

DEVELOPMENT OF OVULATION SYNCHRONISATION PROTOCOLS TO FACILITATE NATURAL MATING AND ARTIFICIAL INSEMINATION BREEDING SYSTEMS

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Executive Summary

Artificial insemination is widely used throughout the pig industry and offers many advantages over natural breeding including increasing health management, genetic improvement and less requirement for boars in the herd. The key to its success is the deposition of viable sperm within an optimal time with respect to ovulation. AI strategies to maximise fertility need to accurately determine oestrus and this normally involves the regular use of boars for stimulation and detection of oestrus. Because the time of ovulation after weaning in sows is variable and dependent on accurate detection of standing oestrus, optimal pregnancy rates using AI normally require the use of 2 or more inseminations. The use of boars for oestrus stimulation and detection requires significant labour commitment and this procedure can account for up to 30% of the labour component in some pig management systems. GnRH analogues have the potential to streamline AI management to allow inseminations to take place at the optimal time by synchronising ovulation without the need for boar heat detection. The aim of this project was to develop an ovulation synchronisation protocol using GnRH analogues to reduce the reliance on boars and allow for a single shot- one insemination AI protocol to increase pregnancy rates and litter sizes in pigs. The GnRH analogue of choice was Gonavet® which is commercially available in Europe and is recommended for use after pre-stimulation of the sow with PMSG.

A total of 77 gilts and post weaned sows at Roseworthy in South Australia were used in replicate experiments designed to determine pregnancy outcome after synchronisation of oestrus and using one fixed-time AI. Based on the results at Roseworthy, a field trial with 380 post weaned sows at a commercial facility in SA were performed to investigate the effect on pregnancy outcome after oestrous synchronisation with Gonavet® and compare single vs double AI protocols. In addition, pre-stimulation of sows with PMSG was investigated at Roseworthy. There were no changes in pregnancy rate or litter size data in sows after synchronising ovulation using the manufacturer's protocol for Gonavet using fixed-time double and single AI. However, using the same protocol at the commercial facility showed a reduction in pregnancy rates (Control 91%; double AI 81%; single AI 74%) in post weaned sows but no change in litter size data. The different outcomes between Roseworthy and the commercial piggery is likely due to the shorter weaning to oestrus interval at the commercial site. The necessity for pre-stimulation using PMSG investigated in a small trial at Roseworthy showed that pregnancy rates and litter sizes were not different when PMSG was omitted from the synchronisation protocol. However, pre-stimulation with PMSG did improve weaning to oestrus intervals in first parity sows.

In conclusion, ovulation synchronisation protocols based on Gonavet can be designed to provide single fixed-time AI without compromising pregnancy rates and number of piglets born alive. It is necessary to determine the weaning to oestrus intervals of the herd to determine the optimal time for Gonavet injection which can be achieved by reviewing mating records of the herd beforehand. Further studies are required to determine the necessity of pre-stimulation with PMSG in a commercial setting. Significant cost savings and profits for the pig industry can be made due to fewer inseminations, less reliance on keeping boars and significant labour reductions due to heat detection.

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1. Introduction

Background and rationale for conducting the research

A major goal of commercial pig production is to maximise reproductive efficiency. Artificial insemination (AI) programs compared to natural mating systems offers considerable advantages to the pig industry by improving genetic diversity, health status and reducing the number of boars of herds without compromising reproductive performance. The success of an AI program is the accurate detection of oestrus in gilts and post weaned sows. This is done by daily oestrous stimulation and detection using boars to determine when the optimal time is for inseminations to take place. Approximately half the labour in breeding units is devoted to the detection of oestrus in sows and gilts. Currently, gonadotrophins including PMSG (Folligon, Intervet) and hCG (Chorulon, Intervet) are used in the pig industry to induce and synchronise oestrus. PG600 (Intervet) consists of a combination of PMSG and hCG for administration as a single injection.

The objective of this project is to develop a protocol for synchronising ovulation using a synthetic Gonadotrophin Releasing Hormone analogue (GnRHa). In mammals, GnRH is produced in the hypothalamus and acts on the anterior pituitary to release luteinizing hormone (LH) and follicle stimulating hormone (FSH). The LH and FSH released induces ovarian follicle maturation and ovulation following the normal physiological pattern. GnRH is a decapeptide consisting of 10 amino acids. Synthetic analogues have amino acid substitutions within the decapeptide and this can lead to an increased potency of action in the body. GnRHa have significant advantages over using PMSG and hCG for synchronising ovulation. Firstly, GnRHa stimulate endogenous production of FSH and LH compared with PMSG and hCG that have FSH-like activity and LH-like activity respectively in the body. Moreover, GnRHa have low toxicity, are rapidly metabolised in the body and are less expensive than the comparable gonadotrophins. Furthermore, due to the small molecular size of GnRH, antibody production is not elicited due to single or multiple uses ensuring efficacy of action for subsequent applications.

There is considerable variation in the time of ovulation in relation to the onset of oestrus between pigs {Kemp, 1996 #65} and the spread of ovulation in pigs can be as much as 6 hours {Soede, 1993 #71}. The use of GnRHa has been proposed as a method whereby the LH surge can be induced thereby synchronising ovulation between animals and also tighten the spread of ovulation after weaning in sows. This provides the opportunity for fixed-time inseminations ensuring viable sperm at the site of fertilisation at an optimal time in relation to ovulation.

GnRHa have been used commercially in cattle and horses to facilitate artificial insemination and there is evidence to suggest that a potent GnRH analogue can be used to synchronise ovulation in pigs (Brussow et al. 1996, Hazeleger et al. 2001). There are many GnRH analogues commercially available overseas, however to date, there are no GnRH analogues registered for use in livestock species in

Australia. At present there is only one synthetic form of the endogenous GnRH (Gonadorelin, Parnell Laboratories, Australia Pty Ltd) available in Australia which has potency well below the analogues available overseas.

This study initially investigated the use of GnRH analogue deslorelin acetate administered in sucrose acetate isobutyrate (SAIB excipient, slow release vehicle) that has been shown to synchronise ovulation in pigs (Lauderdale 2005 Provisional Patent Pub. No. US 2005/ 0130894 Assignee Thorn Bioscience). Investigating the use of deslorelin was stopped after initial experiments in favour of using Gonavet because it is already commercially available in Europe and is provided ready to use in an injectable form.

If using GnRH analogues to synchronise ovulation in gilts and sows can be developed commercially, it has the potential to:

- Increase pregnancy rates and litter size following AI by “tightening” the spread of ovulation between (and within) animals ensuring that spermatozoa are present in the tract before ovulation
- Allow AI to be undertaken without regard to oestrus detection and;
- Allow “one shot” insemination (single AI) to be used without regard to oestrus detection.

To develop this approach for use in pigs we investigated the following hypotheses:

1. Intramuscular injection of 50 µg of GnRH_a induces endogenous FSH secretion and a LH surge to initiate ovulation at a consistent time post injection.
2. Intramuscular injection of GnRH analogue allows for fixed time insemination without the need for heat detection in gilts and sows.
3. Intramuscular injection of GnRH analogue allows for fixed time insemination and increases pregnancy rates and litter sizes in gilts and sows.
4. Intramuscular injection of GnRH_a can be used without pre-stimulation with PMSG to synchronise ovulation when weaning to oestrus interval is known.

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 Australian Pork Farms Group (APFG) piggery at Wasleys in South

Australia. All experiments detailed in this report were approved by The University of Adelaide Animal Ethics Committee. Initially, a series of small replicate experiments were performed at PPPI piggery to investigate the efficacy of GnRH analogues to influence ovarian function and synchronise ovulation in gilts and parity one and parity two sows. These experiments were followed by a field trial at APFG Wasleys piggery.

2.1 Treatments

All GnRH and GnRHa treatments were administered by intramuscular (im) injection 96 h after cessation of Regumate treatment (in gilts) or 96 h after weaning (in post weaned sows). In experiments involving the use of PMSG (Folligon), PMSG was given by im injection 24 h after weaning. Treatments consisted of:

- (1) Deslorelin acetate (D-Trp⁶-,Des-Gly¹⁰-LHRH) a potent GnRH analogue was provided (gift) by Peptech Animal Health Pty. Ltd. The original stock was manufactured by Bachem AG, Switzerland. Experimental formulation of Deslorelin for use as an intramuscular injection, was prepared by weighing and mixing deslorelin in propylene carbonate (Merck, Germany; cat no. 807051) and then mixing with sucrose acetate isobutyrate (SAIB) provided (gift) by Multichem Pty. Ltd. (Melbourne, Australia) to give a final concentration of 10 µg/ml. The deslorelin treatment consisted of 5 ml injection of 50µg of deslorelin, the diluting solvent composition was SAIB : Polycarbonate 70 : 30 v/v. For experiments 1 and 2, the control groups received im injection of SAIB/polycarbonate vehicle without deslorelin. Deslorelin is reported to be 144 times more potent than natural occurring GnRH (Wenzel et al. 2002).
- (2) Gonadorelin, synthetic form of endogenous naturally occurring GnRH was purchased from Parnell Laboratories Pty. Ltd (Aust) and commercially available as 'GONAbreed' for use in cattle. The dose used was 200 µg in 2 ml.
- (3) Gonavet, a GnRH analogue was provided (gift) by Veyx GmbH (Germany) and is commercially available in Europe for oestrus synchronisation in pigs. Gonavet is a commercial preparation consisting of GnRH analogue Depherelin (D-Phe⁶-LHRH). Gonavet is in use in German pig production units and is provided ready to use. All gilts and post-weaned sows received 1 ml im injection consisting of 50 µg of Gonavet. Gonavet Veyx ® Depherelin is reported to be approximately 10 times more potent than natural occurring GnRH (Zaremba et al. 2005).
- (4) 1000 iu of Folligon (PMSG; Intervet, Holland) was used to induce ovarian follicle growth. Post-weaned sows were given Folligon 24 h after weaning.

2.2 Measurement of ovarian follicle growth and ovulation

The effect of treatment on ovarian follicle growth and detection of ovulation was assessed using trans-rectal real-time ultrasound using 3.5 MHz Sector probe (Scanner 200; Esaote Pie Medical, Maastricht, The Netherlands). Ultrasound scanning took place on all sows within the Roseworthy replicates only and were performed 24, 84, 96, 106, 118, 130, 142, 156 and 166 hours after Regumate treatment in gilts or after weaning in post-weaned sows. All scanning and ovarian measurements were made by Ms. Emmy Bouwmann from The University of Adelaide. Ovarian follicle size of four to six of the largest follicles were recorded for each sow. The time of ovulation was determined by the absence of large follicles and the presence of corpora lutea (Figure 1).



Figure 1: Real-time ultrasound scanning images of ovarian follicle growth and corpora lutea in a sow. The above image demonstrates how measurements of ovarian follicle size can be taken by ultrasound scanning at time points after weaning. In this ultrasound image, follicles of approximately 3 mm in diameter can be seen after weaning (A.), follicles of between 6-8 mm in diameter 86 to 96 h after weaning (B.) and corpora lutea 106 h after weaning (C.).

2.3 Roseworthy Experiments

The experiments at Roseworthy piggery investigated the efficacy of GnRH analogues, Deslorelin, Gonavet and synthetic GnRH Gonadorelin to stimulate FSH and LH secretion and synchronise ovulation between sows. Blood samples (approximately 10 ml) were taken by means of a chronic indwelling ear vein catheter and plasma was collected after centrifugation and stored at -20°C until assayed for FSH and LH by radioimmunoassay (Diagnostic System Laboratories, USA). Blood samples were taken before treatment and at 15 minute intervals for 2 collections, 30 minute intervals for two collections, 60 minute intervals for 6 collections, 2 hourly intervals for 3 collections, 4 hourly for 6 collection and then 2 collections 24 h apart.

The number and parity of sows within each replicate and each treatment group are presented in table 1.

Exptl. Replicate	Number of sows (parity:number)	piggery	treatment	AI	Outcome measured
1	15 (gilts)	Rwthy	Deslorelin Gonadorelin	Single	H profile, pregnancy
2	12 (gilts)	Rwthy	Deslorelin Gonavet	Single	H profile, pregnancy
3	26 (P1:9, P2:8, P3:3, P4:6)	Rwthy	Goanvet +/- PMSG	Single	Pregnancy, litter data
4	27 (P1:9, P2:9, P3:7, P4:1, P5:1)	Rwthy	Goanvet +/- PMSG	Single	Pregnancy, litter data
5	25 (P1:10, P2:9, P3:6)	Rwthy	Goanvet +/- PMSG	Single	Pregnancy, litter data
Field trial 1	69 (P1:57, P2:12)	APFG	PMSG/Gonavet	Single vs double	Pregnancy, litter data
Field trial 2	57 (P1:33, P2:24)	APFG	PMSG/Gonavet	Single vs double	Pregnancy, litter data
Field trial 3	48 (P1:16, P2:32)	APFG	PMSG/Gonavet	Single vs double	Pregnancy, litter data
Field trial 4	29 (P1:14, P2:15)	APFG	PMSG/Gonavet	Single vs double	Pregnancy, litter data
Field trial 5	42 (P1:33, P2:9)	APFG	PMSG/Gonavet	Single vs double	Pregnancy, litter data
Field trial 6	60 (P1:29, P2:31)	APFG	PMSG/Gonavet	Single vs double	Pregnancy, litter data
Field trial 7	53 (P1:45, P2:8)	APFG	PMSG/Gonavet	Single vs double	Pregnancy, litter data
Field trial 8	24 (P1:15, P2:9)	APFG	PMSG/Gonavet	Single vs double	Pregnancy, litter data

Table 2.1 Experimental replicates for Roseworthy (Rwthy) and APFG Wasley piggeries. Within each replicate sows were randomly allocated into treatment group and insemination group (AI). H: hormone profile - measure of FSH and LH content in plasma.

Proposed strategy to be followed

To demonstrate proof of concepts i.e. that litter is similar or increased using this approach, different insemination protocols using these GnRH analogs were compared with normal management practice i.e. heat detection using boar stimulation and double AI. Experiments were performed in weaned sows at Roseworthy PPPI Piggery and for field trials at APFG commercial piggery in Wasleys S.A.

Aim 1. Determine the size of the LH surge in animals injected with 50ug of GnRH analogue administered in SAIB excipient via intramuscular injection.

The potency of various GnRH analogues has been measured in different ways both in vitro and in vivo. Very little is known about the pharmacokinetics of these compounds in pigs. For example it has been suggested that Deslorelin can double the size of the LH surge (Lauderdale 2007) however this has not been compared directly in pigs. Furthermore there is little evidence to demonstrate that an excipient is necessary for the use of these analogues. As such, the first experiment examined the size of the LH surge in animals treated with 50µg Deslorelin injected intramuscularly with and without SAIB excipient and compared this to the natural LH surge. This result provides a basis (i.e. bioassay) for examining the efficacy of other analogues.

Experiment 1. Characterisation of LH and FSH production in animals treated with Deslorelin with and without excipient (carrier).

At approximately 28 weeks of age 15 gilts commenced Regumate treatment for 15 days. On day 4 (96 h) after cessation of Regumate treatment, gilts received 50µg of deslorelin acetate in saline, 50µg of deslorelin in SAIB excipient or 200µg of gonadorelin. Gilts in the control group received no treatment for synchronisation of ovulation.

- Control: 3 unsynchronised gilts injected with 5 ml of SAIB
- Treatment 1: 4 gilts injected with 50µg Deslorelin in saline
- Treatment 2: 4 gilts injected with 50µg Deslorelin in SAIB excipient.
- Treatment 3: 4 gilts injected with 200µg gonadorelin

Experiment 2. Characterisation of FSH secretion and LH surge in gilts treated with Gonavet.

Experiment 1 was repeated except the treatment using gonadorelin was replaced with Gonavet. No pre-stimulation with PMSG was used with any of the treatments in expt. 1 or expt.2.

Experiment 3. The effect of Gonavet on FSH and LH secretion with and without pre-stimulation with PMSG.

The manufacturer's recommended protocol involves the pre-stimulation of gilts and sows with PMSG. Omitting PMSG from the ovulation synchronisation protocol is in line with the goal to have a 'one shot - one insemination' protocol and this experiment was designed to investigate whether leaving out pre-stimulation with

PMSG would influence the profile of FSH and LH secretion after Gonavet treatment.

Aim 2: Determine the effect of ovulation synchronisation and fixed time insemination on conception and pregnancy outcome

Experiment 4. The effect of Gonavet treatment to synchronise ovulation with and without PMSG on ovarian follicle growth, ovulation time, and efficacy of single vs double AI.

The manufacturer's protocol for synchronising ovulation using Gonavet was investigated in 3 replicate experiments performed at Roseworthy piggery involving 78 first and second parity sows in replicates 3, 4 and 5 (Table 1). Outcomes measured in this series of experiments included measuring ovarian follicle growth after Gonavet administration, the effect with or without pre-stimulation with PMSG and the effect of advancing Gonavet treatment by 24 h (84 h vs 96 h after weaning). Pregnancy rate was determined by ultrasound scanning on day 21 post AI and litter size data was collected after farrowing.

Wasley Experiments

A field trial was designed based on the preliminary experimental results from the Roseworthy experiments and performed at APFG commercial piggery in Wasley in South Australia. The experiments consisted of 8 replicates consisting of 382 first and second parity post weaned sows (Table 1) and was designed to investigate the effect of one versus two fixed-time inseminations.

The ovulation synchronisation protocol (Recommended by the manufacturer) consisted of giving intramuscular injection of 1000 iu of PMSG (Folligon) 24 h after weaning followed by 50µg of Gonavet given 96 h after weaning. The insemination protocol was designed to accommodate the operating procedures at the commercial facility and involved fixed-time inseminations consisting of 3×10^9 sperm given the following day after Gonavet treatment. For the double insemination group AI was given at approximately 7.30am and 3.00pm, while for the single insemination group AI was given at 12.00 noon (Fig 2.1). Pregnancy rates were determined by ultrasound scanning 21 days after inseminations and litter size data was collected after farrowing. Weaning, PMSG and Gonavet injection took place from 7.30am in the morning.

The treatment groups (fixed-time inseminations) were compared to an untreated control group that received daily boar stimulation and at least two AI in the presence of a boar. The treatment groups received no boar stimulation and were inseminated in the detection-mating area (DMA) where they could have access to nose-nose boar contact. All sows within the treatment groups were inseminated at the fixed-time regardless of exhibiting signs of standing oestrus.

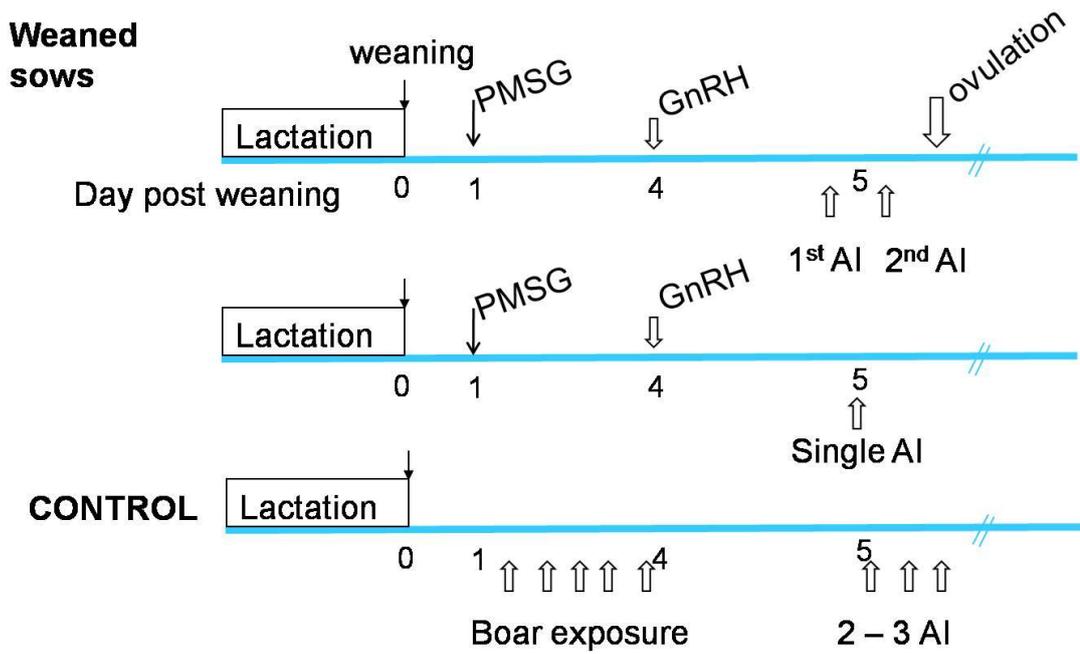


Figure 2.1 Experimental protocol for experiment 5 at APFG (Wasley) investigating the effect of fixed-time inseminations after synchronizing ovulation in post weaned sows with 1000 iu PMSG and 50 µg Gonavet. Control group received no hormone treatments and received daily boar stimulation for heat detection and 2 and in some cases 3 AI.

3. Outcomes

3.1 Roseworthy experiments

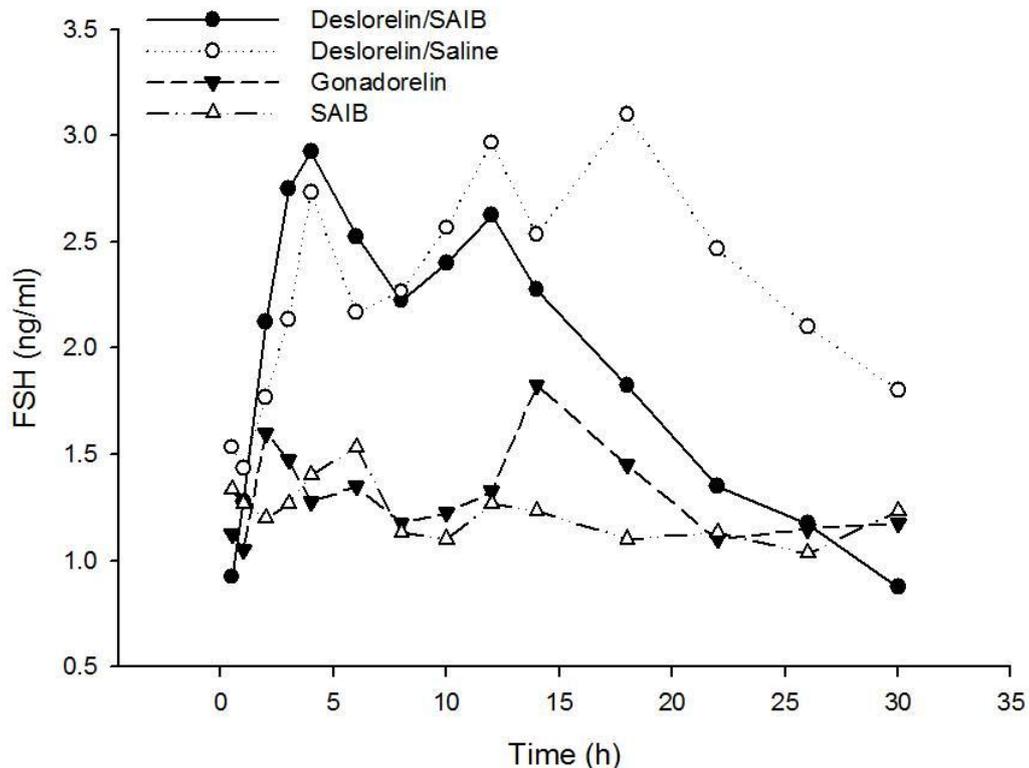
Experiment 1 : The effect of GnRHa Deslorelin on synchronizing ovulation and pregnancy in gilts

After treatment with Regumate (oral progestagen) for 15 days 15 gilts were randomly allocated into 4 treatment groups consisting of a single intramuscular injection of

1. Deslorelin /SAIB
2. Deslorelin/Saline
3. Gonadorelin
4. SAIB control

Ear vein catheterization to allow multiple blood sampling was performed 48 h after the end of regumate treatment and intramuscular injections were given 96 h after the end of Regumate treatment. A single AI was given regardless of standing oestrus behavior 24 h after treatments.

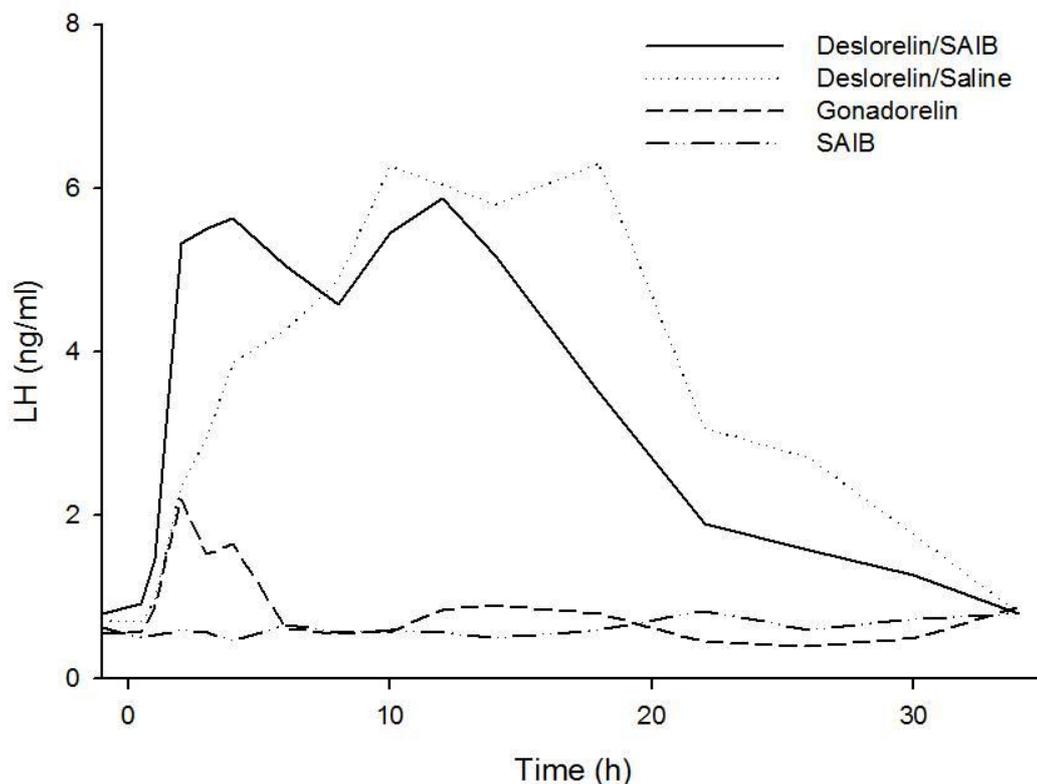
The effect of GnRH on FSH production in gilts



3.1 The effect of GnRH analogue Deslorelin on FSH secretion in gilts. Gilts were given intramuscular injection of 50µg of deslorelin in SAIB (n=4), 50µg deslorelin in saline (n=4), 200µg of Gonadorelin (n=4) and 5 ml of SAIB vehicle (n= 3). Time = 0 at injection.

Intramuscular injection of 50 μg of deslorelin acetate increased secretion of FSH in gilts. The FSH secretion induced by Deslorelin commenced shortly after the injection (within 1 h) and peaked at approximately 3 ng/ml approximately 5 h after injection. This level of secretion was maintained for between 15-20 h post administration. 200 μg of Gonadorelin did not significantly increase FSH production in this small number of gilts, however there was a rise shortly after administration and another peak approximately after 15 h although it is not clear if this was due to the treatment or the natural FSH production profile in these gilts. Injection of SAIB excipient control did not increase FSH secretion for the time period of this experiment. Moreover, there was no significant difference in the secretion of FSH when deslorelin was administered in conjunction with SAIB excipient (Fig 3.1).

The effect of GnRH on LH production in gilts



3.2 The effect of GnRH analogue Deslorelin on LH secretion in gilts. Gilts were given intramuscular injection of 50 μg of deslorelin in SAIB (n=4), 50 μg deslorelin in saline (n=4), 200 μg of Gonadorelin (n= 4) and 5 ml of SAIB vehicle (n=3). Time = 0 at injection.

Intramuscular injection of deslorelin acetate induced LH secretion and displayed a similar profile to the FSH secretion after treatment. There was an immediate rise in LH secretion after treatment and LH secretion peaked at approximately 6 ng/ml 12-15 hours after treatment. Synthetic GnRH (Gonadorelin) also induce an immediate rise in LH secretion which was comparatively short lived and peaked at

approximately 1 h after administration and returned to pre-treatment levels 7 h after treatment.

Experiment 2. Characterisation of FSH secretion and LH surge in gilts treated with Gonavet.

As for experiment 1, blood samples were taken via chronic indwelling ear vein catheters and plasma was analysed for FSH and LH content after treatment with Deslorelin, deslorelin with SAIB, Gonavet and SAIB alone.

The effect of GnRHa on FSH production in gilts

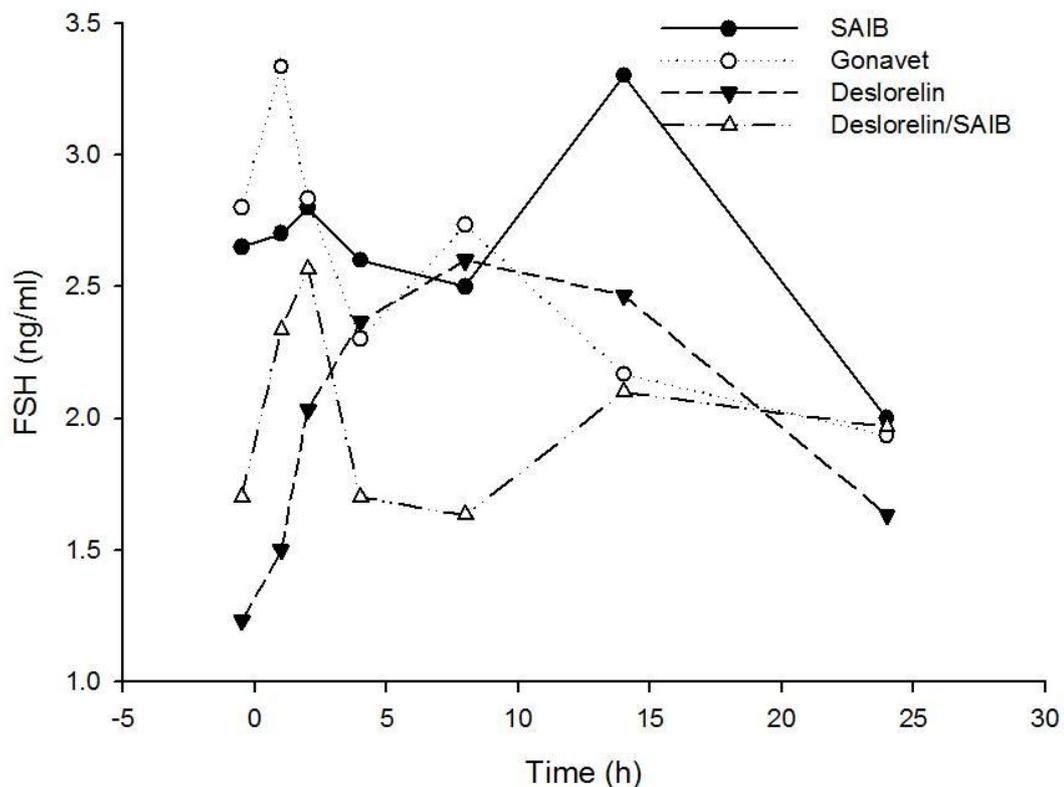


Figure 3.3 The effect of a single intramuscular injection of 50 µg GnRH analogue on FSH secretion. Treatment was given 96 h after cessation of Regumate treatment in gilts wt approximately 30 weeks of age (n=4 per treatment group).

In this experiment, 50 µg of deslorelin acetate given 96 h after cessation of Regumate treatment induced secretion of FSH immediately after treatment. In contrast to expt 1., the peak secretion was approximately 2.6 ng/ml and the duration of increased FSH secretion was 4 h in the deslorelin/SAIB group (Fig. 3.3). Pre-treatment levels of FSH in the Gonavet and SAIB-alone treatment groups were significantly higher than those for the deslorelin-treated groups. Due to the levels of FSH in this group of gilts we are unable to determine definitively an effect of treatment and the FSH profiles, although clearly influenced by treatment are more reflective of the cyclic nature of FSH secretion at this stage of oestrus post Regumate treatment.

The effect of GnRH on LH production in gilts

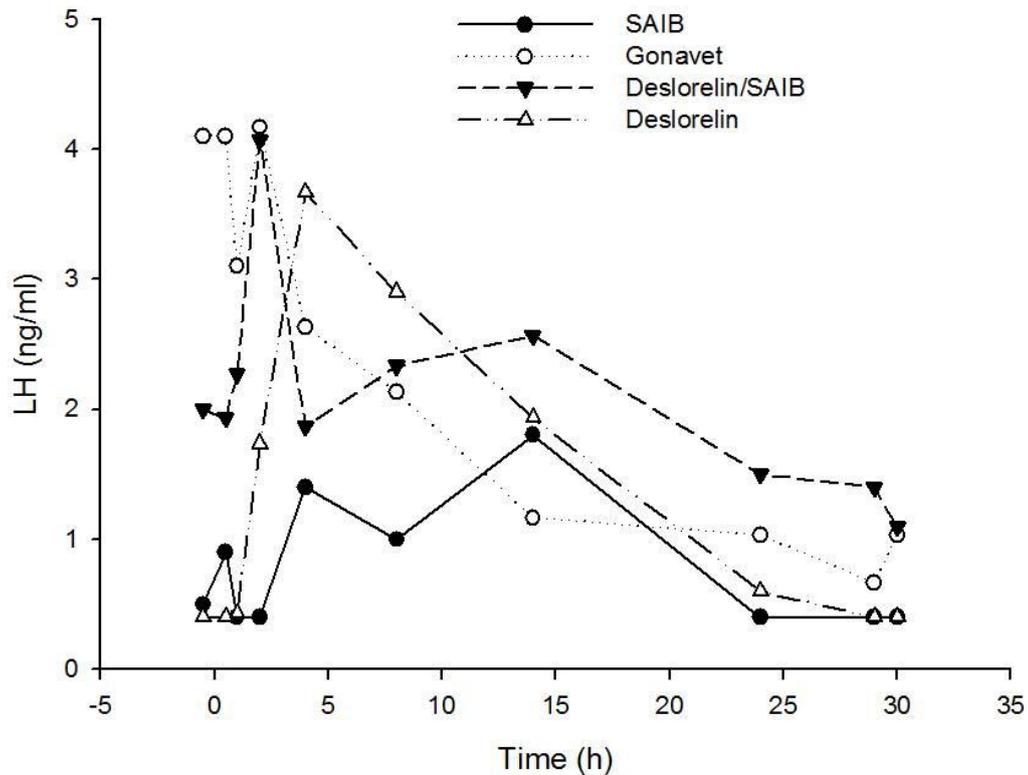


Figure 3.4 The effect of GnRH analogue on inducing LH secretion in gilts. Treatment consisting of 50 µg of GnRH_a were given 96 h after cessation of Regumate in gilts (n= 4 per group).

A similar profile of secretion was seen for LH (Fig 3.4) as was observed for FSH (Fig 3.3) in this cohort of gilts from Roseworthy piggery. The pre-treatment levels of plasma LH in the Gonavet treatment group were high and possibly reflective of these gilts already in the midst of a LH surge and were soon to ovulate. Treatment with Deslorelin induced LH secretion immediately after treatment and there was no significant difference in pattern of expression when Deslorelin was given with SAIB excipient.

Interestingly, the negative control group that received SAIB alone showed an increase in LH secretion within 5 h of treatment that was sustained until 15 h after treatment. This result likely reflects an earlier ovulation time after cessation of Regumate for the Gonavet and SAIB-alone treatment groups compared with the Deslorelin groups. Alternatively, all the gilts within this cohort of animals may have a shorter duration of cessation of Regumate to ovulation interval and this was masked in the Deslorelin groups due to the higher potency of action of this GnRH analogue.

Experiment 3. The effect of Gonavet on FSH and LH secretion with and without pre-stimulation with PMSG.

As for the previous experiments, blood samples were collected and assayed for FSH and LH content after treatment with Gonavet.

The effect of pre-stimulation with PMSG on FSH production after Gonavet treatment in gilts

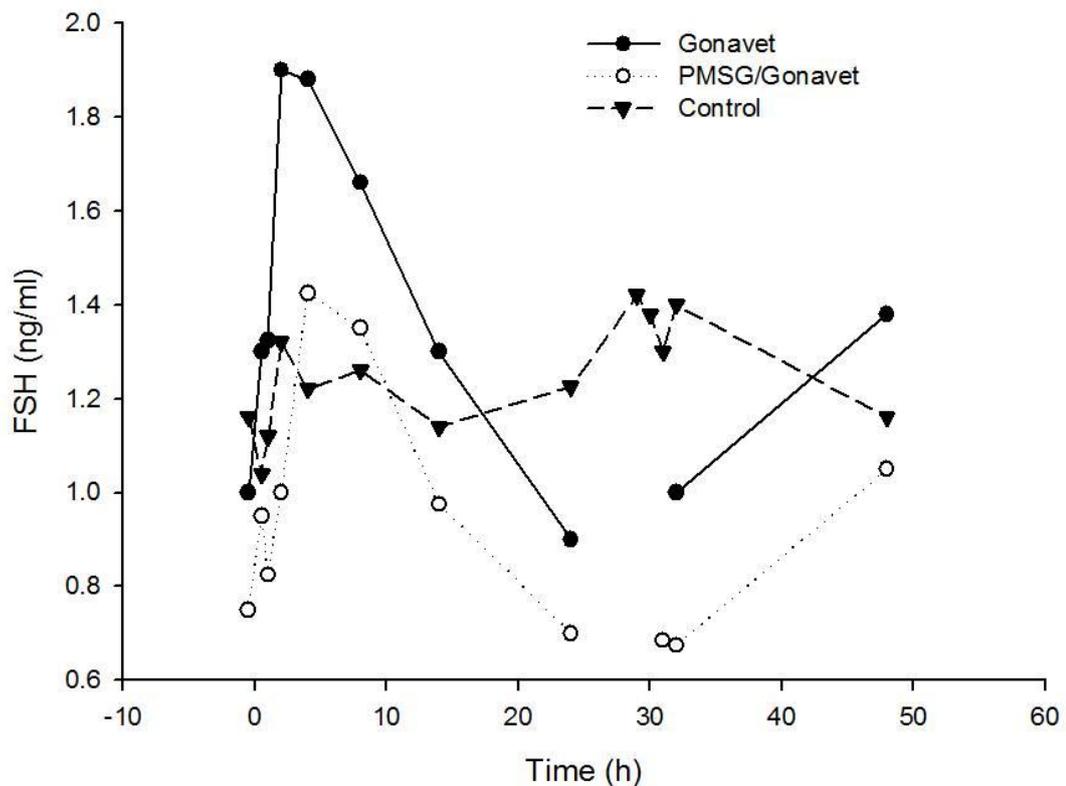


Figure 3.5 The effect of pre-stimulation with PMSG 24 h after cessation of Regumate treatment on FSH secretion after Gonavet treatment in gilts.

Intramuscular injection of 50 mg of Gonavet induced secretion of FSH 96 h after cessation of Regumate treatment in gilts. There was no significant difference in the plasma FSH levels due to pre-stimulation with PMSG or the duration of FSH secretion (Fig. 3.5). The control group (saline injection) displayed a relatively high level of FSH secretion and reflects the cyclic nature of FSH secretion for this stage of oestrus after cessation of Regumate. There were no significant differences in FSH secretion between the control gilts and the PMSG/Gonavet group in the first 10 h after treatment.

The effect of pre-stimulation with PMSG on LH production after Gonavet treatment in gilts

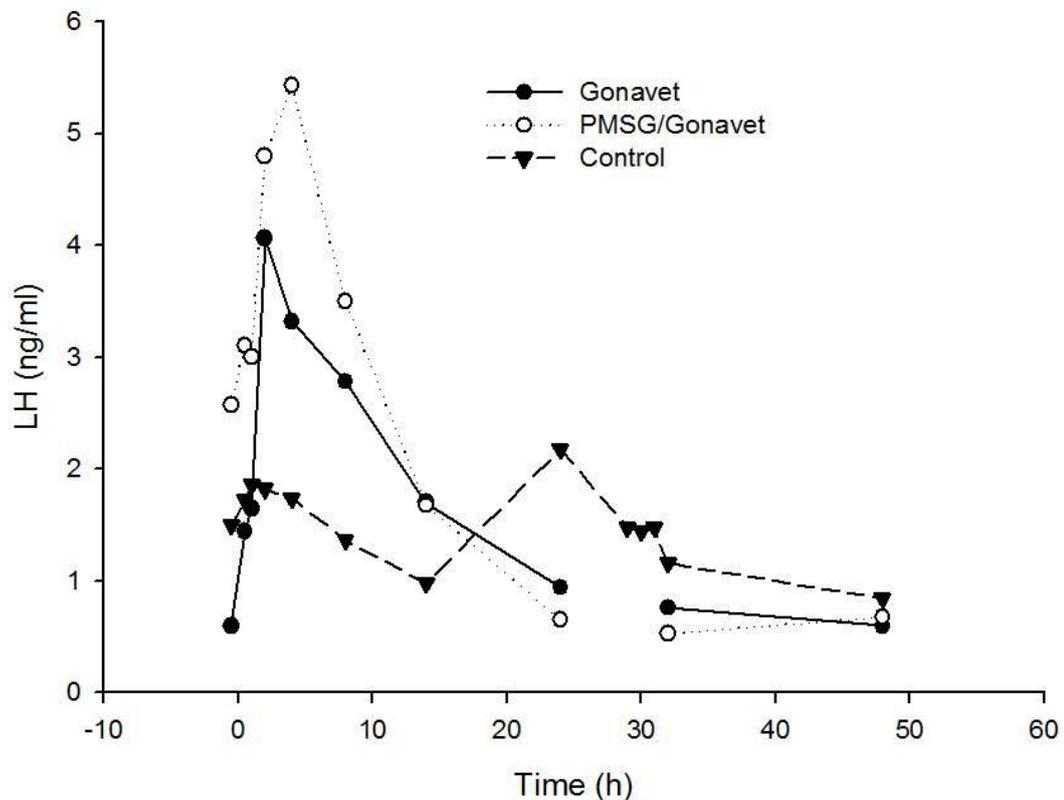


Figure 3.6 The effect of pre-stimulation with PMSG 24 h after cessation of Regumate on secretion of LH in gilts after Gonavet treatment.

Intramuscular injection of Gonavet given 96 h after cessation of Regumate treatment immediately induced secretion of LH in gilts. Peak secretion was observed before 5 h after treatment at approximately 4 to 5.5 ng/ml and pre-treatment levels were observed after 15 h (Fig. 3.6). There was no significant effect on LH secretion due to pre-stimulation with PMSG.

3.3 The effect of pre-stimulation with PMSG and time of Gonavet administration after weaning on time of ovulation and pregnancy rates in sows

Two factors effecting pregnancy rates and litter sizes in gilts and sows are the time of insemination and weaning to oestrus interval. Insemination with good quality sperm must be performed between 28 h before to 4 h after ovulation (Nissen et al. 1997). Inseminations outside of this period lead to lower pregnancy rates and smaller litter sizes. Weaning to oestrus intervals vary due to management of sow lactation, season, parity and may vary between herds and is typically between 4 and 8 days in length (Dewey et al. 1994). If the weaning to oestrus interval of a herd is 4 days and these sows are given Gonavet on day 4 (viz

96 h after weaning) then a single AI given 24 h later could fall outside the optimal time for insemination.

The objective of this study was to investigate the need for priming sows with PMSG 24 h after weaning and to determine whether earlier administration of Gonavet may improve pregnancy rates in post-weaned sows. A series of experiments using 4 different synchronisation regimens were performed. All sows in the oestrous synchronised groups received a single AI 24 h after Gonavet injection and did not receive any boar stimulation. Real-time transrectal ultrasound scanning was performed on all sows to determine the time of ovulation after Gonavet treatment. Pregnancy rate (PR) was determined using ultrasound 21 days after AI. In total, 77 first and second parity sows were randomly allocated into 5 groups:

- 1) PMSG at 24 h after weaning followed by Gonavet at 84 h after weaning. Single AI given 24 h after Gonavet injection.
- 2) Gonavet 84 h after weaning. Single AI given 24 h after Gonavet injection.
- 3) PMSG at 24 h after weaning followed by Gonavet at 96 h after weaning. Single AI given 24 h after Gonavet injection.
- 4) Gonavet 96 h after weaning. Single AI given 24 h after Gonavet injection.
- 5) Control group where sows were exposed to boar stimulation daily after weaning and inseminated with 2 to 3 AI after standing oestrus was detected.

Advancing the injection of Gonavet by 24 h (84 vs 96 h after weaning) improves the synchronising effect of oestrus in this cohort of sows at Roseworthy piggery (Table 2). Not taking PMSG treatment into account, there were fewer sows that ovulated before the optimal time before 34 h after Gonavet (84 h 9.3 % vs 96 h 29%) and a higher PR in sows that ovulated during the optimal time compared with those sows in the 96 h group (84 h 95% vs 96 h 81%).

Oestrus synchronisation treatment (n)	Ovulation after Gonavet administration					Sows that did not respond (follicles at WOI > 7 d)*
	22-34 h (early)		34-46 h (optimal)		46-58 h (late)	
	n	PR (%) [†]	n	PR (%)		
PMSG/Gon 84 h (15)	3	2/3 (67%)	10	9/9 (100%)	0	2 (first parity)
Gon 84 h (17)	0		13	12/13 (92%)	0	4 (first parity)
PMSG/Gon 96 h (15)	6	5/6 (83%)	9	7/8 (88%)	0	0
Gon 96 h (16)	3	3/3 (100%)	9	6/8 (75%)	0	4 (3/4 first parity)
Control (14)			13	13/13 (100%)		1 (first parity)

Table 3.1 The effect of oestrus synchronisation on pregnancy rate (PR) and the time of ovulation after Gonavet administration in post-weaned sows. Oestrus in post-weaned sows was synchronised using PMSG 24 h after weaning and Gonavet given at either 84 h or 96 h after weaning (PMSG/Gon). The need for pre-stimulation with PMSG was investigated by omitting this treatment (Gon). The time of ovulation after Gonavet was determined by real-time ultrasound scanning of the ovaries, *number of sows that did not respond to treatment and had immature follicles present for more than 7 days after weaning. The control group consisted of sows that received boar exposure from 24 h post weaning up until second and in some cases third AI.[†]PR was determined as the % of sows with a positive pregnancy diagnosis by ultrasound scan 21 days after AI.

The ‘one shot one insemination’ protocol (no PMSG pre-stimulation) using this relatively small number of animals did not show conclusively an improvement in the time of ovulation after Gonavet treatment nor a significant difference in PR. However, the need for pre-stimulation with PMSG 24 h after weaning is very likely to be needed for first parity sows as all the sows that did not respond to treatment were first parity in the 84 h time group and 3 out of 4 sows that did not respond to treatment in the 96 h were first parity (Table 3.1). All the sows in the control group, that received no hormonal treatment and received boar stimulation and double AI, ovulated during the optimal time post detection of standing oestrus although considering the number of animals within each group this is unlikely to be significant in a commercial setting.

Treatment (n)	culls*	Farrowing rate	Total born	Born alive	Stillborn
Control (14)		86% (12/14)	11.3 ± 2.6	10.8 ± 2.6	0.6 ± 0.9
Gonvet 84 h (17)		65% (11/17)	14.9 ± 1.6	13.2 ± 1.5	0.9 ± 0.9
Gonavet 96 h (15)	1	80% (12/15)	12.0 ± 3.3	10.5 ± 3.2	1.4 ± 1.9
PMSG/Gon 84 h (14)	1	86% (12/14)	12.7 ± 3.2	11.1 ± 2.8	1.7 ± 1.9
PMSG/Gon 96 h (14)	1	86% (12/14)	13.0 ± 3.2	12.2 ± 2.8	1.0 ± 0.3

Table 3.2 The effect of oestrous synchronisation on pregnancy and litter size in parity 1 & 2 sows after single fixed-time AI. Control sows were mated with 2 AI without oestrous synchronisation, and after boar stimulation and detection of oestrus. Data are mean ± SD. Litter size data were compared using One Way Anova (SPSS) with Bionferoni correction; no significant differences between groups detected. Pregnancy rate data were compared using CHI Square test (Excel) no significant difference found; $p > 0.05$. *culls: number of sows that were taken out of the trial and culled due to lameness, abortion or unrecorded reason (these sows were not included in the farrowing rate calculation).

Single fixed-time insemination following ovulation synchronisation with Gonavet at 84 h or 96 h after weaning did not affect the numbers of total born or born alive piglets (Table 3.2). In this cohort of animals, pregnancy rate was 21% lower compared to controls and other treatment groups when Gonavet was given at 84 h post weaning and without pre-stimulation with PMSG. This relatively small study supports single fixed-time insemination after ovulation synchronisation with Gonavet. As mentioned previously with regard to ovulation time relative to insemination, pre-stimulation with PMSG is most likely not required for parity 2 and above sows but helpful (and possibly necessary) in developing ovarian follicle growth in first parity sows.

Experiment 5. The effect of single versus double AI on pregnancy rate in sows after oestrous synchronization with PMSG / Gonavet (Wasley field study)

In a field trial at a commercial piggery in SA, oestrous synchronisation with PMSG 24 h after weaning followed by Gonavet 96 h after weaning influenced pregnancy rate using fixed-time single and double AI (Table 1). Pregnancy rate dropped between 10 and 17 % using fixed-time the following day after Gonavet injection. The drop in pregnancy rate is most likely due to the average weaning to oestrus interval at this piggery of only 4 days after weaning and hence more inseminations in the fixed-time AI groups were given after ovulation. Therefore, Gonavet was given on the day when a large number of these sows would have normally ovulated. The litter size data was not significantly different between the fixed-time AI groups and the controls (Table 3.3).

	Wasleys herd average (mixed parity)	Control (parity 1+2)	Double A.I. (parity 1+2)	Single A.I. (parity 1+2)
	(n= 285)	(n= 131)	(n= 125)	(n = 124)
NIP	33	11	24	33
Did not come into oestrus	8	1	0	0
Pregnant	244	119	101	91
% Pregnant	86% ^a	91% ^a	81% ^b	74% ^b

Table 3.3 The effect of fixed-time single vs double AI on pregnancy rate. After synchronisation of oestrus with PMSG given 24 h after weaning and Gonavet 96 h after weaning, pregnancy rates were determined in parity 1 & 2 sows. Pregnant: number of sows with a pregnancy diagnosis after ultrasound on day 28 post AI. Data was compared using CHI Square (Excel) a:b <0.05.

Reps 1-8	Control (Parity 1+2)	Double A.I. (parity 1+2)	Single A.I. (parity 1+2)
Farrowed	n=104	n= 92	n= 81
% farrowed*	87%	79%	71%
Culled, died, destroyed*	n= 9 (can't find 2)	n = 7 (can't find 2)	n = 9 (can't find 1)
Total born	9.8 ± 3.0	11.2 ± 2.8	11.0 ± 3.5
Born alive	9.1 ± 3.1	10.1 ± 2.9	10.5 ± 3.2

Table 3.5 The effect of fixed-time single vs double AI on numbers of piglets born per litter. After synchronisation of oestrus with PMSG given 24 h after weaning and Gonavet 96 h after weaning litter size data was recorded in parity 1 & 2 sows. * Sows that returned a positive pregnancy after day 28 but were culled later for reasons including lameness, vaginal discharge, body condition and NIP after day 28; can't find were sows with double ID, or ID not present in computer records at piggery. Data were compared using One Way Anova (SPSS) with Bionferoni correction; no significant differences between groups detected. *% farrowed did not include the number of sows that were culled or those where data was not found.

The field trial performed at a commercial piggery at Wasley in South Australia provided valuable information with regard to designing and implementing an ovulation synchronization program involving fixed-timed inseminations. Firstly, the time of Gonavet treatment must be made cognisant of the weaning to oestrus interval for the herd for the time of year. Piggery records can be easily accessed to assess this for parity and for season. Knowledge of the weaning to oestrus interval allows the determination of the time for Gonavet treatment after weaning. However, the results here do not negate the use of fixed-time AI in a commercial setting. Gonavet administration on day 4 after weaning for sows with a weaning to oestrus interval of 4 days would mean that the fixed-time insemination was between 12 and 24 h after the optimal time for insemination. This situation for a significant number of sows has led to the reduction of

pregnancy rates in the single and double insemination groups compared with control and with the mixed parity herd average.

4. Application of Research

The research contained within this report investigated the effects of an ovulation synchronization program to facilitate single fixed-time inseminations in gilts and sows. Although results of fixed-time inseminations reduced pregnancy rates in some replicates (and overall farrowing rate at the commercial facility), the findings contain herein do provide a method of using fixed-time single AI without compromising litter sizes and together with prior knowledge of herd weaning to oestrus intervals will provide comparable pregnancy rates to current piggery mating protocols.

The benefits to the pig industry of using an ovulation synchronization protocol are significant. The cost of production will be dramatically reduced due to labour savings involved with heat stimulation and detection and the reduced number of boars that need to be kept in the herd. Also, the direct savings involved in using at least one less insemination per mating cycle are considerable for larger piggeries. Veyx GmbH manufacturers of Gonavet have provided promotional material indicating that use of their products Maprelin (promoted to replace PMSG) and Gonavet increase farrowing rate and reduce weaning to oestrus interval and therefore provide less 'empty' days per year within the herd. The less 'empty' days provide considerable savings in feed costs and allow for more piglets per year. If we just use the observations contained within this report for 'one shot single insemination' with the same pregnancy rate and litter size as currently employed methods of mating produce, then the savings to a piggery of 3000 sows conservatively would be in the vicinity of \$50K per year for less AI doses alone. Add to this figure less labour required for heat detection and more sows per worker in the mating sheds then this saving to cost of production would increase significantly above this figure. Moreover, less boars that need to be kept also contribute to considerable savings per year. The cost of purchasing Gonavet in Australia is not known at present but is expected to be approximately \$4 to \$6 per treatment. The cost of PMSG is approximately \$10 per treatment however Veyx have Maprelin which has considerable advantages over using PMSG and is less expensive.

4.1 Opportunities uncovered by the research

Fixed time inseminations offer considerable benefits to the pig industry

and there are a number of products that may assist this. Gonavet has considerable advantages over developing our own formulations for controlling and synchronizing ovulation in gilts and post weaned sows. Gonavet is already being used in commercial piggeries in Germany and toxicity and efficacy studies have already been done with this product. The registration of Gonavet for use in Australia is likely to be accomplished with relative ease and within a relatively short period of time. This means adoption of this product and a fixed-time insemination protocol by the Australian pig industry should be possible in a timely manner. Veyx GmbH has also committed assistance to this process and has been supportive of this project since its inception.

4.2 Commercialization/Adoption Strategies

After facilitating the registration of Veyx's Gonavet and Maprelin for use in Australia, the adoption strategy would be to promote the use of fixed-time AI in a controlled way in larger piggeries. This would involve an education promotion strategy regarding ovulation synchronization and the use of hormones in this process. Also, this would involve the importance of knowing the weaning to oestrous intervals of a herd taking into account parity and season.

- Potential benefits to cost of production

As mentioned above in Section 4.1, significant reductions in cost of production can be made utilizing fixed-time single insemination protocols.

- Ease of adoption by producers

The pig industry is familiar with the use of hormones and their application in oestrus synchronization. The reduction in the use of boars for oestrus detection may not be accepted by all piggery managers and may take longer for the industry to accept. In addition, insemination in sows not exhibiting strong oestrous behavior may take time for some piggeries to adapt to. These are issues can easily be addressed.

- Impact of the research

This is the first ovulation synchronization study involving fixed time inseminations performed in Australia. In addition, we have collected physiological responses regarding hormonal profiles and ovarian follicle growth. The data contained herein contributes to growing bank of information regarding the control of ovarian function in pigs and how this can be applied directly to industry. Furthermore, this research will generate further research refining and improving mating protocols based on ovarian physiology for the pig industry and the results of this research are expected to be published in peer reviewed journals.

5. Conclusion

Closing summary of Research

The results described in this report and in previously submitted progress reports demonstrate the effect of using GnRH analogues to synchronise ovulation to facilitate single fixed-time inseminations in gilts and sows. This involved measuring the ovarian responses to different synchronization protocols and the efficacy of fixed-time inseminations without heat detection. The GnRH analogue of choice was decided to be Gonavet manufactured in Germany and commercially available in Europe. Gonavet is a relatively inexpensive product designed to be used in gilts and weaned sows and its use with fixed-time inseminations can produce pregnancy rates and numbers piglets born comparable to presently used labour-intensive protocols used in the pig industry.

The results contained herein and potential benefits to industry do provide the basis to continue research with Gonavet. Our preliminary results suggest the pre-stimulation with PMSG may not be required for parity 2 and above sows. To limit repeated use of PMSG has considerable benefits including reducing the cost of this treatment. In addition, the experiments contained herein are expected to facilitate the registration process for Gonavet to allow use of this product in Australia.

6. Limitations/Risks

To the application of the research findings

The research reported here highlight the economic benefits involved in using ovulation synchronization protocols to facilitate fixed time AI. However, with most experiments variability between animals and between herds may impact of the clear effectiveness of our protocols. The weaning to oestrus intervals are different from the Roseworthy herd and the Wasley piggery and management regimens differ and impact differently with regard to pregnancy rates in commercial herds compared with smaller research based herds. Notwithstanding, there were clear repeatable effects of treatment at both sites and the benefits outweigh the risks (which would be identified quickly) in the use of GnRH analogues.

Although not investigated within this study, sows that did not respond to Gonavet in the first instance are not likely to suffer any negative effects which may affect return to oestrus or subsequent ovarian function. This is due to the nature of GnRH analogues having short duration of action in the body and their use not influencing the immune status of the sow.

7. Recommendations

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

- (1) Investigate the use of boar stimulation at the time of fixed-time insemination. This is likely to increase the oestrus behavior and uptake of semen at the time of insemination. This will also improve confidence in the AI technician that the mating has been or is likely to be successful.
- (2) Investigate increasing the concentration of spermatozoa in the fixed-time single AI dose. A proposal has been submitted with regard to this as an increase in pregnancy rate was observed in double vs single AI. The double inseminations took place on the same day and the increase in PR may have been due to more spermatozoa present in the tract before ovulation.
- (3) Further investigate the use of pre-stimulation with PMSG at a commercial facility. This would be done after determination of the weaning to oestrus interval of the herd.
- (4) Commence the Australian registration of Gonavet and Maprelin with the Australian Pesticide and Veterinarian Authority to facilitate adoption by the pig industry in the shortest period of time.
- (5) Investigate the use of Maprelin which is also a GnRH analogue promoted to replace the use of PMSG. Maprelin has considerable advantages over PMSG with regard to repeated use (in successive oestrous cycles) in sows.

8. References

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Appendix 1 - Notes

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Appendices

Appendix 1: