Determining the effects of season on timing of ovulation and luteal function

5A-103

Report prepared for the Co-operative Research Centre for High Integrity Australian Pork

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June 2013

An Australian Government Initiative

Established and supported under the Australian Government's Cooperative Research Centres Program

Executive Summary

During summer and early autumn it is common for domestic sows to experience a depression in fertility (seasonal infertility), which is frequently manifested a reduction in farrowing rates and litter size. Seasonal infertility is caused primarily by the reduction in melatonin secretion that occurs during long photoperiods. This reduction in melatonin alters the pattern of luteinizing hormone release, thus impairing pre-ovulatory maturation of the ovarian follicle – oocyte complex, and possibly altering formation, development and functionality of the corpora lutea (CL). Alterations in maturation of the follicle-oocyte can impair the capacity of ovulated oocytes to develop into viable embryos, whilst impaired CL function will also reduce embryo survival and pregnancy maintenance.

Previously, we have demonstrated that post-ovulatory progesterone (P4) profiles differ between summer and winter. Specifically, comparisons of progesterone levels for pregnant sows mated during summer and winter were compared, with P4 levels significantly higher on day 3 and 7 during summer compared to winter. Previous work has also indicated a reduction in the developmental competence of oocytes collected from weaned sows during summer compared to winter. The aims of the current project were to extend these data by conducting two studies. Study one, determined the effect of season (summer / early autumn versus winter) and moderate nutritional restriction on the developmental competence of peri-ovulatory oocytes collected from cycling gilts. The effect of moderate nutritional restriction was included in the trial to mimic the effect of elevated temperatures on feed intake in group housed gilts. Study two, determined the effect of season (summer / autumn versus winter) on the timing of ovulation and peri-ovulatory profiles of LH and P4 in weaned sows. The latter was achieved through the collection of blood samples and detection of oestrus behaviour at 6 hourly intervals, as well as regular ultrasound imaging of the ovary.

The first study demonstrated no effect of season or reducing feed intake from 2.5 to 1.5 times maintenance on oocyte developmental competence (as measured by capacity to reach metaphase II). However, the dynamics of the peri-ovulatory follicle pool did differ between summer/autumn and winter, with fewer follicles growing beyond 6 mm in summer. Interestingly, no effect of moderate nutritional restriction on oocyte competence was observed. When combined with previous studies, this finding suggests a severe restriction in nutrition (feeding at maintenance levels) must occur before the ability of oocytes to reach metaphase II is impaired.

The second study provided some interesting data regarding the effect of season on LH release around ovulation, the timing of ovulation and the pattern of P4 secretion post-ovulation. Specifically, basal LH was lower in summer compared to winter, confirming the negative effect of season on LH release; however the peak of the LH surge and the relative rise in LH was significantly higher in summer compared to winter. Together, these data suggest that while basal LH secretion is reduced during summer, the LH production by the pituitary is no reduced, resulting in a greater release in response to the pre-ovulatory surge of gonadotrophin releasing hormone (GnRH). Similar to our previous data, P4 levels were significantly higher in summer compared to winter, with levels increasing more rapidly from 72 hours post-oestrus detection onwards. However, from a commercial point of view the major outcome from this study was the earlier ovulation relative to oestrus detection that was observed in summer compared to winter. Specifically, sows weaned during summer ovulated approximately 10 hours earlier during oestrus than those weaned in winter, resulting in alterations in the interval from insemination to ovulation. Specifically, assuming oestrus detection occurs every 24 hours, and sows are inseminated at first detection of oestrus and again 24 hours later, the first insemination would occur 18 hours prior to ovulation, with the second one occurring after ovulation. It could, therefore, be suggested that this alteration in the timing of ovulation and hence inseminations occurring outside the optimal period relative to ovulation (i.e. 0 – 12 hours pre-ovulation) could be partly responsible for the reduced fertility and fecundity of sows mated during summer.

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1. Introduction

It is common during summer and early autumn for domestic sows to experience a depression in fertility (seasonal infertility), which is frequently manifested as a reduction in farrowing rates and litter size. Alterations in day length, mediated by changing levels of melatonin secretion, are ultimately responsible for seasonal infertility (Peltoniemi et al., 2000). Specifically, melatonin production is reduced during exposure to long photoperiods (i.e. during the summer months), essentially increasing the negative feedback effects of oestrogen on the GnRH pulse generator. In this way, the pattern of luteinizing hormone (LH) release is altered, thus impairing pre-ovulatory maturation of the follicle-oocyte complex, and possibly altering formation, development and functionality of the corpora lutea (CL). Further, the negative impact of reduced melatonin on activity of the hypothalamic-pituitary-ovarian axis appear to be exacerbated by exposure to elevated ambient temperature (heat stress), either directly via elevations in body temperature, or indirectly via reductions in feed intake and thus altered metabolic status.

Towards identifying seasonal differences in the timing and nature of pregnancy loss and CL function, blood samples collected from sows every 4 days from day 3 to day 31 post-mating (111 and 113 sows in summer and winter) were assayed for progesterone (Pork CRC project 2D-110). To our knowledge, this is the first study conducted in Australia characterizing progesterone profiles during the first 31 days post-mating. A number of key findings were obtained from this study. The first key finding relates to accurate detection of mating outcomes and the timing of pregnancy loss, with enormous discrepancies found to exist between actual pregnancy outcomes based on progesterone profiles compared to transcutaneous ultrasound. Specifically, a considerable proportion of animals were falsely identified as pregnant by ultrasound on day 35, demonstrating that progesterone profiles are a more accurate method of determining incidences and the nature of pregnancy failures. The current findings, demonstrate that early pregnancy loss remains the primary cause of reduced farrowing rates during summer, with early disruption of pregnancy (i.e. pregnancy loss after the 1st signal for maternal recognition of pregnancy) associated with increased concentrations of plasma homocysteine and alterations in insulin-like growth factor-I (IGF-I) and IGF binding proteins. These data provide preliminary evidence that alterations in metabolism or nutritional intake may be involved in early disruption of pregnancy, most likely via alterations in oocyte developmental competence or altered CL function.

The second key finding was that samples collected from pregnant animals demonstrate that progesterone levels are significantly higher ($P < 0.05$) on days 3 and 7 for summer ($n = 88$) compared to winter ($n = 95$) mated sows, equating to a difference of 162% and 140% on day 3 and 7 respectively. Further, on days 15, 19 and 23 post-mating, plasma progesterone levels were significantly lower for summer compared to winter mated sows. A premature rise in progesterone after mating can be attributed to alterations in the timing of follicle luteinisation relative to the pre-ovulatory LH surge, or an earlier ovulation relative to the onset of behavioural oestrus. In cattle, heat stress stimulates premature luteinisation of follicle cells in vitro (Bridges et al., 2005). Studies in pigs, demonstrate that alterations in the timing of the rise in progesterone can impair early embryo development and implantation due to alterations in the uterine environment (van Wettere and Hughes, 2007). Equally important, an earlier ovulation relative to detection of behavioural oestrus may result in inappropriate timing of artificial insemination relative to ovulation, requiring AI practices to be altered in summer. The interval from AI to ovulation has been shown to influence embryo development, and therefore, embryo mortality and litter size. Therefore, inappropriate timing of AI relative to ovulation could explain the increased incidences of conception failure and lower litter sizes typical of seasonal infertility.

Based on the literature and our recent data, two studies were conducted. Study one determined the effect of moderate dietary restriction during the second oestrous cycle on the developmental competence of peri-ovulatory oocytes collected from gilts. The second

study investigated the effect of season (summer versus winter) on the timing of ovulation and peri-ovulatory rise in progesterone in weaned sows.

2. Methodology

Experiment One: The effect of moderate feed restriction and season on oocyte quality

All animal procedures were conducted at the University of Adelaide piggery, Roseworthy, South Australia, with the approval from the Animal Ethics Committee of the University of Adelaide. The experimental design was a 2x2 factorial comparing the main effects of season and feed intake on oocyte developmental competence in cycling gilts. The two seasonal blocks were SUMMER; S (15th February to 10th March) versus WINTER; W (7th July to 4th August) and two feeding levels (2.5 times maintenance (HIGH) which was the control, versus a moderate restriction diet of 1.5 times maintenance (MOD). Throughout the trial period, from selection until slaughter gilts were fed the same commercial diet containing 16% crude protein and 13.0 MJ DE (Table 1). Maintenance feed intake was calculated as $0.444^{LW, 0.75}$ (Feeding Standards for Australian Livestock Pigs).

Animal housing and management

Eighty Large White x Landrace terminal sire line gilts (n = 40 gilts/ season) were stimulated to attain puberty at 178 days (107.2 ± 1.0 kg) using a single intra-muscular injection of PG600 (400 IU pregnant mare serum gonadotrophin and 200 IU human chorionic gonadotrophin (Intervet Australia PTY LTD)) and fifteen minutes of daily, full physical contact with a mature boar (> 11 months of age). Puberty occurred at 182.4 ± 3.7 days of age, with oestrus defined as the exhibition of a standing reflex, either in response to the manual application of pressure to the gilt's back (back pressure test), or mounting by the boar as described by Bartlett *et al*. (2009).

Following puberty attainment, gilts were allocated into their treatment groups based on their liveweight (LW) and predicted day of second oestrus to attain similar mean LW and predicted slaughter days between the treatment groups. Until their second oestrus, gilts were group housed and had *ad libitum* access to food and water.

From the onset of their second oestrus, gilts were individually housed and fed their experimental diets at either HIGH (mean 3.3 kg/day) or MOD (mean 1.87 kg/day) feeding levels from day one until day nineteen post second oestrus (Day 0 was determined as the first expression of behavioural oestrus). Gilts received fifteen minutes fence line boar exposure daily during the trial period. All gilts were weighed on day 0, 7 and 14 to monitor changes in LW and adjust experimental feed intake accordingly and on day 19 prior to slaughter.

Table 1 - Main ingredients and chemical analysis of commercial diet fed to gilts

Ingredients	%	Chemical Analysis	
Barley	15.00	Dry Material (%)	90.26
Wheat	41.49	Digestible energy (MJ)	12.99
Millrun	20.00	Protein (%)	16.00
Peas	7.40	Fat (%)	3.60
Canola expeller	8.40	Fibre (%)	5.10
Meat meal	3.13	Calcium (%)	0.85
Tallow	0.50		
Limestone	1.25		

Ingredients	%	Chemical Analysis
Sodium betonite	1.10	
Sodium chloride	0.20	
Lysine sulphate	0.45	
Theronine	0.11	
Avizyme 2100	0.02	
Betaine Liquid	0.20	
Lienerchrom 400 PMX	0.05	
HP Grower PMX	0.15	
Biofix Select	0.05	

Maintenance feed intake ($0.444^{LW, 0.75}$) calculated using "Feeding Standards for Australian Livestock Pigs"

Ovaries and reproductive tract collection

Nineteen days post second oestrus gilts were slaughtered at a commercial abattoir with reproductive tracts collected within 20 minutes post-exsanguination. Ovaries were separated from the uterine horns, placed into 50 mL tubes (Falcon) containing 35 mL phosphate buffered saline (PBS) and maintained at 34ºC during transport to the laboratory. The uterus was subsequently trimmed of mesentery and weighed.

Collection of follicle cells and cumulus-oocyte complex (COC)

At the laboratory, ovaries were washed with PBS (34ºC) and maintained at 37ºC until processed. Individual ovaries were weighed and the diameter of all surface antral follicles greater than 1 mm in diameter were individually measured and recorded within size categories (small: < 4 mm; medium: 4 mm to 5.99 mm; and large: ≥ 6 mm). The follicular contents were aspirated from all follicles ≥ 4mm on both ovaries, into a 15 mL tube (Falcon) using an 18-gauge needle and a vacuum pump (Cook Australia) with a pressure equivalent to 25 mm Hg. The follicular contents from both ovaries were pooled and processed for each individual animal.

Following aspiration, cumulus-oocyte complexes (COCs) were recovered from the aspirate using a dissecting microscope (40 x magnification) and washed 3 times in sodium bicarbonate-buffered TCM-199 supplemented with follicle-stimulating hormone (FSH) (5 µg mL^{-1}), luteinising hormone (LH) (5 µg mL^{-1}), cysteamine (100 µM), epidermal growth factor (10 ng mL^{-1}), oestradiol (1 µg mL^{-1}), 100 µg mL^{-1} streptomycin, 100 IU mL^{-1} penicillin G and 20% v/v sow follicular fluid (maturation media (IVM)). Sow follicular fluid used in the IVM medium was harvested in bulk from culled sow ovaries sourced from a local abattoir. The sow follicular fluid was collected from antral follicles ≥ 3 mm, pooled prior to aliquot freezing then stored frozen at -80ºC. The COCs were placed in 4 well dishes (Nunc) (maximum 25 COCs/well) containing 600 µL of maturation medium under 300 µL of mineral oil and incubated for 42-44 hours at 38.6ºC under humidified 5% CO_2 in air.

Upon removal of the COCs, the remaining follicular contents were centrifuged for 5 minutes at 2000 rpm. The supernatant (follicular fluid) was separated from the debris and stored frozen in 1.5 mL eppendorf tubes at -20ºC until analysis.

Oocyte developmental competence

After maturation, the cumulus cells were removed from the COC using hyaluronidase and a fine bore pipette. The oocytes were stained with Hoescht 33342 and nuclear maturation status was assessed using a fluorescent microscope. Oocytes were classified as either

germinal vesicle (GV), germinal vesicle breakdown (GVBD), metaphase I (MI) or metaphase II (MII) as described by Kikuchi *et al*. (2008).

Hormone analysis

Follicular fluid analysis was conducted by the Adelaide Research Assay and Services, University of Adelaide.

Oestradiol

Oestradiol was determined in the Adelaide Research Assay Facility by double antibody radioimmunoassay in duplicate (DSL4800; Beckman Coulter, Brea, CA, USA) after diluting samples 100 fold with PBS, according to the manufacturer's instructions.

Progesterone

Progesterone was determined by coated tube radioimmunoassay (IM1188; Beckman Coulter, Brea, CA, USA) in duplicate after diluting samples 100 fold with PBS, according to manufacturer's instructions.

Luteinizing Hormone

Luteinizing hormone (LH) was assayed by double antibody RIA 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. 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, 100ul sample (either neat or diluted 10 fold with PBS) or standard was incubated with 400ul antibody for 24 hours, followed by the addition of 100ul 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; 50ul) was added, the tubes vortexed and left at 4C for 20 minutes. One millilitre of ice cold water was added and the tubes immediately centrifuged at 4000rpm for 20 minutes. The supernatant was aspirated, the radioactivity determined in a gamma counter and levels of LH calculated by interpolation from the standard curve.

Statistical Analysis

All values in text are expressed as mean with pooled SEM, unless otherwise stated. Using a general linear model, a two-way analysis of variance (ANOVA) was used to examine differences between the main effects and interaction between both. Season and dietary differences were examined on trial LW; average daily liveweight gain (ADG calculated by the change in LW over the trial period); oestrous cycle length (measured by the days from first to second oestrus); absolute uterine and ovary weight; relative uterine and ovary weight (% liveweight); and follicular fluid hormone concentrations of E2, P4 and LH. The number of total visible follicles, the proportion of follicles within each size category and the proportion of fixed oocytes at different nuclear maturation stages were also tested. All proportions were transformed by arcsine square root prior to analysis. Differences were examined using least significant difference, with significance set at the 0.05 level ($P < 0.05$). All statistical analyses were conducted using IBM SPSS version 19.

Experiment Two: The effect of season on the timing of ovulation and peri-ovulatory hormone profiles of weaned sows

This study was conducted on a 5 weekly batch farrowing facility using five batches of weaned sows. Two of these were summer batches, sows weaned on the 19th of January and 22nd of February 2012), and three were winter batches, sows weaned on 12th June, 17th July and 1st September 2012.

On the day after weaning, ear vein catheters were inserted non-surgically into all sows, with blood samples collected every 6 hours (7 am, 1 pm, 7 pm and 1 am) from day 3 post-weaning until the end of oestrus and every 12 hours (7 am and 7 pm) from the end of oestrus until 168 hours after first detection of oestrus. Commencing on day 3 post-weaning

at 7 am, sows received 10 minutes of fence line contact with two mature boars in a detection mating immediately after collection of blood samples until oestrus behaviour was no longer observed. The onset of oestrus was defined as the first exhibition of a standing reflex in response to boar stimulation, minus 3 hours (half way between time at which standing reflex was observed and the time at which it was not observed). The end of oestrus was defined as the first time point at which oestrus behaviour was no longer observed minus 3 hours. Sows were artificially inseminated 12 hours after first detection of oestrus and again every 24 hours until oestrus behaviour was no longer observed. In addition, trans-rectal ultrasound was conducted every 12 hours after first detection of oestrus until oestrus behaviour was no longer observed. The size of the four largest follicles present were measured (to the nearest 0.1 mm), with the occurrence of ovulation defined as the time when no follicles greater than 4 mm were observed on either ovary minus 3 hours (half the time to the former scan; as described by Gerritsen et al., 2008).

Only hormone profiles from sows maintaining their pregnancy and farrowing a litter were included in the analysis, equating to hormone profiles from 24 sows in summer and 17 sows in winter. Samples collected from 7 am on day 3 post-weaning until the end of oestrus were assayed for luteinizing hormone (LH), with samples collected from the 6 hours prior to oestrus detection until 168 hours (7 days) post-first oestrus detection assayed for progesterone (P4).

Hormone analysis

Progesterone

Progesterone was determined by coated tube radioimmunoassay (IM1188; Beckman Coulter, Brea, CA, USA) in duplicate after diluting samples 100 fold with PBS, according to manufacturer's instructions.

Luteinizing Hormone

Luteinizing hormone (LH) was assayed by double antibody RIA 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. 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, 100ul sample (either neat or diluted 10 fold with PBS) or standard was incubated with 400ul antibody for 24 hours, followed by the addition of 100ul 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; 50ul) was added, the tubes vortexed and left at 4C for 20 minutes. One millilitre of ice cold water was added and the tubes immediately centrifuged at 4000rpm for 20 minutes. The supernatant was aspirated, the radioactivity determined in a gamma counter and levels of LH calculated by interpolation from the standard curve.

Statistical Analysis

All values in text are expressed as mean with pooled SEM, unless otherwise stated. Using a general linear model, a two-way analysis of variance (ANOVA) (unbalanced design) was used to determine the effect of season on all reproductive measures recorded. Differences were examined using least significant difference, with significance set at the 0.05 level ($P < 0.05$). All analysis was done using Genstat, 10th Edition (Rothamsted Experimental Station, Harpenden). Probability values < 0.05 were described as significant.

3. Outcomes

Experiment One: The effect of moderate feed restriction and season on oocyte quality

Of the 80 gilts that were allocated into this trial, 19 were removed as a result of ill health or failure to attain first or second oestrus. Of the 40 gilts allocated to the Summer block, 7 did not attain pubertal oestrus seven days post PG600. Of the 33 remaining, 4 did not

attain second oestrus, resulting in a final number of 29 gilts on trial feed during summer (n = HIGH: 14, MOD: 15). Of the 40 gilts allocated to the Winter block, 7 did not attain pubertal oestrus within seven days post PG600, 1 gilt was sick and removed from the trial, leaving a final number of 32 gilts on trial feed during winter (n = HIGH: 16, MOD: 16).

Growth characteristics

Prior to dietary treatment (LW d0) the starting weights for each of the seasonal and dietary treatment groups were similar (Table 2). At slaughter (d19) there was no difference in LW between the seasonal blocks (P > 0.05); however, MOD gilts were lighter (P = 0.004) and had a lower ADG than the HIGH gilts (P = 0.001, Table 2).

Table 2 - Main effect of season and diet on day 0 and 19 liveweight (LW), average daily liveweight gain (ADG). Data expressed as mean with pooled SEM.

	Season				Diet			
	Summer	Winter	Pooled SEM	*P* Value	High	Mod	Pooled SEM	*P* Value
n	29	32			30	31		
LW d0 (kg)	125	127	1.3	0.314	126	126	1.3	0.757
LW d19 (kg)	138	134	3.6	0.130	141	131	3.3	<0.01
ADG (kg)	0.68	0.39	0.4	<0.01	0.81	0.25	0.4	<0.01

* indicates values across rows differ significantly (p < 0.05)

Reproductive Characteristics

All gilts were considered to be cycling based on the presence of visible corpora albicantia on the surface of the ovaries.

Ovaries, Uterus and Oestrus Cycle Length

MOD gilts had lighter ovaries than the HIGH gilts (P = 0.003) and gilts in summer had lower relative ovarian weight than those in winter (P = 0.004). There were no diet or season or interaction differences for the absolute and relative uterine weights (P > 0.05) or for the number of days between the first and second oestrus periods between the gilts (P > 0.05, Table 3).

Table 3 - Main effects of diet and season on oestrus cycle length, absolute and relative uterine and ovary weight. Data represented as mean with pooled SEM.

	Season				Diet			
	Summer	Winter	Pooled SEM	*P* Value	High	Mod	Pooled SEM	*P* Value
Oestrus cycle length (days)	21.9	21.4	0.29	0.833	21.6	21.3	0.28	0.283
Uterine wt (g)	675.2	699.1	33.08	0.613	709.9	667.3	32.30	0.254
Relative uterine wt (%, LW)	0.0049	0.0053	0.0002	0.772	0.0050	0.0051	0.0002	0.268
Ovarian wt (g)	14.0	15.0	0.50	0.003*	15.6	13.5	0.45	0.184

* indicates values across rows differ significantly (p < 0.05)

Ovarian Follicle Population

Gilts in summer had a higher proportion of medium antral follicles (0.22 ± 0.03 versus 0.13 ± 0.01, P = 0.006) and a lower proportion of large antral follicles (0.23 ± 0.03 versus 0.38 ± 0.03, P = 0.001) compared to gilts in winter. There were no diet or interaction effects for

the number or proportion of medium and large antral follicles (P > 0.05) or on the total number of follicles on the ovary, the number and the proportion of small follicles or between the mean size and the size range of total aspirated follicles (P > 0.05, Table 4).

Table 4 - Main effects of diet and season on the total follicle number, number and proportion of aspirated follicles (≥4 mm), the mean aspirated follicle diameter and range of aspirated follicle size, total follicle population and proportion of follicles within each size cohort. Data represented as mean with pooled SEM.

	Season				Diet			
	Summer	Winter	Pooled SEM	*P* Value	High	Mod	Pooled SEM	*P* Value
Total follicles/gilt (*n*)	58.9	51.4	4.3	0.607	56.6	53.4	4.4	0.240
Total aspirated follicles (≥4 mm)	24.0	22.8	1.2	0.194	24.5	22.3	1.2	0.454
Proportion of total follicles aspirated (≥4 mm)	0.45	0.51	0.03	0.745	0.49	0.48	0.03	0.188
Aspirated Follicles (mm)								
Mean diameter	6.04	6.29	1.8	0.706	6.12	6.22	1.5	0.352
Min	4.37	4.32		0.712	4.35	4.32		0.623
Max	7.88	8.33		0.637	8.05	8.19		0.201
Follicle Size Population (*n*)								
Small (<4 mm)	34.8	28.6	3.7	0.294	32.2	31.0	3.7	0.864
Medium (≥ 4 -5.99 mm)	13.3	7.3	1.2	<0.01	10.7	9.5	1.2	0.606
Large (≥6 mm)	10.8	15.5	0.9	<0.01	13.8	12.7	1.1	0.453

Oocyte Nuclear Maturation

One gilt from the HIGH group in summer had ovulated prior to oocyte collection and therefore no oocyte data was collected from this animal. The number of aspirated oocytes fixed was greater in summer compared to winter (P = 0.04, Table 5). Gilts in summer had a lower proportion of oocytes at GV (0.06 ± 0.02) following *in vitro* maturation compared to gilts in winter (0.08 ± 0.01, P = 0.026). The proportion of oocytes at the other meiotic stages (GVBD, MI or MII) was unaffected by season, diet or the interaction between the two (P > 0.05).

Table 5 - Main effects of season and diet on the nuclear maturation status. Germinal vesicle (GV); germinal vesicle breakdown (GVBD); metaphase I (MI); metaphase II (MII). Data represented as mean proportion with pooled SEM.

	Season				Diet			
	Summer	Winter	Pooled SEM	P Value	High	Mod	Pooled SEM	P Value
Fixed Oocytes (*n*)	529	437		0.197	522	444		0.04
GV	0.06	0.08	0.02	0.482	0.07	0.08	0.02	0.026*
GVBD	0.12	0.14	0.03	0.631	0.15	0.13	0.03	0.540

	Season				Diet			
	Summer	Winter	Pooled SEM	P Value	High	Mod	Pooled SEM	P Value
MI	0.10	0.09	0.02	0.739	0.09	0.10	0.01	0.382
MII	0.72	0.69	0.04	0.997	0.69	0.69	0.04	0.904

* indicates values across rows differ significantly (P < 0.05)

Hormone Analysis

Seasonal differences were found for the concentrations of LH and E2 (P <0.001, Table 6). Gilts in summer had a higher concentration of LH and a lower concentration of E2 compared to gilts in winter. There was no season or dietary effect on P4 concentration (P > 0.05); however, there was a significant interaction between season and diet on P4 concentration (P = 0.035, Table 6). The P4:E2 ratio was greater in winter gilts compared to summer gilts (P< 0.001). There were no differences found between diet and interaction on the P4:E2 ratio.

Table 6 - Main effects and treatment effects on reproductive hormones luteinizing hormones (LH), progesterone (P4) and oestradiol (E2) in gilt pooled follicular fluid from aspirated follicles ≥ 4mm. Data expressed as mean concentration with pooled SEM.

	Season				Diet				Treatment$_1$					
	Summer	Winter	Pooled SEM	*P Value*	High	Mod	Pooled SEM	*P Value*	SH	SM	WH	WM	Pooled SEM	*P Value*
P4 (ng/ml^{-1})	220.3	162.8	36.77	0.265	184.9	196.2	37.12	0.912	274.4^{a}	169.7^{ab}	106.7^{b}	222.6^{ab}	49.31	0.035*
E2 (pg/ml^{-1})	4.2	27.7	3.44	<0.001*	16.1	16.1	4.14	0.948	7.4	1.2	24.3	31.1	4.42	0.197
LH (ng/ml^{-1})	40.3	11.6	4.02	<0.001*	24.7	29.2	4.92	0.385	39.9	40.6	6.9	16.0	5.49	0.449
E2:P4	0.08	0.37	0.070	<0.001*	0.31	0.14	0.02	0.405	0.15	0.02	0.46	0.27	0.09	0.392

* indicates values across rows differ significantly (P < 0.05)
[a,b]Values with different superscripts differ significantly within treatment groups (P < 0.05)-maybe across treatment groups or within a row
1 Summer High (SH); Summer Moderate (SM); Winter High (WH); Winter Moderate (WM)

Experiment Two: The effect of season on the timing of ovulation and peri-ovulatory hormone profiles of weaned sows

Progesterone profiles were significantly different in summer compared to winter (Figure 1), with concentrations rising more rapidly after 72 hours post-first detection of oestrus during summer. When averaged over each 24 hour period following ovulation P4 levels were higher ($P < 0.01$) on days 3 to 7 for sows weaned in summer compared to winter (Figure 2).

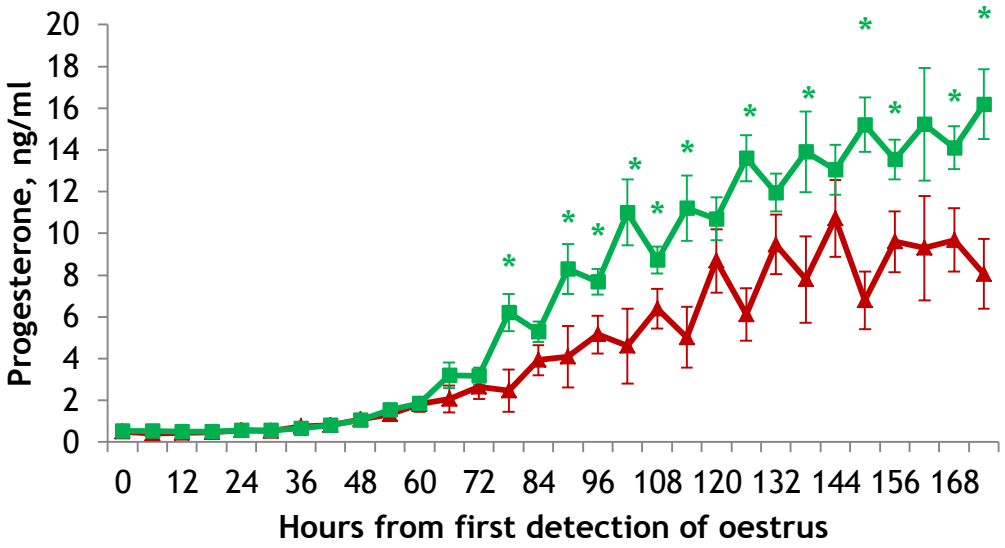

Figure 1 - Post-oestrus detection progesterone profiles for sows weaned and ovulating during summer (■) and winter (▲) (where 0 = 6 hours prior to first observation of a standing reflex in response to boar stimulation). * indicate significant differences between sum r and winter; $P < 0.05$.

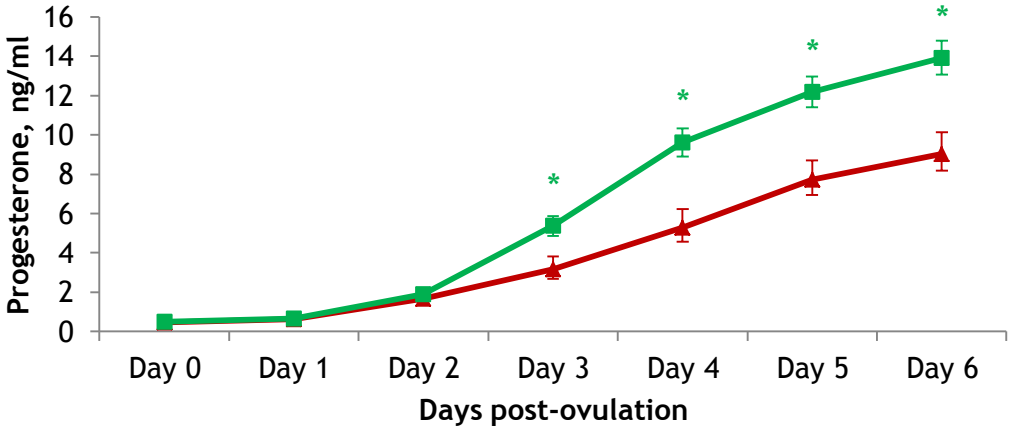

Figure 2 - Post-ovulatory progesterone profiles for sows weaned and ovulating during summer (■) and winter (▲) (where day 0 = first 24 hours after completion of ovulation) * indicate significant differences between summer and winter; $P < 0.05$.

The effects of season, summer versus winter, on LH profiles are presented in Figure 3. The peak of the pre-ovulatory LH surge was higher in summer compared to winter (14.9 ± 1.21 versus 10.7 ± 1.44 ng / ml; Figure 3), whilst basal LH was significantly lower in summer compared to winter (2.64 ± 0.26 versus 3.54 ± 0.34 ng/ml). Consequently the relative rise in LH during the pre-ovulatory surge was significantly higher in summer compared to winter (582.4 ± 37.37 versus 337.6 ± 47.9%). Sows ovulating during winter had higher LH 48 and 54 hours post-LH peak (Figure 3). There was no effect of season (summer versus winter) on subsequent litter size; total born (12.5 ± 0.74 and 11.6 ± 0.98), born alive (11.9 ± 0.69 and 11.1 ± 0.91) and still born (0.68 ± 0.26 and 0.60 ± 0.36).

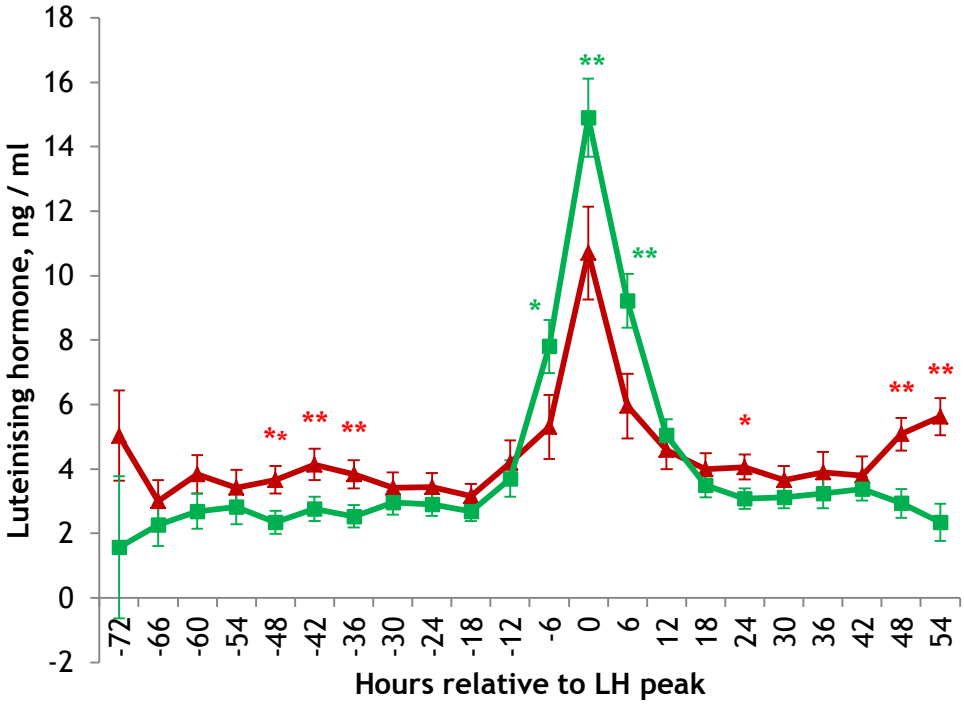

Figure 3 - Luteinising hormone (LH) profiles (expressed relative to the LH peak) for sows weaned and ovulating during summer (■) and winter (▲) (where 0 = the LH peak). Differences between means at a specific time point are indicated by *P < 0.06 and **P < 0.05

Timing of ovulation relative to when oestrus was first detected was significantly affected by season (Table 7), with sows ovulating earlier in summer compared to winter. Based on 6 hourly oestrus detection, sows ovulated approximately 9 hours earlier in summer than in winter. Based on 24 hourly oestrus detections sows ovulated 10 hours earlier in summer than in winter. The timing of ovulation relative to oestrus duration was also earlier, with ovulation occurring 51% of the way through oestrus in summer compared to 65% in winter (Table 7; P < 0.05). There was no effect of season on the LH peak to ovulation or ovulation to rise in PG (> 1ng/ml) intervals (Table 7). The timing of ovulation within each cohort is presented as a cumulative proportion of sows ovulating relative to when oestrus was first detected (6 hourly detections as conducted during the trial; Figure 5) or would have been detected if oestrus only conducted every 24 hours (Figure 6).

The interval from artificial insemination to ovulation has been calculated in two ways. First, based on the actual intervals observed in the current trial with the first insemination occurring 12 hours after first detection of oestrus (1stAI) and again at 24 hourly intervals (2ndAI) until oestrus no longer observed (referred to as the ACTUAL intervals). Second, based on oestrus detection being conducted at 24 hourly intervals, with the AI at first detection of oestrus (0AI), 6 hours after first oestrus detection (6hrAI) and again 24 hours after first detection of oestrus (24hrAI) (referred to as COMMERCIAL intervals). For the COMMERCIAL intervals, there was a significant effect of season (summer versus winter; $P < 0.05$) on the 0AI to ovulation interval (21.8 ± 3.30 versus 31.4 ± 3.30 hours), the 6hrAI to ovulation interval (15.8 ± 2.73 versus 25.4 ± 3.30 hours), and the 24hrAI to ovulation interval (-2.2 ± 2.73 versus 7.39 ± 3.30 hours). For the ACTUAL intervals, there was also a significant effect of season (summer versus winter; $P < 0.05$) on the interval from 1st AI to ovulation (18.2 ± 2.59 versus 27.5 ± 3.13 hours) and from 2nd AI to ovulation (-5.84 ± 2.59 versus 3.46 ± 3.13 hours).

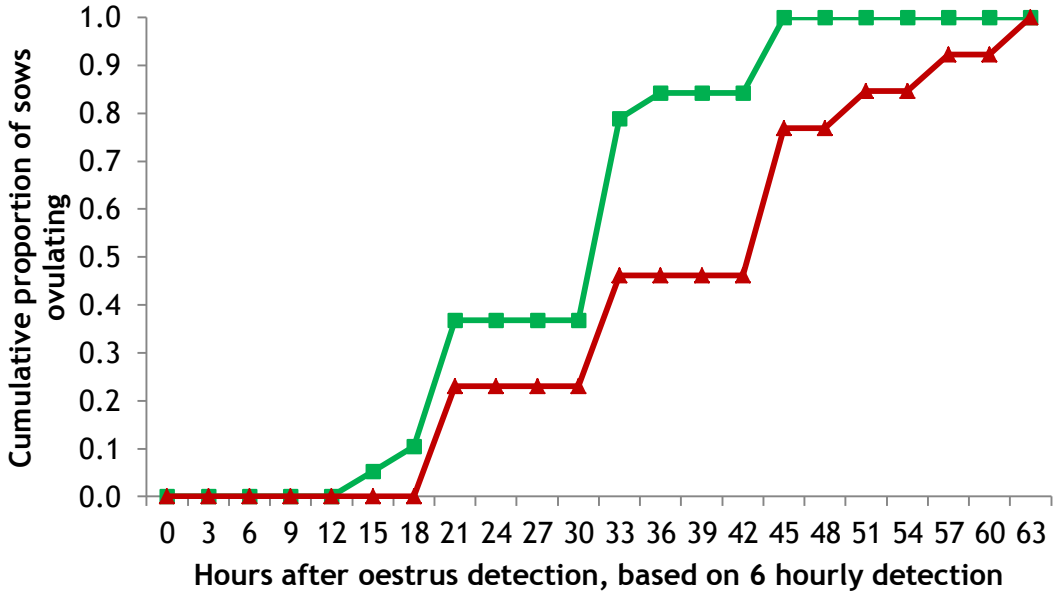

Figure 4 - Timing of ovulation (Cumulative proportion) after first detection of oestrus for sows weaned during summer (■) and winter (▲), based on oestrus checking occurring at 6 hourly intervals.

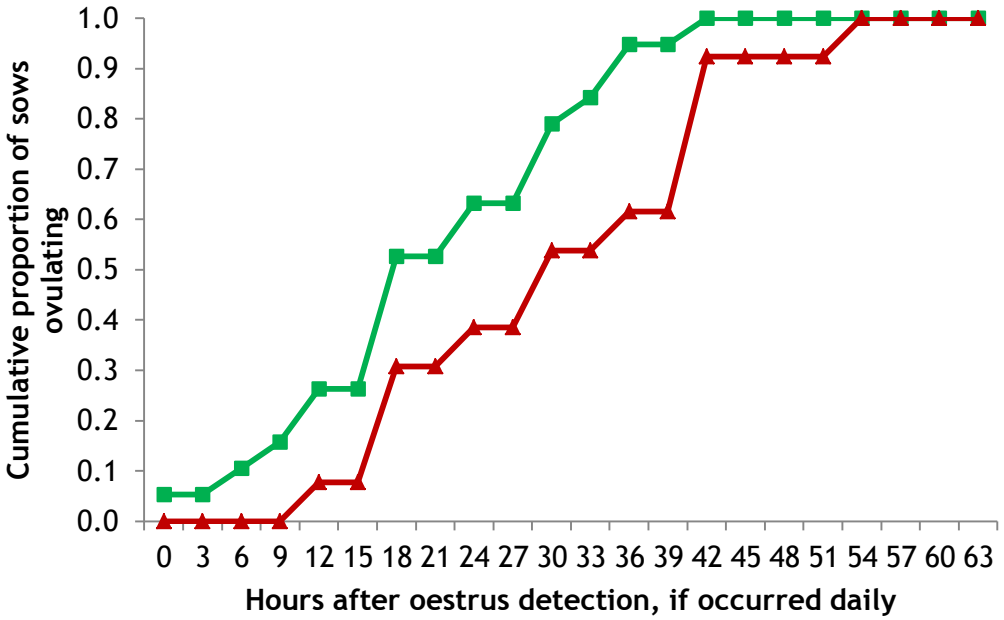

Figure 5 - Timing of ovulation (Cumulative proportion) after first detection of oestrus for sows weaned during summer (■) and winter (▲), based on oestrus checking occurring on a daily basis at 8 am in the morning.

Table 7 - Effect of season (summer versus winter) on the timing and duration of oestrus, timing of ovulation, the occurrence of the LH peak and the time at which progesterone rises above 1 ng / ml in weaned sows (data expressed based on 6 hourly observations, as well as if oestrus detection only occurred once daily at 7 am).

	Season of weaning		
	Summer	Winter	P value
Interval (based on 6 hourly checks) from:			
Weaning to oestrus (hours)	88.3 ± 4.08	83.1 ± 4.84	0.422
Start of oestrus to ovulation (hours)	30.2 ± 2.72	39.5 ± 2.97	0.029
Duration of oestrus (hours)	57.5 ± 2.43	55.8 ± 2.89	0.648
Interval (based on daily checks) from:			
Weaning to oestrus (hours)	97.0 ± 4.62	93.2 ± 5.49	0.597
Start oestrus to ovulation (daily checks)[1]	21.8 ± 3.26	31.4 ± 3.30	0.033
Interval (hours)from:			
LH peak to rise in P4 (> 1 ng/ml)	40.5 ± 2.85	38.8 ± 3.38	0.707
LH peak to ovulation	19.3 ± 2.89	24.5 ± 3.22	0.224
Ovulation to rise in P4 (>1 ng /ml)	22.4 ± 3.64	16.6 ± 3.94	0.328
Oestrus to LH peak	14.5 ± 1.91	17.7 ± 2.27	0.295
Timing of ovulation relative to oestrus duration (proportion)	0.51 ± 0.04	0.65 ± 0.05	0.031

[1] Based on oestrus checks occurring every 24 hours at 7 am

4. Application of Research

Overall, the current project has determined two key findings with regard to the effect of season and moderate nutritional modification on oocyte developmental competence and peri-ovulatory follicle growth in cycling gilts. Previous studies have demonstrated that severe dietary restriction impaired the capacity of peri-ovulatory oocytes collected from cycling gilts to reach metaphase II in vitro (i.e. Ferguson et al., 2003), whilst moderate nutritional restriction of pre-pubertal gilts reduced the proportion of oocytes reaching MII in vitro. In contrast, the current data demonstrated no effect of moderate nutritional restriction on MII rates in cycling gilts, suggesting that nutritional restriction needs to be severe before oocyte quality is impaired. However, future work is required to determine the effects of moderate nutritional restriction on embryo survival in vivo.

Interestingly, and in contrast to studies in weaned sows, no effect of season on the capacity of peri-ovulatory oocytes to reach MII was observed. It is, therefore, suggested the impaired competence of oocytes recovered from weaned sows during summer reflects the interaction between season and lactation induced metabolic demands on ovarian function. Specifically, photoperiod (and temperature) induced reductions in LH release combined with the negative energy balance typical of lactation are responsible for the impaired oocyte competence of sows weaned during summer / autumn. In contrast, cycling gilts are not metabolically challenged and as a result endocrine support for ovarian function is less likely to be affected, resulting in less of an effect on oocyte development. However, our previous work indicated reduced embryo survival in cycling gilts mated at the same time of year as oocytes were collected in the current trial. It could, therefore, be possible that the negative effects of season on oocyte development may be manifested as differences in early embryonic development. Support for an effect of season on ovarian follicle growth is provided by the observed differences in the dynamics of the peri-ovulatory follicle pool. Specifically, fewer follicles grew beyond 6 mm in diameter by day 19 of the oestrous cycle in summer / autumn compared to winter, suggesting that ovarian follicle growth may be reduced in summer/autumn compared to winter, or that the timing of ovulation may be delayed. It is, therefore, suggested that further research be conducted to investigate the effect of season on embryo development and survival?

The data from the second study in this project, confirms our previous finding that peri- and post- ovulatory progesterone profiles are different in summer compared to winter. From 72 hours onwards post-first detection of oestrus, progesterone concentrations were significantly higher in summer compared to winter, which is consistent with data from our previous studies in other commercial facilities (Pork CRC Final Report 2D-110 - *Determining the effects of season on ovarian development and early pregnancy returns*). It was interesting that the timing of the rise in progesterone relative to the peak of the pre-ovulatory LH surge and ovulation were similar in summer compared to winter. These data suggest that differences in the secretory capacity of the corpora lutea differ between summer and winter. Differences in feed intake, and thus metabolic clearance rate, could alter peripheral progesterone levels; however, no differences in feed intake were observed. Although an objective measure of feed intake was not recorded, feed refusals were monitored, and all sows were offered a quantity of feed they would have been able to consume comfortably (2.5 kg). Despite this, future research should be conducted to determine whether progesterone secretion by the corpora

lutea is different during summer, which would be best achieved through *in vitro* studies involving luteal cells.

Prior to the onset of the pre-ovulatory LH surge, basal LH was significantly lower in summer compared to winter, which is consistent with a photoperiod induced suppression on LH release. However, the peak of the LH surge and relative rise in LH concentrations were significantly higher in summer compared to winter. Together, these data suggest the reduction in basal LH release resulted in increased pituitary stores which were then released in response to the pre-ovulatory surge in gonadotrophin releasing hormone (GnRH). Although early development of the corpora lutea (CL) is believed to occur independently of LH, there is limited evidence that LH may play a supportive role early during CL development. It is, therefore, interesting that LH concentrations were higher 48 and 54 hours post-LH peak during winter compared to summer, providing further support for photoperiod induced impairment of LH release.

From a commercial perspective, one of the most relevant outcomes from study two was the alteration in the timing of ovulation relative to first detection of oestrus and the duration of ovulation. Specifically, sows weaned and expressing oestrus during summer ovulated approximately 10 hours earlier relative to oestrus detection compared to their winter counterparts. When these data was applied to a once daily oestrus detection protocol (fairly standard commercial strategy), ovulation occurred on average 22 and 31 hours post-oestrus detection in summer and winter, respectively. Based on inseminating sows at first detection of oestrus and again 24 hours later, the interval from 1st insemination to ovulation was 18 hours versus 28 hours (summer versus winter) and from 2nd insemination to ovulation was -2 versus plus 7 hours (summer versus winter). Although there is some debate as to the optimal timing of insemination relative to ovulation (eg Waberski et al., 1994; Nissen et al., 1997; Bertolozzo et al., 2005), it is generally agreed that optimal timing of semen delivery is prior to ovulation. The optimal duration between insemination and ovulation has been reported as 24 hours, 16 hours or 12 hours by Waberski et al., (1994) Nissen et al (1997) and Bertolozzo et al., (2005) respectively. The optimal duration from insemination to ovulation during summer to maximize conception and farrowing rates, as well as litter sizes is unknown. However, taking into account previous evidence that oocyte quality is reduced in sows weaned during summer and that sperm quality may also be impaired, it can only be assumed that optimal fertility requires semen to be inseminated with 12 hours prior to ovulation. It is, therefore, possible that the reduced fertility of sows mated during summer may reflect alterations in the timing of ovulation, and semen being delivered outside the optimal window. Future research should, therefore, focus on determining the optimal insemination strategy for sows mated during summer and whether improvements in fertility can be achieved by increasing the frequency of oestrus detection to ensure oestrus is detected as early as possible. Although experimental validation is required, preferably on a commercial scale with large numbers, the current data suggests that providing an additional insemination approximately 6 – 12 hours after first oestrus detection may result in a more appropriate interval from insemination to ovulation. Such a strategy may improve fertility and fecundity of sows mated during summer. Additional work should also investigate the effect of season on semen viability (how long it should be used for post-collection) and investigate the potential benefits of controlling the timing of ovulation using exogenous gonadotrophins.

5. Conclusion

In summary, ovarian follicle growth appears to be altered during summer compared to winter in cycling gilts; however, no differences in oocyte developmental competence were observed. Equally, the current data demonstrated no effect of mild nutritional restriction (as might be observed commercially in group housing situations) on follicle growth or oocyte developmental competence.

Importantly, our data demonstrated that ovulation occurred earlier relative to first oestrus detection in sows weaned during summer compared to winter. This finding has implications for insemination protocols during summer. Specifically, based on a standard commercial insemination protocol, both the first and second insemination would occur outside the optimal window for semen delivery. Further work is, therefore, required to determine if more accurate and rapid detection of oestrus by increasing the frequency of heat detection combined with alterations in insemination protocols can alleviate seasonally induced fertility suppression of weaned sows.

6. Limitations/Risks

The primary limitation to study one was the use of metaphase II as the marker of oocyte developmental competence. The capacity of oocytes to reach the blastocyst stage in vitro is widely accepted as a superior measure; however, fertilization is required which would have resulted in the confounding effect of seasonal variation in semen quality.

The main limitation to the data from study two is the small number of animals used. This can easily be overcome either by investigating timing of ovulation on a larger scale using a variety of genotypes. However, the currently observed seasonal differences in progesterone in summer compared to winter are supported by our previous data from two separate commercial facilities and genotypes. Alternatively, the better, and perhaps simpler option, would be to conduct a commercial trial to determine the effect of an altered insemination protocol and increased frequency of oestrus detection post-weaning on fertility and fecundity.

7. Recommendations

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

- Ovarian function and oocyte developmental competence of cycling gilts is unaffected by commercially relevant suppression of feed intake or indeed season.
- Ovulation occurs earlier in summer compared to winter, emphasizing the need to conduct trials to develop an optimal oestrus and insemination protocol for the summer / autumn period.

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