embryo weight was higher in sows that gained weight after oestrus ( $1.6 \pm 0.1$  versus  $1.4 \pm 0.1$  g;  $P < 0.01$ ) and placental weight tended to be higher in sows that gained weight ( $25.0 \pm 1.0$  versus  $21.5 \pm 1.3$  g;  $P < 0.1$ ). There was no effect of litter weight or size at any time point on embryo survival or embryo weight.

**Table 18 Percent sow body weight change from day 1 post parturition to lactation oestrus expression on subsequent reproductive measures**

	Lost > 5% BW			Lost > 7% BW			Lost > 10% BW		
	Yes	No	P value	Yes	No	P value	Yes	No	P value
<i>n</i>	33	14		21	26		9	38	
Pregnancy rate (%)	88	57	< 0.05	90	69	> 0.05	100	74	> 0.05
Ovulation rate	22.9 ± 0.8	22.0 ± 1.3	0.290	23.5 ± 1.0	22.3 ± 0.9	0.912	22.8 ± 1.4	22.7 ± 0.8	0.413
# embryos	12.7 ± 0.7	13.7 ± 1.0	0.110	13.6 ± 0.9	13.3 ± 0.8	0.119	14.4 ± 1.5	13.4 ± 0.8	0.576
Embryo survival (%)	57.0 ± 3.4	64.5 ± 5.2	0.034	59.3 ± 3.9	61.4 ± 3.6	0.075	65.2 ± 7.6	60.5 ± 4.3	0.459
Embryo weight (g)	1.5 ± 0.1	1.6 ± 0.1	0.517	1.6 ± 0.1	1.5 ± 0.1	0.878	1.5 ± 0.0	1.6 ± 0.1	0.865
Placenta weight (g)	24.2 ± 1.3	24.8 ± 1.9	0.423	24.1 ± 1.6	24.1 ± 1.5	0.839	23.3 ± 1.8	24.0 ± 1.0	0.615

### ***Hormone analysis***

There was no effect of treatment on plasma insulin concentration on days three or 18 post parturition nor was there any effect on progesterone at any time point (Table 19). Plasma LH concentration at lactation oestrus was higher in SW 7 mated and OES SW 7 mated sows compared to control mated sows; and it tended to be higher in SW 7 mated sows compared to OES SW 7 mated sows (Table 19).

**Table 19 Plasma insulin, progesterone and LH concentrations over the period of lactation, lactation oestrus and post mating.**

	Treatment			P value
	Control mated	SW 7 mated	OES SW 7 mated	
<b>Day 3 post parturition</b>				
Insulin (uU/mL)	54.03 ± 11.02	69.32 ± 12.11	49.27 ± 11.52	0.655
<b>Day 18 post parturition</b>				
Insulin (uU/mL)	30.95 ± 4.43	35.53 ± 4.66	39.07 ± 4.74	0.843
Progesterone (ng/mL) <sup>1</sup>	0.76 ± 0.07	0.46 ± 0.06	0.65 ± 0.07	0.196
<b>Oestrus</b>				
Luteinising hormone (ng/mL)	1.43 ± 0.75 <sup>a</sup>	5.18 ± 0.58 <sup>b d</sup>	3.71 ± 0.56 <sup>b e</sup>	0.025
Progesterone (ng/mL)	0.63 ± 0.11	0.67 ± 0.08	0.70 ± 0.08	0.244
<b>Progesterone (ng/mL)</b>				
3 days post AI	2.11 ± 0.34	3.94 ± 0.33	4.10 ± 0.32	0.550
10 days post AI	16.88 ± 2.24	16.90 ± 1.95	19.84 ± 1.84	0.827
15 days post AI	24.64 ± 3.11	25.68 ± 3.17	32.23 ± 3.34	0.428
30 days post AI	24.91 ± 2.70	28.05 ± 2.67	24.08 ± 2.63	0.592

<sup>abc</sup> Within a row, means without a common superscript differ (P < 0.05)

<sup>xyz</sup> Within a row, means without a common superscript differ (P < 0.01)

<sup>def</sup> Within a row, means without a common superscript tended to differ (P < 0.1)

<sup>1</sup> Excluding sows on heat on day 18 post parturition

Of sows that failed to express oestrus and ovulate during lactation ( $n = 12$ ), there was no effect of treatment on plasma concentration of progesterone at weaning (average concentration  $0.59 \pm 0.10$  ng/mL;  $P > 0.05$ ). However, there was one sow that ovulated in the absence of behavioural oestrus as indicated by progesterone concentration at weaning and this sow was also found to have high progesterone on day 18 post parturition (5.0 ng/mL) indicating she ovulated prior to day 18. This sow was in the control mated treatment but was not inseminated. Of sows that expressed lactation oestrus, one control mated sow and one SW 7 mated sow did not subsequently ovulate. Both of these sows were also not pregnant at day 30 of gestation. Five sows had a progesterone concentration > 1.0 ng/mL on day 18 post parturition and four of these sows expressed lactation oestrus, two of which were on heat on day 18. These two sows were not pregnant at day 30 of gestation.

### **Part Two: Effect of split weaning on embryo survival**

#### **Sow liveweight and body composition**

Liveweight and P2 backfat were similar for both treatments on days 1, 18 and 21 of lactation (Table 20). Liveweight and P2 backfat change from days 1 to 18 and days 18 to 21 post-parturition did not differ between treatments. Irrespective of treatment, 56% of sows lost more than 5% of their body weight from day 1 to 21 of lactation, 38% of sows lost more than 7% while 15% of sows lost more than 10%.

**Table 20 Effects of split-weaning at day 18 post parturition on liveweight (LW) and P2 backfat on days 1, 18 and 21 post-parturition, and LW and P2 change from day 1 to 18, and from day 18 to 21 post parturition.**

Parameter	Treatment	
	Control	SW 7
<i>N</i>	20	19
<b>Sow liveweight, kg</b>		
Day 1 post-parturition	279.4 ± 8.1	286.0 ± 8.3
Day 18 post-parturition	265.3 ± 7.5	273.0 ± 7.7
Day 21 post-parturition	264.2 ± 7.7	270.5 ± 8.0
<b>Sow liveweight change, kg</b>		
Days 1-18	-14.1 ± 2.3	-13.3 ± 2.4
Days 18-21	-1.1 ± 1.2	-2.5 ± 1.2
Days 1-21	-15.2 ± 3.0	-15.8 ± 3.1
<b>Sow backfat, mm</b>		
Day 1 post-parturition	25.1 ± 1.2	24.2 ± 1.2
Day 18 post-parturition	23.4 ± 1.4	22.2 ± 1.5
Day 21 post-parturition	23.6 ± 1.2	21.8 ± 1.3
<b>Sow backfat change, mm</b>		
Days 1-18	-1.7 ± 0.7	-2.0 ± 0.7
Days 18-21	0.2 ± 0.5	-0.3 ± 0.5
Days 1-21	-1.5 ± 0.8	-2.3 ± 0.8

### ***Litter characteristics***

On day 3 post parturition, total litter weight, average piglet weight and litter size did not differ between treatments (Table 21). At day 18 post parturition litter characteristics were similar between treatments, however, once SW 7 sows had their litter sizes reduced to 7 piglets by weaning the heaviest piglets, total litter weight and average piglet weight was significantly lower in SW 7 sows (Table 21). At day 21 post parturition, SW 7 sows still had lower total litter weight; however, the average weight of their piglets was not different from piglets suckled by control sows (Table 21). Piglet growth rate did not differ between treatments for any time period during the trial. On average, piglets gained 268 ± 5.0 g each day from three days of age until weaning at 21 days of age.

**Table 21 Litter size, total litter weight and average piglet weight on days 3 and 21 post parturition, and total litter weight and average piglet weights of pre and post split-weaning on day 18 post parturition.**

	Treatment	
	Control	SW 7
<b>Day 3 post-parturition</b>		
Litter weight, kg	23.0 ± 0.8	20.7 ± 0.8
# piglets	11.0 ± 0.1	11.0 ± 0.5
Average piglet weight, kg	2.1 ± 0.1	1.9 ± 0.1
<b>Day 18 post-parturition</b>		
Average piglet weight prior to split-weaning, kg	6.1 ± 0.2	6.0 ± 0.2
# piglets weaned	NA <sup>1</sup>	3.6 ± 0.1
Litter weight weaned, kg	NA <sup>1</sup>	24.7 ± 0.8
Average piglet weight weaned, kg	NA <sup>1</sup>	7.0 ± 0.2
Litter weight post split-weaning, kg	66.2 ± 1.7 <sup>x</sup>	38.5 ± 1.8 <sup>y</sup>
# piglets post split-weaning	10.8 ± 0.1 <sup>x</sup>	7.0 ± 0.0 <sup>y</sup>
Average piglet weight post split-weaning, kg	6.1 ± 0.2 <sup>a</sup>	5.5 ± 0.2 <sup>b</sup>
<b>Day of weaning, day 21 post-parturition</b>		
Litter weight, kg	75.0 ± 1.7 <sup>x</sup>	46.8 ± 1.8 <sup>y</sup>
# piglets	10.8 ± 0.1 <sup>x</sup>	7.0 ± 0.1 <sup>y</sup>
Average piglet weight, kg	6.9 ± 0.2	6.7 ± 0.2

<sup>a,b</sup> P<0.05; <sup>x,y</sup> P<0.01; <sup>1</sup>NA: not applicable. No piglets were weaned from control sows on day 18 of lactation.

### ***Ovarian follicle growth***

The average diameter of the three largest follicles across both ovaries was assessed by transrectal ultrasound at days 14, 18 and 20 post-parturition. There were no significant differences between groups at any time point, although average follicle diameter tended to be lower in the SW 7 sows at day 14 and day 20 post-parturition (Table 22). Follicle diameter change between days 14-18 did not differ between groups, but tended (P < 0.1) to be reduced in the SW 7 sows compared to control sows, between days 18-20 (Table 22).

**Table 22 Average diameter of the three largest follicles across both ovaries on days 14, 18 and 20 post parturition**

<i>n</i>	Treatment		P value
	Control	SW 7	
	20	19	
Average follicle diameter (mm)			
Day 14 post parturition	2.17 ± 0.08	2.02 ± 0.10	0.066
Day 18 post parturition	3.13 ± 0.10	3.13 ± 0.12	0.752
Day 20 post parturition	4.38 ± 0.22	3.57 ± 0.27	0.084
Day 14-18 change	1.05 ± 0.17	0.77 ± 0.23	0.274
Day 18-20 change	1.10 ± 0.18	0.45 ± 0.21	0.058

### ***Reproductive parameters***

At slaughter on day 21 ± 0 post parturition, there was no effect of treatment on ovarian weight; however, uterine weight was significantly heavier in SW 7 sows (Table 23). Of the presumed pre-ovulatory follicles, there was no difference in the average diameter of preovulatory follicles (larger than 4 mm), and no effect of treatment on the proportion of follicles across three size categories (4 - 5.9 mm, 6 - 7.9 mm, or > 8 mm) (Figure 5). However, independent of treatment, the greatest proportion of follicles were in the 4 - 5.9 mm category (0.53 ± 0.05) followed by the 6 - 7.9 mm category (0.30 ± 0.03) with the lowest proportion in the > 8 mm category (0.17 ± 0.03) (P < 0.05).

Split weaning did not affect any measure of oocyte quality. There were no differences between groups in embryo cleavage or blastocyst development rates following *in vitro* oocyte maturation and fertilisation, and no differences in total cell number in each of the blastocyst categories (Table 24). However, a greater proportion of oocytes from control sows had developed to the early blastocyst stage, when compared to oocytes from SW 7 sows (Table 24).

Furthermore, regression analyses, independent of treatment, found no relationship between, a) average follicle size and blastocyst development rates, or b) blastocyst development rates and blastocyst cell numbers (P > 0.05).

The effect of sow body weight loss on reproductive parameters was also assessed. Sow body weight loss during the first 21 days of lactation did not alter follicle characteristics, but did have a significant effect on blastocyst development rates *in vitro* (Table 25). Sows that lost more than 5%, 7% and 10% of their day one post-parturition body weight had less oocytes progress to blastocyst stage following fertilisations compared to sows that lost less than 5%, 7% and 10%.

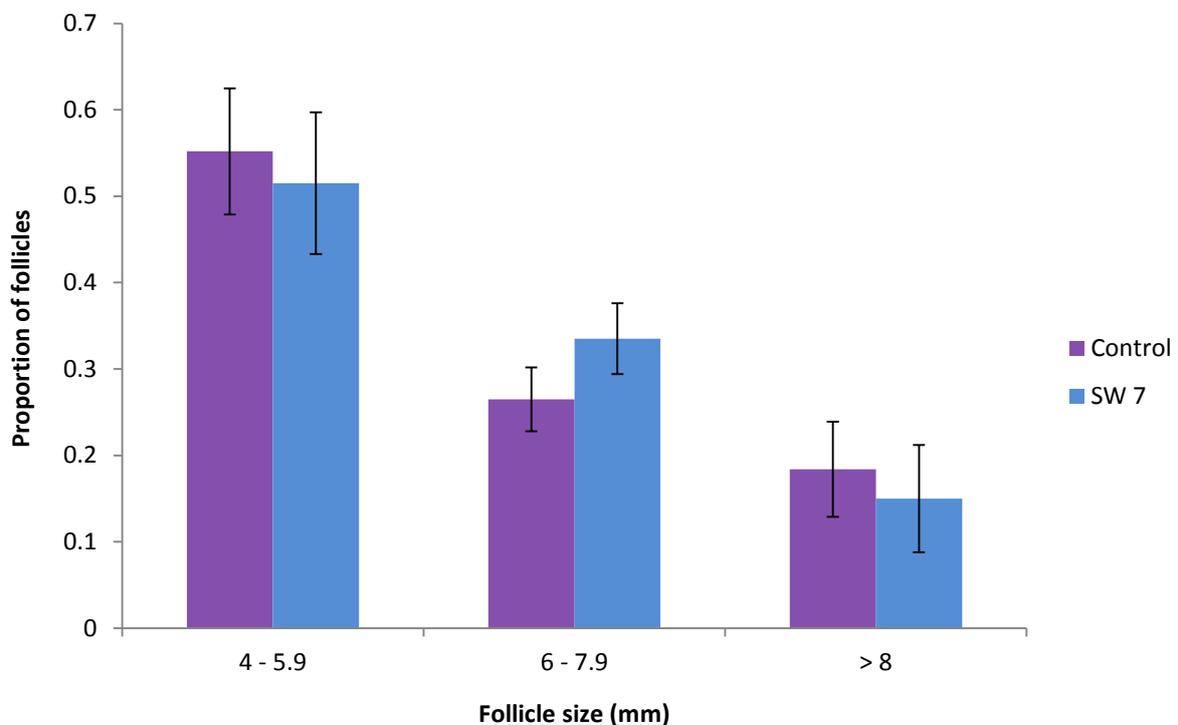
**Table 23 The effects of split-weaning on uterine and ovarian weight and ovarian follicle dynamics**

	Treatment	
	Control	SW 7
n	20	18 <sup>1</sup>
Uterine weight, g	798.8 ± 43.9 <sup>a</sup>	1005.9 ± 47.0 <sup>b</sup>
Average ovarian weight <sup>2</sup> , g	7.10 ± 0.42	7.55 ± 0.46
# follicles < 4 mm	34.16 ± 4.69	27.60 ± 5.37
# follicles 4 - 5.9 mm	17.04 ± 2.21	16.66 ± 2.41
# follicles 6 - 7.9 mm	7.79 ± 1.05	9.97 ± 1.15
# follicles > 8 mm	5.18 ± 1.59	4.31 ± 1.74
Total # follicles > 4 mm	30.03 ± 2.20	30.90 ± 2.40
Average follicle size > 4 mm	6.21 ± 0.27	6.16 ± 0.29

<sup>a,b</sup> P<0.05

<sup>1</sup> One SW 7 sow had ovulated at the time of collection and was consequently removed from the analysis

<sup>2</sup> Average weight of individual left and right ovaries



**Figure 5 The proportion of ovarian follicles distributed across three size categories in control and SW 7 sows at day 21 post parturition.**

**Table 24 Effect of split weaning on the *in vitro* development of oocytes collected on day 21 post parturition and embryo total cell counts**

	Treatment		P value
	Control	SW 7	
<i>n</i> COCs	297	332	
% cleaved <sup>1</sup>	68.1 ± 7.5	71.9 ± 8.6	0.808
% blastocysts / cleaved <sup>2</sup>	47.4 ± 4.9	54.5 ± 5.6	0.830
% blastocysts / total <sup>3</sup>	33.4 ± 3.1	37.7 ± 3.5	0.187
<b># blastocysts in each category</b>			
Early blastocyst	22	9	-
Blastocyst	25	35	-
Expanded blastocyst	48	75	-
Hatching blastocyst	2	4	-
Hatched blastocyst	1	0	-
<b>Proportion of blastocysts in each category</b>			
Early blastocyst	0.22	0.07	< 0.05
Blastocyst	0.26	0.28	> 0.05
Expanded blastocyst	0.49	0.61	> 0.05
Hatching blastocyst	0.02	0.03	> 0.05
Hatched blastocyst	0.01	0.00	> 0.05
<b>Average cell number</b>			
Blastocyst	30.1 ± 2.7	29.1 ± 2.4	0.102
Expanded blastocyst	44.2 ± 3.4	49.5 ± 3.7	0.186

<sup>1</sup>The percentage of COC's that progressed to 2-cell stage post fertilisation

<sup>2</sup>The percentage of cleaved zygotes that then progressed to blastocyst stage (all blastocyst categories included)

<sup>3</sup>The percentage of COC's that progressed to blastocyst stage (all blastocyst categories included)

**Table 25 Effect of sow percent body weight loss from day 1 to 21 of lactation on the number and size of follicles > 4mm, cleavage and blastocyst development rates *in vitro* on day 21 of lactation.**

	Lost > 5 % BW			Lost > 7 % BW			Lost > 10 % BW		
	Yes	No	P value	Yes	No	P value	Yes	No	P value
<i>n</i> sows	22	17		15	24		6	33	
# follicles <sup>1</sup>	30.9 ± 2.2	28.6 ± 2.6	0.828	30.8 ± 2.4	28.8 ± 2.0	0.419	29.1 ± 2.9	30.7 ± 1.4	0.917
Average follicle size <sup>1</sup>	6.2 ± 0.2	6.2 ± 0.3	0.507	6.0 ± 0.2	6.3 ± 0.2	0.968	6.2 ± 0.4	6.2 ± 0.2	0.647
% cleaved	69.7 ± 6.4	71.9 ± 7.2	0.066	73.5 ± 8.2	70.5 ± 6.8	0.505	67.4 ± 12.1	71.3 ± 5.7	0.269
% blastocyst / cleaved	46.0 ± 4.2	58.2 ± 4.8	0.138	38.4 ± 3.8	58.6 ± 3.2	0.004	24.7 ± 7.7	55.0 ± 3.6	0.014
% blastocyst / total	31.8 ± 3.8	42.2 ± 4.3	0.006	29.4 ± 4.3	41.5 ± 3.6	0.027	16.5 ± 7.5	39.2 ± 3.5	0.024

<sup>1</sup> Only for antral follicles > 4 mm

### ***Plasma and follicular fluid hormone concentrations***

Plasma insulin concentration was higher on day 3 post-parturition, when compared to day 18 (56.6 ± 5.63 and 33.3 ± 3.72 ng/mL respectively;  $P < 0.01$ ) when analysed irrespective of treatment. Plasma insulin concentrations on day 3 and 18 post parturition did not differ in between control and SW 7 sows (Table 26). Furthermore, irrespective of treatment, insulin concentration on either day was not related to sow liveweight, P2 backfat on day 1, 18 or 21 post parturition or changes in liveweight and P2 backfat ( $P > 0.05$ ).

Plasma progesterone concentration on day 18 post parturition was higher in SW 7 sows, compared to control sows. Plasma luteinising hormone (LH) on day 21 post parturition was unaffected by treatment (Table 26). At day 21 post-parturition, follicular fluid concentrations of LH and progesterone did not differ between treatments; however, the concentration of oestradiol in follicular fluid was increased in SW 7 sows (Table 26). Embryo cleavage and development rates were not related to plasma or follicular fluid levels of any of the hormones measured.

Irrespective of treatment, sows that lost more than seven percent ( $n = 15$ ) of their total body weight by day 21 of lactation had significantly lower plasma insulin concentration on day 3 post parturition (49.44 ± 7.61 ng/mL) than sows that gained weight or lost less than seven percent body weight ( $n = 24$ ) (64.93 ± 6.24 ng/mL) ( $P = 0.015$ ). Sows that lost more than seven percent of their body weight also had lower plasma LH on day 21 of lactation (1.50 ± 0.57 ng/mL) compared to all other sows (2.59 ± 0.47 ng/mL) ( $P = 0.043$ ). Plasma LH

also tended to be lower in sows that lost more than ten percent body weight (n = 6) ( $1.24 \pm 0.80$  ng/mL) compared to all other sows (n = 33) ( $2.34 \pm 0.40$  ng/mL) (P = 0.063).

**Table 26 Plasma insulin, progesterone and luteinising hormone (LH), and follicular fluid (FF) LH, oestradiol and progesterone concentrations<sup>1</sup>.**

	Treatment		P value
	Control	SW 7	
Day 3 post parturition			
Plasma insulin (uU/mL)	52.16 ± 8.90	63.81 ± 9.35	0.900
Day 18 post parturition			
Insulin (uU/mL)	29.00 ± 6.23	31.47 ± 6.17	0.607
Progesterone (ng/mL)	0.53 ± 0.04 <sup>a</sup>	0.93 ± 0.04 <sup>b</sup>	0.002
Day 21 post parturition			
Plasma luteinising hormone (ng/mL)	1.75 ± 0.16	2.72 ± 0.16	0.138
FF luteinising hormone (ng/mL)	4.63 ± 2.49	2.04 ± 2.81	0.613
FF oestradiol (ng/mL)	17.05 ± 2.06 <sup>a</sup>	22.39 ± 2.32 <sup>b</sup>	0.003
FF progesterone (ng/mL)	352.31 ± 50.92	583.41 ± 57.52	0.164

<sup>1</sup>P values presented are based on Log10 transformed data however means and SEM in table are untransformed concentrations.

#### Experiment Four: Effect of group housing during lactation and fenceline boar contact on lactation oestrus expression

The incidence of lactation oestrus expression was highest (0.81; P < 0.05) for sows housed in groups and receiving daily, fenceline boar exposure (Table 27). Boar exposure increased (P < 0.05) the incidence of lactation oestrus in crate housed sows (0.50) compared to non-boar exposed crate (0.00) and group housed sows (0.14) (Table 27). The timing of oestrus during lactation and post-weaned was unaffected by treatment. However, the mean interval from farrowing to oestrus was lowest for the boar-exposed group housed sows, followed by the boar-exposed crate housed sows, with the longest intervals observed in the two non-boar exposed treatments (Table 27). There was no effect of treatment on subsequent total born (Table 27). There was no effect of the treatment on sow liveweight on day 18 of lactation ( $265.9 \pm 3.20$  kg; P = 0.69) or weaning ( $268.7 \pm 3.00$  kg; P = 0.60), or weight change during lactation ( $1.88 \pm 1.84$  kg; P = 0.39).

**Table 27 Treatment effects on timing and expression of oestrus and subsequent litter size**

	Farrowing crate		Multi-suckle pens	
	No boar exposure	Boar exposure	No boar exposure	Boar exposure
Proportion sows in oestrus:				
During lactation	0.00 <sup>a</sup>	0.50 <sup>b</sup>	0.14 <sup>a</sup>	0.81 <sup>c</sup>
Post weaning	0.83 <sup>c</sup>	0.39 <sup>b</sup>	0.75 <sup>c</sup>	0.03 <sup>a</sup>
Total	0.83	0.89	0.89	0.84
Days from farrowing to oestrus:				
During lactation	-	25.4 ± 0.68	23.0 ± 0.91	24.5 ± 0.38
Post-weaning	32.7 ± 0.82	32.3 ± 1.20	34.0 ± 0.61	31.7 ± 1.84
All	32.7 ± 0.82 <sup>c</sup>	27.4 ± 1.11 <sup>b</sup>	32.2 ± 0.79 <sup>c</sup>	24.4 ± 0.79 <sup>a</sup>
Subsequent total born	11.3 ± 1.07	11.4 ± 1.34	11.8 ± 0.86	9.5 ± 0.79

<sup>abc</sup> within row indicates significant difference;  $P < 0.05$

The mean interval from farrowing to first oestrus was shorter ( $P < 0.05$ ) for sows expressing oestrus during as opposed to after lactation ( $23.5 \pm 0.45$  versus  $33.3 \pm 0.41$  days). Subsequent litter size tended ( $P = 0.09$ ) to be lower for sows mated during as opposed to after lactation ( $9.9 \pm 0.71$  versus  $11.6 \pm 0.65$  piglets total born). The interval from farrowing to first oestrus was similar for farrowing crate and group housed sows ( $30.0 \pm 1.01$  and  $28.3 \pm 0.71$  days;  $P = 0.18$ ). The proportion of sows expressing oestrus during lactation was higher in the group compared to farrowing crate housing (0.49 versus 0.25;  $P < 0.05$ ) and in the boar exposed compared to non-boar exposed treatments (0.70 versus 0.09;  $P < 0.05$ ).

Piglet liveweight change between day 18 and 28 of post-natal life was lower ( $P < 0.05$ ) for the multi-suckle boar exposure treatment compared to the two farrowing crate treatments (Table 28). Otherwise, there were no individual treatment effects on any other aspect of piglet performance or survival during lactation (Table 28). Sows expressing oestrus during lactation suckled a similar number of piglets to those which expressed oestrus post-weaning ( $10.07 \pm 0.30$  and  $9.66 \pm 0.25$  piglets), and produced a similar litter weight at weaning ( $80.53 \pm 3.01$  and  $76.31 \pm 2.49$  kg). Piglets reared in multi-suckle pens from day 18 to 28 of post-natal gained less ( $P < 0.05$ ) weight during that period ( $2.22 \pm 0.12$  versus  $2.86 \pm 0.17$  kg), and tended ( $P = 0.1$ ) to be lighter at weaning ( $8.01 \pm 0.17$  versus  $8.50 \pm 0.24$  kg). There was also a tendency ( $P = 0.09$ ) for more piglets to die when housed in multi-suckle pens compared to a farrowing crate ( $2.63 \pm 0.65$  versus  $0.64 \pm 0.93$ , piglets).

**Table 28 Treatment effects on litter size and weight, as well as piglet weight, mortality and weight change**

	Farrowing crate		Multi-suckle pens	
	No boar exposure	Boar exposure	No boar exposure	Boar exposure
Litter size, day 18	8.78 ± 0.45	10.61 ± 0.45	9.89 ± 0.32	9.89 ± 0.32
Litter size, day 28	8.78 ± 0.47	10.50 ± 0.47	9.57 ± 0.34	9.69 ± 0.33
Piglet mortality, %	0.00 ± 0.00	1.45 ± 1.35	3.26 ± 0.93	2.06 ± 0.92
Piglet weight (kg), day 18	5.47 ± 0.25	5.78 ± 0.25	5.70 ± 0.17	5.89 ± 0.17
Piglet weight (kg), day 28	8.23 ± 0.34	8.72 ± 0.35	8.13 ± 0.24	7.93 ± 0.23
Piglet weight change (kg)	2.76 ± 0.24 <sup>b</sup>	2.94 ± 0.24 <sup>b</sup>	2.43 ± 0.17 <sup>ab</sup>	2.03 ± 0.16 <sup>a</sup>
Litter weight (kg), day 18	51.97 ± 2.34	56.38 ± 2.37	55.68 ± 1.64	57.73 ± 1.61
Litter weight (kg), day 28	78.41 ± 3.50	83.70 ± 3.55	77.17 ± 2.45	75.98 ± 2.42

<sup>ab</sup> within row indicates significant difference; P < 0.05

## 4. Application of Research

The data from the first study demonstrated that daily full boar exposure is an effective stimulant of lactation oestrus in primiparous and multiparous sows within a commercial setting. Multiparous sows had a greater incidence of lactation oestrus, compared to primiparous sows, with oestrus expression further enhanced in both multiparous and primiparous sows when litter sizes were reduced to 7 piglets at about day 17 of lactation. The current data supports previous findings from this group where a combination of BE and split weaning was used to bring about a lactation oestrus in a high proportion of multiparous sows (Terry et al., 2013). Reducing the litter size to seven piglets also allowed daily piglet growth to be increased between day 17 and weaning. Reducing the litter sizes of multi- and primi-parous sows mated during lactation resulted in a decreased farrowing rate compared to sows mated post-weaning. Further, when parity and treatment were pooled, mating in lactation decreased the subsequent total born and born alive. Previously, mating in lactation after day 21 post-partum and weaning within 7 days of mating did not negatively affect the subsequent reproduction (Gaustad-Aas et al., 2004; Soede et al., 2012). However, although not significant, multi-parous sows which were split weaned and mated post-weaning had 2.1 more total born piglets at the subsequent litter than MP sows which had no litter reduction. These results may suggest that follicular growth could be improved in sows which are split weaned and mated post-weaning (van Leeuwen et al., 2012). Consequently, a reduction in litter size prior to complete weaning, by removing the larger piglets, represents a possible strategy to increase subsequent litter sizes of sows, and increase the weaning weights of lighter piglets.

It is evident from the second study that a high proportion of sows will ovulate prior to weaning in response to three days of sow and piglet separation, in conjunction with fence-line boar contact commencing on day 17 post-partum. This finding is supported by previous evidence (Gerritsen et al., 2008; Soede et al., 2012) that daily, extended periods of interrupted suckling which are continued through to weaning or insemination stimulated a high proportion of sows to ovulate whilst still lactating. However, to the best of our knowledge, this is one of the first studies to compare two durations of interrupted suckling and to resume 24 hour suckling after three days, and it is evident that 16 hours of sow and piglet separation was the most effective method of stimulating lactation oestrus. However, 16 hours of sow-piglet separation resulted in a lower farrowing rate and impaired piglet

growth compared to zero and seven hours of separation. The increased proportion of sows farrowing a litter in the seven hour separation regime, combined with the lack of a negative effect on piglet growth, indicate that this may be a more commercially attractive option. Interestingly, when comparing the productivity of the three protocols investigated, it is evident that the 7 hour separation protocol results in significantly higher productivity. When the number of piglets produced per 100 sows farrowing prior to the oestrus stimulation protocol was calculated, the 7 hour sow-piglet separation protocol increased the number of piglets produced by 185 compared to the conventional weaning protocol.

The data from study three indicate that split weaning on day 18 post-parturition did not improve the capacity of oocytes, collected on day 21 post-parturition, to be fertilized and develop to the blastocyst stage *in vitro*. However, reducing litter size to seven piglets on day 18 post parturition improved embryo survival to day 30 of gestation *in vivo*, when sows were mated on day 22.4 of lactation, compared to sows which suckled a full litter through to weaning or had their litter reduced to seven piglets at first detection of oestrus. Interestingly, split weaning either four days prior to lactation oestrus or coincident with lactation oestrus improved both pregnancy rates and embryo weight at day 30 of gestation. These results suggest that the improvements in subsequent litter size observed by Terry et al. (2013) in sows that were split weaned on day 18 post parturition and mated at 22.4 days post parturition, were due to an improved pre-mating as opposed to post-mating environment. However, split weaning at oestrus was still beneficial as pregnancy rates were improved and embryo weight was increased compared to sows that suckled a full litter for the duration of lactation. This indicates that mating in lactation and maintaining a large suckling litter post mating has negative impacts on the subsequent litter. Despite split-weaning having no significant effect on oocyte quality, a numerical increase in blastocyst development rates and expanded blastocyst cell number were observed in oocytes collected from split-weaned sows. It is, therefore possible that subtle improvements in oocyte development resulting from the reduction in suckling load pre-mating, may have been responsible for the improved embryo survival observed *in vivo*. Importantly, the current study also found that sows which lost more than five percent of their body weight over the course of lactation had reduced blastocyst development rates *in vitro*, when compared to sows that lost less than five percent body weight and/or gained weight in lactation. This finding confirms the importance of minimising lactation weight loss in order to maximise the output of sows mated during lactation. Considering weight losses in excess of 10% are normally required before the reproductive output of weaned, multiparous sows is impaired (Thacker and Bilkei, 2005), it might be suggested that the reproductive output of sows mated during lactation is more sensitive to lactation weight loss.

The data from the final study provided some interesting insights into the impact of group-lactation housing on lactation oestrus expression and piglet performance. Overall, it is clear that housing sows in groups for the last ten days of a 28 day lactation does not markedly increase the incidence of lactation oestrus. However, providing group-housed sows with twenty minutes of fenceline contact with a boar while in their home pen resulted in a lactation oestrus rate of 81%. Interestingly, and consistent with the data from the first study mating sows in lactation did appear to result in lower subsequent litter sizes compared to animals mated post-weaning. Further, while lactation oestrus expression and boar exposure did not affect the performance of the suckling piglets, group housing of sows and litters resulted in reduced liveweight gain and a tendency for greater piglet mortality during this period of group housing in late lactation.

When taken together, it is evident that sow-piglet separation, full physical boar exposure in a detection mating area and fenceline boar exposure to group housed sows, can result in a high proportion of sows expressing oestrus and ovulating during lactation and conceiving

when mated during lactation. It is also clear that a permanent reduction in suckled litter size increases the capacity of sows to ovulate during lactation, and positively effects both embryo survival and development. It is also evident that while three days of seven hours of sow-piglet separation only resulted in 50% of sows ovulating during lactation, it was shown to provide significant improvements to reproductive output (litter size and piglets born/100 sows) when the performance of the sows mated post-weaning was also considered. Similarly, a permanent reduction in suckled litter size prior to complete weaning improved the reproductive output of sows mated post-weaning. Based on the current data it is suggested that strategies which reliably stimulate lactation oestrus may also improve the performance of those sows which do not ovulate until after weaning. It is likely, that this reflects positive effects on the metabolic status of the sow as well as an increase in positive inputs (boar pheromones) into the hypothalamic-pituitary axis, which improve ovarian follicle growth, and possibly oocyte quality, prior to weaning.

## 5. Conclusion

In conclusion, the data from the four studies presented here demonstrates the following key points:

- Full boar exposure is an effective stimulus of lactation oestrus
- Reducing the suckling load increased the incidence of lactation oestrus
- First lactation sows are less likely to express oestrus during lactation than multiparous sows
- A reduction in suckled litter size prior to mating will increase embryo survival
- A reduction in suckled litter post-mating may also benefit embryonic development
- Three days of sow-piglet separation for seven hours has the potential to increase the productivity of the breeding herd by increasing the number of piglets produced per 100 sows and increasing subsequent litter size.
- Three days of sow-piglet separation for 16 hours was effective at stimulating lactation oestrus, but resulted in very low pregnancy rates.
- Group housing sows from day 18 of lactation in combination with fenceline boar exposure is an effective method of stimulating lactation oestrus.
- Group lactation housing systems can increase piglet deaths and reduce piglet liveweight gains

## 6. Limitations/Risks

While the efficacy of the various strategies to stimulate lactation oestrus is evident, the cost-benefit analysis needs to be conducted before commercial piggeries can really evaluate their benefit. Specifically, the increased labour needs to be offset against the reduced farrowing to farrowing intervals and the potential improvements in piglet performance post-weaning which may arise from extended lactation.

## 7. Recommendations

As a result of the outcomes in this study the following recommendations have been made:

- Conducting full, physical boar exposure in a detection mating area from day 18 of lactation onwards is an effective method of stimulating farrowing crate housed sows to ovulate during lactation
- If sows are housed in groups during lactation, fenceline boar exposure can be used to stimulate a high proportion of sows to ovulate prior to weaning
- Separating sows and piglets for seven hours for three days during lactation (day 17 to 20), combined with physical nose to nose contact with a mature boar in the farrowing shed, may be an effective method of increase the productivity of the sow herd
- A reduction in litter size prior to complete weaning appears to be an effective method of improving the reproductive performance of sows mated post-weaning

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## **9. Appendix 1 - Notes**

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## **10. Appendices**

### ***Appendix 1:***