USING GNRH ANALOGUES TO ADDRESS SEASONAL INFERTILITY IN PIGS

Report prepared for the Co-operative Research Centre for an Internationally Competitive Pork Industry

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Executive Summary

Seasonal infertility in the domestic pig is manifested by delayed puberty in gilts, reduced pregnancy rates, smaller litter sizes and increased weaning to oestrus intervals during the summer-autumn period. The environmental factors leading to seasonal infertility are still being fully elucidated but high ambient temperatures and photoperiod are thought to be the main factors altering the neurological and endocrinology of reproductive processes in the sow. It is difficult to fully evaluate the loss in cost of production due to seasonal infertility as the effect is different between farms, from year to year and between different locations but is likely to cost the pig industry millions of dollars in lost production each year in Australia. The major component determining litter size in sows is embryo mortality which occurs before day 30 of gestation and is higher during periods of seasonal infertility leading to pregnancy loss and lower litter sizes. The high level of embryo loss during this period is reported to be due to environmental conditions decreasing progesterone production during this critical period of pregnancy. The aim of this project was to provide a treatment using intramuscular injections on day 10 of pregnancy of Gonadotrophin releasing hormone (GnRH) analogue Gonavet and hormones including PMSG/hCG and PG600 to increase progesterone levels in early pregnancy and increase litter sizes by reducing the impact of embryo mortality during the period of seasonal infertility.

The experiments described here used a total of 162 sows at Roseworthy in South Australia in replicate experiments throughout the year. Based on results at Roseworthy a field study in southern Queensland with 643 sows at two sites and during two periods of reported normal fertility and seasonal infertility was performed using treatment with PMSG/hCG only. Periods of clear seasonal infertility on pregnancy outcome were not identified at Roseworthy or Queensland during the course of this project. Treatments did not increase litter size at either sites during the reported periods of infertility. However at Roseworthy, treatment with PMSG/hCG increased progesterone production ($P < 0.05$) in early pregnancy and increased total number of piglets born ($P < 0.05$) without regard to season. Treatments with Gonavet and PG600 were marginal in increasing progesterone and ineffective with regard to improving reproductive performance at Roseworthy. Treatment with PMSG/hCG in Queensland lessened the decline in pregnancy rate between periods, however this did not meet statistical significance due to variability between treatments and sites in the period of reported normal fertility.

In conclusion, the environmental factors influencing seasonal infertility in pigs are multifactorial and vary between piggeries. We were unable to address seasonal infertility using a hormonal treatment in this project, likely due to limitations regarding changes in management at Roseworthy, and temperature and photoperiod differences in Queensland.

However, we were able to confirm the role of progesterone in early pregnancy in addressing embryo mortality in the pig with resultant effects on total number of piglets born. Furthermore we have identified a new application for PMSG/hCG to increase litter size in pigs and that increasing endogenous levels of progesterone during early pregnancy has the potential to improve reproductive outcome in sows and address lower pregnancy rates during periods of environmental stress in gilts.

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1. Introduction

Background and rationale for conducting the research

Seasonal infertility in pigs is manifested by reduced farrowing rates, delayed puberty in gilts, extended weaning to oestrus intervals, early disruption of pregnancy and a reduction in litter sizes during the summer-autumn period (Peltoniemi et al. 1999). Gilts and sows affected by seasonal effects conceive after mating but lose embryos during the implantation period and the whole litter is usually lost (Love et al. 1993). This has a major economic consequence to the pig industry due to increased cost of production. The physiological mechanisms behind the reduction in fertility are being determined since the introduction of specific melatonin assays (Peltoniemi et al. 2000). There are a variety of environmental factors that contribute to seasonal infertility with photoperiod being the primary cause (Peltoniemi et al. 2006). However other factors including nutrition, group housing, high ambient temperatures and dominance structures between females can exacerbate the seasonal effect. In the modern day domestic pig, the endocrinological basis of seasonal infertility still remains to be fully elucidated. However, it is now known that luteinising hormone (LH) secretion is reduced during the most sensitive summer-autumn period (Basset et. al., 2001) and this is likely to affect ovarian follicle development and corpus luteum (CL) function leading to downstream effects on the capacity of embryos to provide adequate signalling to maintain CL progesterone production from about day 10 of pregnancy (Love et al. 1993). As a result embryos are lost during the most sensitive pre-implantation stage of pregnancy.

In simple terms, the current belief is that a reduction in LH secretion in the seasonal infertility period results in one or more of the following:

- Reduced oocyte competence
- Sub-optimal progesterone secretion, due to:
    - sub-optimal corpus luteum formation in early pregnancy (days 0-10 post conception)
    - sub-optimal maintenance of CL function from days 10-15 post-conception

Considering the important role of LH in regulation of the oestrous cycle in pigs, it is proposed that suppression of the GnRH-LH axis is the major cause of seasonal infertility. The consequences of the resulting LH deficiency being expressed both during the follicular and luteal phases of the oestrous cycle.

This report documents a series of experiments using treatments of injectable Gonadotrophin releasing Hormone (GnRH) and gonadotrophins to increase endogenous progesterone production from day 10 of pregnancy during the periods of summer infertility in post weaned sows and gilts.

2. Methodology

The studies contained within this report were conducted at The University of Adelaide Pig and Poultry Production Institute (PPPI) piggery in Roseworthy, South Australia and at Cameron Holdings (CHM Alliance) piggeries Pepperina and Lapunyah in south west Queensland. All experiments detailed in this report have been approved by The University of Adelaide Animal Ethics Committee. Initially a series of replicate small scale experiments were performed at Roseworthy PPPI piggery at different times of the year to investigate the effect of treatments to increase progesterone production during early pregnancy in the pig. These experiments were followed by a field trial conducted at CHM Alliance's Pepperina and Lapunyah piggeries during two periods in which seasonal infertility is not reported to occur and during a period when in the past these piggeries experienced seasonal infertility.

2.1. Treatments

All treatments were administered to gilts and sows by intramuscular injection beginning on day 10 after AI. The treatments were chosen for their ability to increase progesterone production by either producing accessory corpora lutea or by enhancing luteal function. The treatments consisted of:

(1) 50 μg Gonavet (Veyx GmbH, Germany) on day 10 after first AI
(2) Single dose of PG600 (400 iu of PMSG, 200 iu of hCG; Intervet, Holland) on day 10 after first AI
(3) 1000 iu of Folligon (PMSG; Intervet, Holland) followed by 750 iu Chorulon (hCG; Intervet, Holland) on day 13 after first AI (for second and higher parity sows the dose of Folligon was increased to 1200iu)
(4) Control group at Roseworthy received an injection of 5 ml of sterile saline on day 10 after first AI. For the field trial in Queensland the control group received no injections (i.e. untreated controls)

All treatments were prepared, stored and administered according to manufacturers' instructions.

2.2. Measurement of ovarian follicle growth

The effect of treatment given to sows on day 10 of pregnancy on ovarian follicle growth was assessed using trans-rectal real-time ultrasound using a 3.5MHz Sector probe (Scanner 200; Esaote Pie Medical, Maastricht, the Netherlands). Ultrasound scanning took place in all sows within the Roseworthy replicates only and were performed before treatment on day 10 and after treatment on day 14 and day 17 of pregnancy. All scanning and measurements were made by Ms. Emmy Bouwmann from The University of Adelaide. Ovarian follicle size of four to six largest follicles on the right ovary was recorded for each sow. Follicles of sizes above 6 mm that were not present on a following scan were assumed to have ovulated and produced accessory corpora lutea.

2.3. Day length and maximum temperatures

Values for length of visible light and maximum daily temperatures were accessed from "weather underground' website: http://www.wunderground.com/global/AU.html and on the Australian Government Bureau of Meteorology website: http://www.bom.gov.au/ . For statistical analysis using day length and maximum temperatures, the average of 21 days of data was used to accommodate the period from mating to day 21 of pregnancy. This gave a single day length and temperature value to represent these parameters during the proposed critical period of pregnancy loss and embryo mortality in the pig. The maximum temperatures during the periods that the sows were entered into the trials are listed in Table 1. The temperatures used in the statistical analyses are mean maximum, mean and mean minimum temperatures for a 21 day period from first AI until day 21 of pregnancy.

2.4. Season

The major contributor to seasonal infertility is reported to be length of visible light (photoperiod) and therefore the term season within this report relates to day length. 'Winter' refers to the period of time (months) between March equinox and September equinox and 'summer' refers to the period between September and March equinoxes (Figure 1). For the field trial in Queensland, the two arms of the experiment were performed during periods that were historically known to be of normal fertility (before Christmas) and of lower fertility attributed to seasonal infertility during the first 3 months of the year. These periods related to Nov/Dec 2008 and Feb/Mar 2009 (Figure 1).

2.5. Roseworthy Experiments

The experiments conducted at Roseworthy piggery investigated the efficacy of GnRH analogues, PG600 and PMSG/hCG to increase endogenous progesterone production after treatment given at day 10 of pregnancy (day 10 post first AI). Blood samples (approximately 10 ml) were taken via jugular venipuncture in heparin coated vacutainers (Becton Dickinson, USA) and plasma was collected after centrifugation and stored at -20^{o}C until assayed for progesterone content by radioimmunoassay (Diagnostic Systems Laboratories, USA). Blood samples were taken before treatment on day 10 and after treatment on day 11, 14, 21 and 28.

162 post-weaned sows were used in total over a period of 24 months. These sows consisted of gilts (n=62) and parity one (n=59), parity two (n= 28), parity three (n=12) and parity four (n=1) post-weaned sows. Transrectal ultrasound scanning was used to detect ovarian follicle growth and approximate time of ovulation of accessory corpora lutea if large follicles were present.

The number of sows within each replicate and the time of year which each replicate took place are presented in Table 1.

Replicates of between 10-20 of predominantly parity 1 and parity 2 sows were entered into the trial within the winter and summer periods. The times and period of the year are listed in Table 1.

2.6. Queensland Experiments

Early replicate experiments performed at Roseworthy piggery showed that treatment with PMSG/hCG from day 10 of pregnancy increases progesterone production (Section 3.1.2). This increase in progesterone was associated with an increase in litter size (total born). To investigate whether this response could be achieved in a larger commercial facility, a field trial involving 643 sows across two piggeries and two time periods was performed in central Queensland near the NSW border.

The field trial consisted of gilts from Pepperina piggery and parity one and two sows from Lapunyah piggery from the 15th of November to the 8th of December 2008, a period that is historically associated with normal fertility at both piggeries and from the 14th of February until the 3rd of April 2009, a period within which seasonal infertility has been reported at these two piggeries. A total of 268 sows (including gilts) were randomly allocated into the treatment group with PMSG/hCG or to a non-treated control group in Nov/Dec group and 375 within Feb/Mar/Apr group (Table 1).

Pepperina piggery is located approximately 20 km north of Goondiwindi and Lapunyah piggery is approximately 120 km north west of Goondiwindi. It was considered that day length and temperature calculations for these two locations would be similar and therefore all reported values from Wunderland and BOM weather websites for Goondiwindi, Queensland were used for statistics involving sows from both locations. Variation in day length between the two experimental periods was approximately 77 minutes and mean temperatures were equivalent between the periods (Table 1 and Figure 1).

Parities for the sows used in this study were not evenly distributed between sites due to a concurrent research trial (Lifetide trial) being performed at Pepperina. Due to this, only gilts were used at Pepperina and parities 1 and 2 were used at Lapunyah.

Table 1: Experimental replicates for the Roseworthy piggery (Rwthy SA) and CHM Pepperina and Lapunyah piggeries

Exptl. replicate	Month/Year	Number of		Piggery	Season*	Day length† (minutes)	Temp.‡ (max/av/min)
		gilts	sows				
1	Aug 2007	0	11	Rwthy SA	Winter	697	30/17/12
2	Oct 2007	0	17	Rwthy SA	Summer	813	35/21/15
3	Nov 2007	0	16	Rwthy SA	Summer	873	26/25/15
4	Jan 2008	6	15	Rwthy SA	Summer	890	40/28/21
5	Feb 2008	6	13	Rwthy SA	Summer	832	35/26/20
6	May 2008	12	0	Rwthy SA	Winter	669	23/18/15
7	July 2008	11	0	Rwthy SA	Winter	678	17/14/11
8	Sept 2008	6	2	Rwthy SA	Winter	749	32/19/13
9	Oct 2008	15	0	Rwthy SA	Summer	818	32/22/15
10	Nov 2008	15	0	Rwthy SA	Summer	891	32/22/15
11	Jan 2009	7	10	Rwthy SA	Summer	891	43/28/21
Field trial	Nov/Dec 08	152	0	Pepperina	Period of normal fertility**	870	37/32/24
Field trial	Nov/Dec 08	0	116	Lapunyah	Period of normal fertility**	870	37/32/24
Field trial	Feb/Mar/Apr 09	150	0	Pepperina	Period of seasonal infertility**	793	37/31/23
Field trial	Feb/Mar/Apr 09	0	225	Lapunyah	Period of seasonal infertility**	793	37/31/23

†: Day length is measured as the period of visible light reported on *Weather Underground* website and the temperatures as recorded on The Australian Government Bureau of Meteorology website for the locations Roseworthy, SA and Goondiwind, QLD. ‡: Temperature is given as maximum highest temperature (max), mean temperature (av) and the minimum daily temperature (min) for the month. * Season refers to the period between winter equinox to summer equinox (winter) and summer equinox to winter equinox (summer). **Period of normal fertility: relates to the period within the year that the piggery historically recorded normal fertility or historically a period of infertility.

Day length measurements and temperature values were sourced from

- *Weather Underground* website:
  Goondiwindi: http://www.wunderground.com/history/station/94530
  Roseworthy: http://www.wunderground.com/global/stations/95671
- Bureau of meteorology website:
  Goondiwindi:
  http://www.bom.gov.au/climate/dwo/IDCJDW4051.latest.shtml
  Roseworthy:
  http://www.bom.gov.au/climate/dwo/IDCJDW5062.latest.shtml

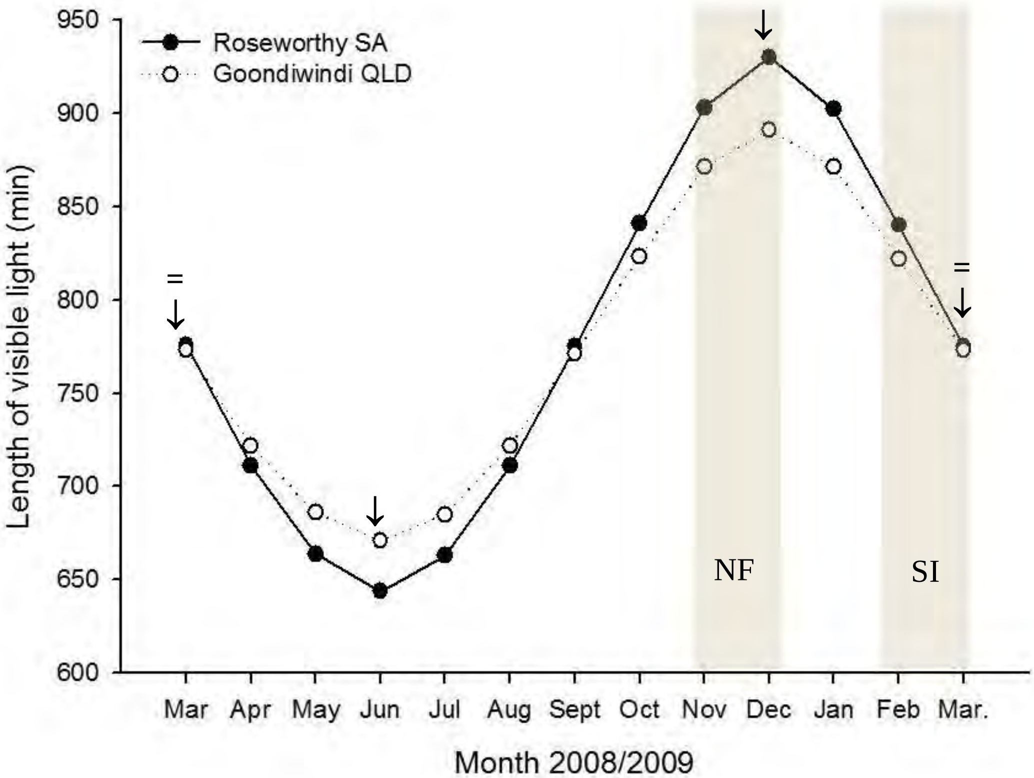

Figure 1: Day length as function of 'length of visible light' recorded for Roseworthy in South Australia and Goondiwindi in Queensland for the period from March equinox 2008 until March equinox 2009. Summer and winter solstice are indicated by ↓ and equinox by the shaded bars indicate the period of reported 'normal fertility (NF)' and 'summer infertility (SI)' at the Queensland piggeries during which the field trials were performed.

3. Outcomes

3.1 Roseworthy experiments

3.1.1. The number of gilts and sows

In total, 63 gilts and 100 parity 1-4 sows were randomly allocated within four treatment groups in 11 replicate experiments between August 2007 and January 2009 (Table 1). Within this cohort of gilts and sows there were no 'return to oestrus' (RTN) recorded and low numbers (between 0 and 10%) of 'not in pig' (NIPs) for each treatment group (Table 2). Gilts and sows were classified as NIPs when an abdominal ultrasound scan performed on or after day 21 were negative for implantation sites. Due to the relatively low numbers of gilts and sows within each treatment group, the numbers of NIPs or abortions (aborts) are not considered significant for comparisons between treatment or parity. Not all animals within each treatment group were scanned to determine ovarian follicle growth and the numbers scanned on each day for follicle growth measurements are listed in Table 3. In this study, it was not possible attain real-time ultrasound analysis for every animal nor was it possible to attain a blood sample from every animal at every time point. Blood samples were predominantly taken from day of treatment to day 21 and on day 28 for gilts and parity one sows that were part of replicate #11 (Table 3). Estradiol content was analysed in plasma collected from a smaller cohort of animals within each treatment except for those within Gonavet treatment. The number of gilts and sows within the Gonavet group is smaller due to early analysis revealing that the effect of treatment on ovarian follicle growth and progesterone production was negligible and this meant we were able to increase the number of animals within the other treatment groups. One gilt from the PG600 treatment was culled for non-reproductive reasons.

Table 2: Roseworthy Treatment groups

	control		PMSG/hCG		PG600		Gonavet	
n	Gilts (18)	Sows (26)	Gilts (20)	Sows (31)	Gilts (19)	Sows (25)	Gilts (6)	Sows (18)
RTN								
NIPs	1	1		3	2	1		1
Aborts				1	1			
Farr	17	25	20	27	16	24	6	17
Ovarian u/s scan	11	22	12	24	9	21	6	13
P4 d10-d21	16	25	20	23	15	24	6	16
P4 d28	2	2	3	3	1	3		
E2 assayed	3	9	3	10	3	9		
culled					1			

The numbers of gilts and sows within each treatment group involved in the Roseworthy series of experiments and the numbers that returned to oestrus (RTN), were 'not in pig' (NIPs), had abortions (Aborts) and farrowed (Farr) after treatment. In addition, the numbers of gilts and sows that undertook real-time ultrasound scanning for ovarian measurements (Ovarian u/s scan) and had blood taken for progesterone analysis from day 10 to day 21 (P4 d10 - d21) and day 28 (P4 d28) are recorded. Total number of gilts and sows within each treatment are presented in parentheses.

3.1.2. The effect of treatment on progesterone production

The acute effects of giving GnRH analogue and gonadotrophins on the production of progesterone during early pregnancy was determined by measuring plasma progesterone content in blood samples taken before treatment on day 10 of pregnancy and then on day 11, day 14, day 21 and day 28 of pregnancy. Intramuscular injection of 1000 iu of PMSG followed 72 h later with 750 iu of hCG increased progesterone production by approximately 30% ($p = 0.002$) on day 21 (11 days after treatment) compared with saline control and intramuscular injection of PG600 and Gonavet (Figure 2, Table 4). Treatment with Gonavet and PG600 had no effect on increasing plasma progesterone levels above those of the saline-treated controls.

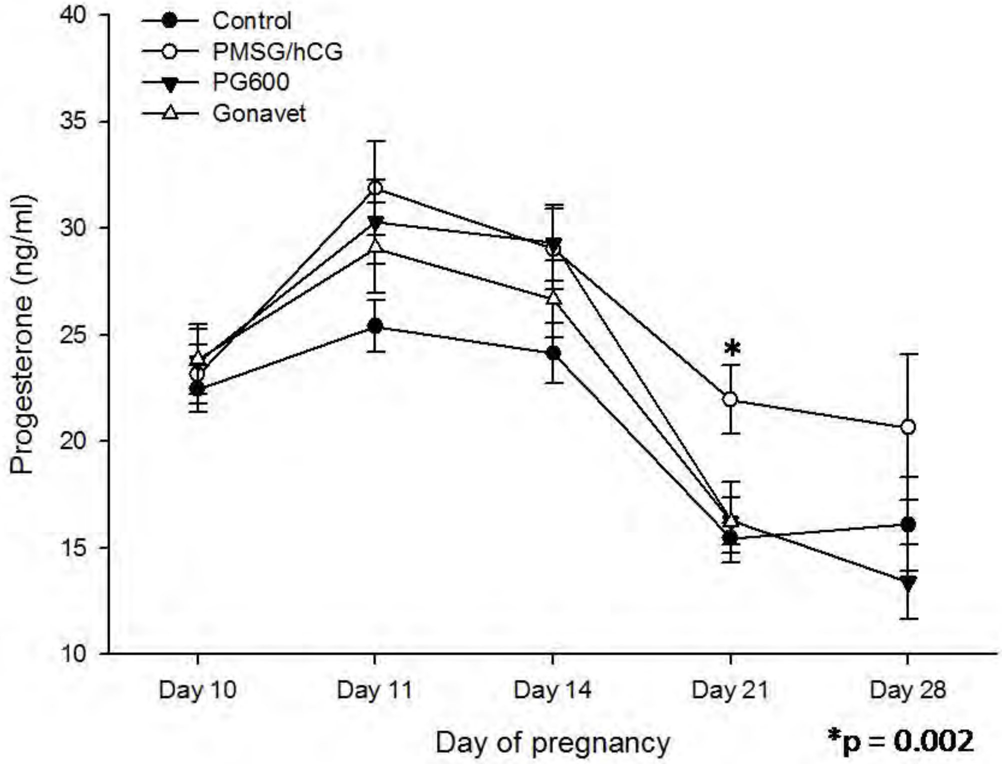

Figure 2: The effect of intramuscular injection of saline (control), 1000 iu of PMSG followed 72 h later by 750 iu of hCG (PMSG/hCG), PG600 and 50μg of Gonavet given at day 10 of pregnancy in sows on progesterone production. Number of sows within each treatment group are given in Table 4. Data are mean ± SEM and the effect of treatment for each time period was compared using Students *t-test* (SPSS v17.0). Only data from pregnant animals that farrowed are included in this analysis.

3.1.3. The effect of treatment on ovarian follicle growth

The acute effects of treatment with PMSG/hCG, PG600 and GnRH analogue Gonavet on ovarian morphology were investigated by measuring the size of four to six largest ovarian follicles using real-time transrectal ultrasound. Ovarian follicle size was recorded before treatment was given on day 10 of pregnancy and then on day 14, day 17 and day 21 of pregnancy. Treatments with PMSG/hCG and PG600 increased mean follicle size by day 14 of pregnancy by approximately 20% compared with follicles measured at day 10 and 52% and 26% for PMSG/hCG and PG600 respectively by day 17 of pregnancy. Follicles over the size of 6mm that were not observed at the next scan were assumed to have ovulated to form accessory corpora lutea.

Using real-time ultrasound, it is not possible to quantify corpora lutea in a meaningful way. Based on the criterion that when large follicles over 6 mm in diameter that do not appear in a following scan have ovulated, most of the large growing follicles observed after treatment with PMSG/hCG resulted in forming accessory corpora lutea and in all PMSG/hCG-treated sows accessory corpora lutea were observed (Table 3). PMSG/hCG was the most effective treatment in increasing follicle growth and forming accessory corpora lutea. Treatment with PG600 caused an increase in follicle growth by day 14 and most of these follicles were still present by day 21. Nevertheless, treatment with PG600 did result in the formation of accessory corpora lutea in approximately 32% of sows treated (Table 3).

Table 3: The effect of treatment on ovarian follicle size (mm) measured by real-time transrectal ultrasound at day 10, day 14, day 17 and day 21 of pregnancy.

Ovarian follicle size (mm)						
	n	Day 10	Day 14	Day 17	Day 21	Acc. CL
Control	31	3.25 ± 0.8^{a}	3.33 ± 0.07^{a}	3.26 ± 0.08^{a}	ND	0/31
PMSG/hCG	37	3.20 ± 0.9^{a}	$5.83 \pm 0.18^{b*}$	$8.12 \pm 0.49^{b,c*}$	$12.88 \pm 2.4^{b,c*}$	37/37
PG600	34	3.30 ± 0.1^{a}	$5.03 \pm 0.18^{b*}$	$4.44 \pm 0.43^{b,c*}$	$5.39 \pm 0.46^{b,c*}$	11/34
Gonavet	16	3.07 ± 0.1^{a}	3.23 ± 0.10^{a}	3.25 ± 0.09^{a}	ND	0/16

Treatments were given at day 10 of pregnancy (before treatment) consisting of intramuscular injections of saline (control), 1000iu of PMSG followed 72 h later by 750iu of hCG (PMSG/hCG), single dose of PG600 (PG600) and 50µg of GnRH analogue (Gonavet). Data are mean ± SEM and were compared by ANOVA (SPSS v17.0), *$p < 0.05$ compared with the day 10 value, different superscripts $p < 0.05$. ND: not detected.

3.1.4. The effect of season and treatment on progesterone and oestradiol production

To investigate the influence of season (day length) on progesterone production in pregnant sows, plasma progesterone content was measured in blood samples taken at day 10 of pregnancy before treatments were given. Progesterone content was compared according to season in which the sample was taken. Season, based on the periods between the summer and winter equinoxes, has no effect ($p = 0.45$) on plasma progesterone level on day 10 of pregnancy (Table 4). Moreover, season had no effect on progesterone levels after treatment on day 11, 14, 21 and 28 of pregnancy regardless of treatment ($p > 0.05$, Table 3).

The effect of month of collection and day length on day 10 (before treatment) plasma progesterone content is shown in Figure 3. Progesterone production is positively correlated ($r^2 = 0.14$; $p = 0.07$) with day length with an approximate 14% increase in plasma level as the photoperiod increases (Figure 4).

Oestradiol was measured in the blood samples to determine if treatments cause increased levels of oestradiol compared with what has been previously reported in non-pregnant sows treated with gonadotrophins (Dial et al. 1984).The level of plasma oestradiol did show a difference due to season however the variability in values (SEM) and the low number of animals present in the winter component may impact on the implication of this result.

In addition to the analysis in section 3.1.2, the effect of treatment on plasma progesterone content was analysed using Univariate analysis (ANOVA) with Bonferroni correction to compare the four treatments. Within 24 h after treatment there was an increase in progesterone production above control although not quite making statistical significance ($p = 0.08$). This result indicates that there was an ovarian response to Gonavet and PG600 (Figure 2, Table 4). This response was short lived in the Gonavet and PG600 group likely due to the comparatively low dose of gonadotrophins in PG600 compared with the PMSG/hCG treatment and the shorter half-life of Gonavet in vivo compared with PG600 and PMSG.

Table 4: The effect of treatment on day 10 of pregnancy on progesterone (ng/ml) and estradiol (pg/ml) production in sows.

	Control	PMSG/hCG	PG600	Gonavet	P value[†]	Winter (Ctl + Gon)	Summer (Ctl + Gon)	P value[‡]
Prog. Day 10	22.4 ± 1.08 (43)	23.14 ± 1.41 (51)	23.74 ± 1.48 (43)	23.86 ± 1.64 (23)	0.90	21.7 ± 1.8 (22)	23.1 ± 2.3 (44)	0.45
Prog. Day 11	25.4 ± 1.2 (44)	31.9 ± 2.2 (49)	30.3 ± 2.0 (43)	29.1 ± 2.1 (23)	0.08	28.0 ± 2.4 (22)	25.6 ± 1.1 (44)	0.30
Prog. Day 14 [a]	24.14 ± 1.4 (43)	29.02 ± 1.9 (51)	29.3 ± 1.7 (43)	26.7 ± 1.8 (23)	0.12	23.8 ± 2.1 (22)	25.4 ± 1.2 (44)	0.49
Prog. Day 21[c]	15.46 ± 0.7 (40)	22.0 ± 1.6 (48)	16.27 ± 1.1 (38)	16.21 ± 1.9 (19)	**0.001**	15.8 ± 0.9 (15)	15.5 ± 0.9 (44)	0.92
Prog. Day 28	16.10 ± 2.2 (4)	20.65 ± 3.4 (6)	13.4 ± 1.8 (5)	ND	0.19	ND	17.0 ± 1.7 (15)	ND
E2 Day 14	3.88 ± 0.2 (10)	4.61 ± 0.3 (11)	4.24 ± 0.1 (9)	4.40 ± 0.3 (8)	0.24	ND	4.11 ± 0.17 (17)	ND
E2 Day 21[b]	7.76 ± 1.7 (11)	5.89 ± 1.2 (12)	9.65 ± 2.2 (11)	5.46 ± 0.3 (9)	0.25	21.4 ± 2.1 (4)	5.4 ± 0.30 (16)	<0.001

Data are mean ± SEM and compared using ANOVA one way analysis followed by Bonferroni *t-test* (SPSS 17.0). ND: not detected. All statistical analysis was compared with parity as a covariate and parity was significant at [a]p = 0.025 for winter vs summer comparison for Prog. Day 14 and [b]p = 0.94 for parity as covariate for E2 day 21. For Prog. Day 21, parity as a covariate [c]p = 0.18. Winter and summer comparisons were made with combined data for the control and Gonavet groups only. All values represent pregnant gilts and sows.

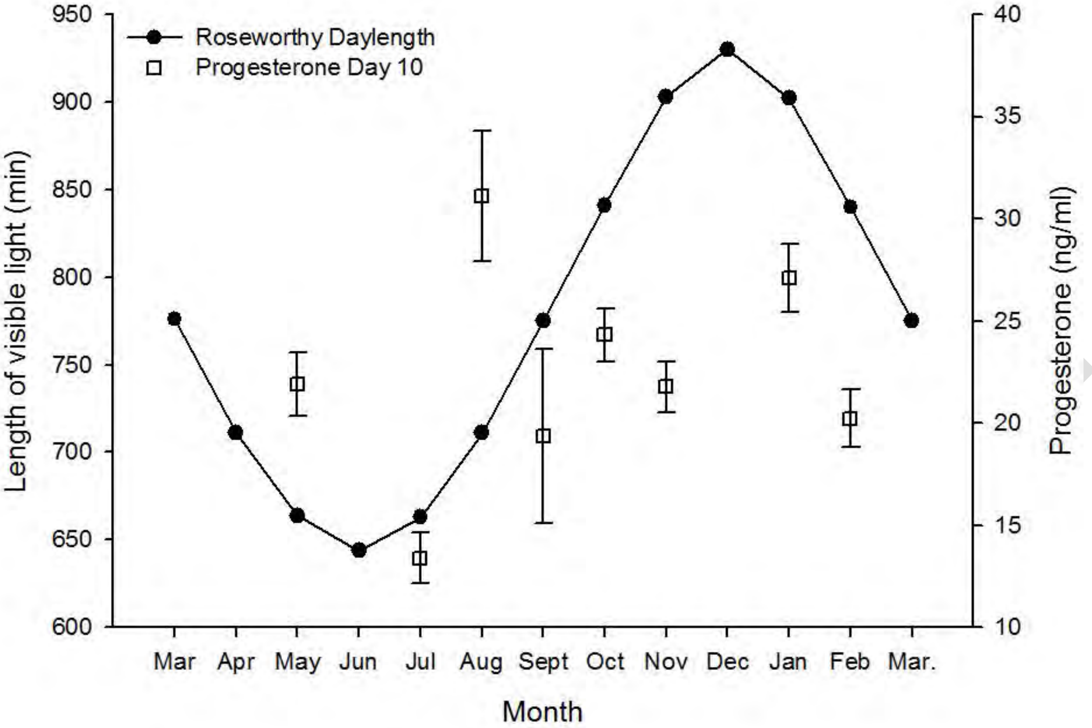

Figure 3: The effect of month of sampling and day length on plasma progesterone content on day 10 of pregnancy in sows. Data are mean ± SEM and compared using Pearson correlation ($r^2 = 0.14$, $p = 0.07$; SPSS v17.0).

3.1.5. The effect of treatment on pregnancy outcome

To examine the effect of treatment on pregnancy outcome, pregnancy rate, total numbers of piglets born per litter (TB), piglets born alive (BA), numbers of still born piglets and mummified fetuses were compared using ANOVA followed by Bonferroni *t-test* (SPSS). Intra-muscular injection of PMSG/hCG increased the numbers of total born piglets by approximately 1 piglet (p = 0.08) when comparing within each of the four treatments (Table 5). Treatments had no effect on pregnancy rate (p = 0.85) nor the number of piglets born alive or the numbers of mummified fetuses (Table 5). However, there was an approximate 2-fold increase in the numbers of stillborn piglets in sows that received the PMSG/hCG treatment (p = 0.03) when compared with the other treatments (Table 5). Furthermore, increasing statistical power by combining the results within control and Gonavet treatment groups that are not significantly different (p = 0.38, Table 6) and comparing to the PMSG/hCG treatment group indicates an increase in the total number of piglets born by approximately 0.9 piglets (p = 0.03; Table 7) and likewise if the control group includes the Gonavet-treated group (p = 0.04; Table 8). The effect of increasing the numbers of control animals however did not significantly change the effect of treatment on the born alive parameter but increased the numbers of stillborn piglets by 0.7 still born piglets (Table 5 and Table 8).

Statistical analysis investigating the effect of treatments on TB, BA, stillborns and mummies showed no significant effects between treatments (p > 0.05) using Univariate analysis with multiple comparisons using Bonferroni t-test (SPSS) (data not shown).

3.1.6. The effect of season on pregnancy outcome

The effect of season was evaluated by calculating the day length during the period in which experimental replicates took place at Roseworthy. Due to the effect of PMSG/hCG treatment on progesterone production and total born and the PG600 treatment on ovarian follicle growth, these two groups of sows were omitted from the analysis. Pregnancy rate was not influenced by season at Roseworthy piggery, nor was the numbers of piglets born alive, born stillborn or the numbers of mummified fetuses (Table 5). Season did influence the total numbers of piglets born with approximately 1.45 piglets per litter extra (p = 0.04) during the summer period (Sept. to March). This result is contrary to what is normally experienced at commercial piggeries and is counter to the hypothesis behind this study. New management practices were introduced during the course of this study, which were likely to be confounding variables and involved:

1. Changing the feeding regimen to decrease body weights of breeding sows and gilts
2. Extending weaning from 3 weeks to 4 weeks of lactation in all sows
3. Introducing stricter criteria for gilt selection
4. Increasing the culling rate to include all gilts and sows that were returns or NIPs

These management strategies were likely to be responsible for the increase in total born per sow figure from 10.2 in 2007 to 11.8 for 2009 (PPPI production figures provided by G. Christian) and may have masked the effects of season in this piggery. Alternatively, the less number of animals within the winter cohort compared with the summer cohort (19 vs 45 respectively) may have biased this result and those for born alive, stillborn and mummies in favour of the summer replicates (Table 5). In addition there were proportionally less gilts in the summer period which could also have accounted for the higher litter size characteristics in summer (eg. May-Aug there were 23 gilts compared with 11 sows, Table 1).

Table 5: The effect of treatment given at day 10 of pregnancy on total born piglets (TB), born alive piglets (BA), still born piglets (SB) and mummified piglets (Mummy)

	Control	PMSG/hCG	PG600	Gonavet	P value[†]	Winter (CTL + Gon)	Summer (CTL + Gon)	P value[‡]
Preg. rate	41/44 (93%)	47/51 (92%)	38/43 (88%)	23/24 (96%)	0.85[a]	17/19 (89%)	43/45 (95%)	0.20[a]
TB	11.07 ± 0.42 (41)	12.02 ± 0.34 (47)	11.50 ± 0.50 (38)	10.48 ± 0.51 (23)	0.08	9.84 ± 0.61 (19)	11.29 ± 0.37 (45)	**0.04**
BA	10.32 ± 0.38 (41)	10.55 ± 0.31 (47)	10.87 ± 0.40 (38)	9.74 ± 0.58 (23)	0.42	9.32 ± 0.50 (19)	10.20 ± 0.41 (45)	0.22
SB	0.61 ± 0.16 (41)	1.28 ± 0.26 (47)	0.55 ± 0.12 (38)	0.57 ± 0.41 (23)	**0.03**	0.42 ± 0.18 (19)	0.65 ± 0.18 (45)	0.44
Mummy	0.15 ± 0.06 (41)	0.19 ± 0.09 (47)	0.08 ± 0.06 (38)	0.17 ± 0.10 (23)	0.89	0.11 ± 0.10 (19)	0.17 ± 0.06 (45)	0.57

Treatments consisted of intramuscular injections of saline (control), 1000 iu of PMSG followed 72 h later by 750 iu of hCG (PMSG/hCG), single dose of PG600 (PG600) and 50μg of GnRH analogue (Gonavet). Number of animals within each treatment are given in parentheses. This table contains data from the Roseworthy piggery replicates only. Data are mean ± SEM and compared using ANOVA followed by Bonferroni *t-test*. † p value represents between treatment comparisons; ‡ p value represents between season comparisons (data from PMSG/hCG and PG600 treatment groups not compared).
[a] comparisons made by Chi Square test.

Table 6: The statistical significance between treatment groups after comparing mean values for total born piglets

Treatment group comparisons	**p value (Total born)**
PMSG/hCG vs PG600	0.33
PMSG/hCG vs Control	0.08
PMSG/hCG vs Gonavet	0.01
PG600 vs Control	0.47
PG600 vs Gonavet	0.13
Gonavet vs Control	0.38

Treatment group comparisons were generated using Student's *t-test* (SPSS v17.0).

Table 7: The effect of treatment on litter size data

	Control (n = 64)	**PMSG/hCG + PG600 (n = 85)**	**P value (ANOVA)**
Total born	10.86 ± 0.32	11.79 ± 0.26	**0.03**
Born alive	10.11 ± 0.32	10.69 ± 0.29	0.18
Stillborn	0.59 ± 0.14	0.95 ± 0.16	0.10
Mummy	0.16 ± 0.06	0.14 ± 0.06	0.85

Data are mean ± SEM and comparisons were made using One way ANOVA (SPSS 17.0). Control group consists of data from the saline and Gonavet groups.

Table 8: The effect of treatment on litter size data

	Ctl + Gon + PG600 (n = 102)	PMSG/hCG (n = 47)	P value (ANOVA)
Total born	11.10 ± 0.26	12.02 ± 0.34	**0.04**
Born alive	10.39 ± 0.25	10.55 ± 0.42	0.73
Stillborn	0.58 ± 0.96	1.28 ± 0.26	**0.003**
Mummy	0.13 ± 0.04	0.19 ± 0.04	0.47

Data are mean ± SEM and comparisons were made using One way ANOVA (SPSS v17.0). Control group consists of saline, Gonavet and PG600 treated sows.

3.1.7. The effect of parity, day length and temperature to litter size data in sows

Pearson's correlation coefficients (r^2) were calculated to investigate the relationship between parity, day length and maximum daily temperatures to progesterone production on day 10 of pregnancy, total number of piglets born, piglets born alive, stillborn piglets and mummified fetuses (Table 9). In this study, progesterone at day 10 of pregnancy had weak correlation with day length (r^2 = 0.14, p = 0.07) but a stronger significant correlation (r^2 = 0.26, p = 0.001) with maximum daily temperature (Table 9). There was no relationship between parity and plasma progesterone content at day 10 of pregnancy.

Significant positive correlations were also observed for total born piglets for parity, day length and maximum daily temperature for sows within the combined control group including Gonavet and PG600-treated sows. Likewise, total born piglets within the PMSG/hCG -treated group had a positive correlation with parity (r^2 = 0.22, p = 0.03) but failed to reach statistical significance for day length and maximum daily temperature (Table 9).

Table 9: Pearson correlations (r2) between parity, day length and average maximum temperatures (T_{max}) with plasma progesterone content at day 10 of pregnancy and litter size parameters number of piglets born (total born), born alive and numbers of still born piglets and mummified fetuses

Parameter	Treatment	n	r^2 parity	p value	r^2 day length	p value	r^2 T_{max}	p value
Progest. day 10	before Tx	160	-0.006	0.94	0.14	0.07	0.26	**0.001**
Total born	Ctl, Gon & PG600	98	0.22	**0.03**	0.30	**0.002**	0.35	**<0.001**
	PMSG/hCG	52	0.31	**0.03**	0.25	0.08	0.17	0.22
Born alive	All	148	-0.04	0.64	0.21	**0.01**	0.24	**0.003**
Stillborn	All	149	-0.03	0.70	0.09	0.26	0.10	0.23
Mummies	All	149	0.03	0.72	-0.06	0.50	0.004	0.96

Significant correlations (p < 0.05) are in bold font, n = number of sows within each treatment (Tx). Total born parameter is compared for two treatments consisting of the all the sows within the control, Gonavet and PG600 groups and separately for the PMSG/hCG group. Pearson correlations were generated by SPSS (v17.0).

3.2. Queensland Experiments

3.2.1. The effect of PMSG/hCG on pregnancy outcome

Intramuscular injection of PMSG on day 10 of pregnancy followed by hCG on day 13 did not significantly influence pregnancy rate at Pepperina (p = 0.64) or Lapunyah (p = 0.30) during the period between November and December 2008. Likewise pregnancy rates were not influenced by treatment during February and April (Table 10 and Table 11). However for Pepperina and Lapunyah piggeries, period did influence pregnancy rate by a reduction of 15 % and 18 % respectively during the period of reported seasonal infertility for control gilts and sows only (Table 11). The change in pregnancy rate in gilts and sows treated with PMSG/hCG is less between the two periods, however this is likely due to the variation between groups during the Nov-Dec period being higher than the variation between groups in the Feb-April period (Table 11). Nonetheless, there was a reduction in pregnancy rates for both piggeries during the period of reported seasonal infertility and treatment with PMSG/hCG was unable to address this.

3.2.2. The effect of period and treatment on pregnancy outcome

The effect of period on the outcome of mating for gilts and sows at Pepperina and Lapunyah piggeries are shown in Table 10 and Table 11. Comparing results individually for each treatment did not show an effect of seasonal infertility in the period Feb-Apr compared with Nov-Dec (Table 10). The effect of PMSG/hCG treatment compared with control for each piggery also did not show an effect except for Lapunyah during Nov-Dec where there was a drop in pregnancy rate (p = 0.06) in PMSG/hCG-treated sows (Table 10). This result reflects the higher number of returns to oestrus within the PMSG/hCG group and is unlikely to be a direct result of treatment as this has not been observed in other experiments within this project. The decline in pregnancy rate between the two periods was less due to PMSG/hCG treatment (Table 11), however this failed to reach statistical significance at Pepperina (p = 0.30) or at Lapunyah (p = 0.13).

Analysing the data by comparing the results of the two piggeries regardless of treatment showed an effect of seasonal infertility with a decrease in pregnancy rate of approximately 13 % in Feb –Apr compared with Nov-Dec (p = 0.005) (Table 10).

Table 10 : The effect of PMSG/hCG treatment on conception and pregnancy parameters between November and December 2008 and February and April 2009

Piggery	period	Tx	n	return	NIPs	Aborts	culls	%pregnant[‡]
Pepperina	Nov-Dec	CTL PMSG/hCG	79 72	4 3	3 5	0 1	0 2	91.1 84.7
		Comb.	151	7	8	1	2	88.1
Lapunyah	Nov-Dec	CTL PMSG/hCG	57 60	1 6	2 3	1 0	1 1	91.2 83.3*
		Comb.	**117**	**7**	**5**	**1**	**2**	**87.2**
Pep + Lap		Comb.	268	14	13	2	4	87.7[a]
Pepperina	Feb-Apr	CTL PMSG/hCG	110 115	8 8	15 22	2 0	2 0	75.5 73.9
		Comb.	225	16	37	2	2	74.7
Lapunyah	Feb-Apr	CTL PMSG/hCG	71 80	6 9	7 8	2 3	4 1	73.2 73.8
		Comb.	**151**	**15**	**15**	**5**	**5**	**73.5**
Pep + Lap		Comb.	376	31	52	7	7	74.2[b]

Data are actual values except for proportions (%) for %pregnant. [‡]Data compared using Chi Square analysis. a:b p = 0.005, * p = 0.06.

Table 11: The effect of treatment with PMSG/hCG and period (Nov-Dec vs Feb-Apr) on pregnancy rate in gilts and sows from Pepperina and Lapunyah piggeries.

Piggery	Treatment	Pregnancy rate (%)			
		Nov-Dec	Feb-Apr	P value (period)	% change
Pepperina	Control	91.1	75.5	**0.006**	15.6
	PMSG/hCG	84.7	73.9	0.08	10.8
Lapunyah	Control	91.2	73.2	**0.009**	18
	PMSG/hCG	83.3	73.8	0.17	9.5

Data are percentages and the raw data was compared using Chi Square analysis. P values are reported for differences between periods. No significant differences were observed between treatment groups in either period.

3.2.3. The effect of treatment on litter size data

Treatment with PMSG/hCG did not influence litter size data from Pepperina or Lapunyah piggeries (Table 11). Differences between total born and born alive for the two sites are likely to reflect the effect of parity on these parameters (Pepperina: gilts vs Lapunyah P1 and P2; p < 0.05) for the Nov-Dec period (Table 12).

Table 12: The effect of treatment with PMSG/hCG given during Nov-Dec 2008 compared with Feb-Apr 2009 on day 10 of pregnancy on litter size data

Piggery	period	Tx	n	Total born	Born alive	Stillborn	Mummy
Pepperina	Nov-Dec	CTL	71	10.14[a]	9.48[a]	0.57	0.10
		PMSG/hCG	59	10.63[a]	9.75[a]	0.63	0.25
Comb.			130	10.40[a]	9.61[a]	0.60	0.17
Lapunyah	Nov-Dec	CTL	52	12.48[b]	11.25[b]	0.88	0.31
		PMSG/hCG	48	12.15[b]	11.02[b]	0.67	0.44
CombT		Pep + Lap	100	12.31[b]	11.14[b]	0.77	0.37
CombTP			230	11.35	10.40	0.69	0.27
Pepperina	Feb-Apr	CTL	80	11.06	10.33	0.56	0.25
		PMSG/hCG	84	10.82	10.12	0.54	0.18
Comb.			164	10.94	10.22	0.55	0.21
Lapunyah	Feb-Apr	CTL	45	11.09	10.42	0.62	0.04
		PMSG/hCG	58	10.78	10.40	0.36	0.02
CombT		Pep + Lap	103	10.94	10.41	0.49	0.03
CombTP			267	10.94	10.31	0.52	0.24

The table presents litter size data for sows at Pepperina (Pep) and Lapunyah (Lap) piggeries during two periods in which historically normal fertility is reported (Nov-Dec) and during which historically these piggeries have experienced seasonal infertility. Treatments (Tx) are non-treated controls (CTL) and intramuscular injection of PMSG/hCG given at day 10/13 of pregnancy. Data for groups are also presented as combined for treatment (CombT) and also combined for treatment plus Period (CombTP). Data are mean ± SEM and compared using Student's *t-test* (SPSS v17.0), different superscripts are different at $p < 0.05$.

Table 13: The effect of treatment on litter size data across period and piggeries

Treatment	n	Total born	Born alive	Stillborn	Mummy
Control	248	11.10 ± 0.18	10.29 ± 0.17	0.65 ± 0.06	0.18 ± 0.03
PMSG/hCG	249	11.02 ± 0.17	10.27 ± 0.17	0.54 ± 0.06	0.21 ± 0.04

Data are mean ± SEM and compared using Student's t-test (SPSS 12.0v). No significant differences were found between treatment groups.

3.2.4. The effect of period on litter size data

The effect of period Nov-Dec vs Feb-Apr also did not influence litter size for total born, born alive, stillborn and mummies ($p > 0.05$, Table 14) when data was combined for treatment. Covariate analysis of maximum temperature and day length data with litter size data using ANOVA (Univariate analysis) also failed to reach statistical significance. Day length during these two periods varied by approximately 77 minutes (Section 2.6) and mean temperatures did not vary between treatments.

Table 14: The effect of period on litter size data across piggeries and treatments

Period	n	Total born	Born alive	Stillborn	Mummy
Nov-Dec	230	11.21 ± 0.19	10.27 ± 0.18	0.68 ± 0.07	0.26 ± 0.04
Feb-Apr	267	10.93 ± 0.17	10.20 ± 0.16	0.52 ± 0.05	0.26 ± 0.04

Data are mean ± SEM and compared using Student’s t-test (SPSS v12.0). No significant differences were found between periods.

4. Application of Research

4.1. Application of the research findings in the commercial world

Increasing plasma progesterone production during early pregnancy in sows by treatment with PMSG/hCG was associated with an increase in litter size (total born) by approximately 1 piglet. This response was observed in experiments at Roseworthy piggery in South Australia. This piggery has not recorded a drop in fertility during summer, however in this research facility the litter sizes and pregnancy rates were often lower than industry averages. The increase in total born was not associated with an increase in born alive. Whether this was due to management strategies within the farrowing sheds at Roseworthy or whether the herd at Roseworthy are close to their genetic potential for uterine capacity (Foxcroft 2009) resulting in an increase in ‘non-viable’ offspring remains to be determined.

However, the results reported in this study do confirm proof-of-concept that increasing progesterone production in early pregnancy improves embryo survival and litter size in pigs. Application of this research in the Australian pig industry has considerable ramifications to improving on-farm profits. Using IMAP (Pork CRC) value estimator software, a modest 40% industry adoption and 30% likelihood of success of an average increase of 0.9 piglet per litter (2 pigs/sow/year) leads to an industry expectation of approximately $4.1M NPV. Although the cost of production increase due to labour and cost of treatment has not been considered, it is likely to be minimal.

4.2. Opportunities uncovered by the research

A patent application has been submitted from the results of this research in March 2009. The basis of the patent application is the ‘invention’ of a method to increase litter size in pigs by increasing endogenous production of progesterone in early pregnancy. The results within this report further support this application.

In addition to using PMSG/hCG to increase progesterone production, other products are available in Europe that are analogues of GnRH, Maprelin and Gonavet manufactured by Veyx, GmbH. These products are promoted by the manufacturer to replace PMSG and hCG. Maprelin and Gonavet are not approved for use in Australia and negotiations are currently underway with Veyx regarding the use of these products in Australia. Although these products would need to be tested, advantages of using GnRH analogues include reducing the potential of antibody production and increasing the effectiveness of treatment in successive pregnancies and these products are less expensive than PMSG and hCG.

Furthermore, progesterone implants are also available from USA that have the potential to replicate the effects of increasing endogenous levels of progesterone during early pregnancy. The implants have significant advantages over the single injections of PMSG and hCG used in this study and may provide a viable treatment option for the pig industry.

Although not meeting statistical significance, treatment with PMSG/hCG reduced the impact of a drop in pregnancy rates between Nov-Dec and Feb-Apr (Table 11). If there was a greater photoperiod difference between the two periods in Queensland perhaps a significant effect would be observed suggesting that this treatment has an application for farms that experience poor pregnancy rates during periods of summer infertility or indeed any other expected environmental stresses that impact on early pregnancy loss.

4.3. Commercialization/Adoption Strategies

The PMSG and hCG treatments used in this study are available commercially in Australia from Intervet Pty. Ltd. as Folligon and Chorulon. The adoption strategy would involve the promotion of these products for use during early pregnancy. Although the manufacturers of Folligon and Chorulon have not been contacted with regard to the use of these products during pregnancy an opportunity to promote this new application exists.

4.4. Potential benefits to cost of production

As mentioned above in Section 4.1, increasing litter size by 2 pigs/sow/year has considerable cost benefits for the pig industry. Currently, the cost of treatment is less than $13.00/sow/pregnancy not including labour costs.

4.5. Ease of adoption by producers

The pig industry is familiar with PMSG/hCG and their application in oestrus synchronisation. As these products are commercially available, adoption by producers would be straight forward and relatively problem free.

4.6. Impact of the research

This research project is the first to show that increasing endogenous progesterone during the implantation period in pigs increases litter size. This increase in litter size is presumably due to reducing embryo mortality. The impact of this research is to highlight the mechanisms that may affect ovarian function during early pregnancy resulting in early pregnancy loss with resultant smaller than potential litters and high rates of returns to oestrus after mating. Furthermore, this research will generate further research into other methods to increase progesterone production in early pregnancy in the pig as well as in other livestock species. The results of this research are expected to be published in peer reviewed scientific journals.

5. Conclusion

The results described in this report and in previously submitted progress reports demonstrate the effect of treatments with GnRH analogue Gonavet, PMSG/hCG and PG600 on addressing seasonal infertility in pigs. To do this we measured the effects of treatments during early pregnancy in the pig in periods of known seasonal infertility and compared them with responses in periods of normal fertility. These include:

1. effect on ovarian follicle growth
2. effect on progesterone production
3. effect on pregnancy rate
4. effect on litter size including total born, born alive, number of still born piglets and mummified foetuses

The unifying hypothesis of identifying a treatment to address seasonal infertility in pigs was not fully supported by this research. Intramuscular injections of PMSG on day 10 of pregnancy followed by hCG on day 13 alleviated the effect on pregnancy rate in two large piggeries in southern Queensland to some degree although did not meet statistical significance in this study. However, this treatment increased litter size (total born) in a series of replicate experiments in South Australia but failed to increase numbers of piglets born alive. The piggery in South Australia did not experience typical seasonal infertility within the period of this research and performed better in the parameters of total born and born alive during the typical seasonal infertility period. Likewise in the Queensland experiment, the piggeries did not experience a drop in litter size data within the period of known seasonal infertility. Treatments with Gonavet and PG600 in the South Australian experiments had no effect on litter size or pregnancy rates.

Despite the lack of effect demonstrated to address seasonal infertility, we are confident that this work has practical application in the strategic development of:

1. treatment to increase endogenous progesterone production in early pregnancy
2. treatment to increase pregnancy rates during periods of environmental stress including seasonal infertility
3. commercial collaborations with manufactures of gonadotrophins and GnRH analogs to introduce new effective treatments to increase litter size and pregnancy rates here in Australia
4. new applications with existing commercial treatments for use in early pregnancy to increase litter size

Further research will facilitate the development of products including progesterone implants to complement current strategies to improve reproductive outcome, and profitability in the pig industry.

6. Limitations/Risks

The research reported here provided some positive responses to treatment which have considerable potential for increasing profits for the pig industry. However, with most experiments variability exists that impacts on the clear effectiveness of treatment. For example in the Roseworthy experiments many changes in the management of the sow herd were experienced and were likely to mask the effect of seasonal infertility including the lack of effect of treatments on pregnancy rates. The Queensland ‘control’ period of normal fertility did not vary significantly in mean temperatures and day length to indicate an effect of these parameters on litter size data. However, the physiological responses to treatments were clearly demonstrated at Roseworthy and considering the response of PMSG/hCG on the formation of accessory corpora lutea, progesterone production and litter size (total born) the risk of application of these research findings that were produced under less than ideal experimental conditions would be reasonably low. Considering this, the risk of these results not being applicable to the pig industry would be in the vicinity of 20-30% and the limitations would be negligible.

7. Recommendations

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

1. Continue the patent application process involving increasing endogenous levels of progesterone during early pregnancy to increase embryo survival and litter size
2. Further investigate the effect of new products that can replace the use of PMSG and hCG to increase progesterone production in early pregnancy
3. Establish closer relationships between manufacturers of gonadotrophins (Intervet, Bioniche) and GnRH analogues (Veyx, Peptech) for promoting another application for their products and supporting the access of their products in Australia
4. Investigate more rigorously the effect of progesterone (and ovarian function) during early pregnancy (progesterone implants Pork CRC proposal - O'Leary)
5. Investigate the relationship between increasing embryo survival and uterine capacity. Does increasing total born lead to an increase in numbers of stillborn piglets or is there a need to change farrowing management, or increase nutrition demands in late gestation to ensure that increases in total born result in increases in weaned pigs (Thorup, APSA, 2009)?

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