Influence of birth litter size and suckled litter size on gilt ovarian development

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Executive Summary

Background. The sub-optimal reproductive performance of sows (i.e. low litter size and reduced farrowing frequency) and insufficient longevity of breeding sows continue to constrain the efficiency and profitability of pig production. This problem persists despite a considerable body of work focussing on post-natal strategies (i.e. gilt management) and lactation strategies (i.e. nutritional interventions) to improve sow longevity. It is, therefore, evident that a new approach to solve reproductive insufficiencies and failures is required. It is evident in ruminant species that conditions experienced pre-natally determine the reproductive potential and longevity of the breeding female. Further, there is preliminary evidence that the pre- and neo-natal environment affect the fertility and fecundity of gilts. It is, therefore, suggested that improvements in sow reproduction and longevity require optimisation of the pre- and neo-natal environment to ensure that ovarian development and responsiveness to endogenous gonadotrophins are maximised. The body of work reported here had two aims; one, to determine the effect of pre-natal conditions and neonatal conditions on ovarian development and responsiveness to boar stimulation at a young age; two, to determine whether plasma levels of Anti-Mullerian hormone early in life were predictive of ovarian development and responsiveness to boar stimulation at a young age.

Methods. To achieve the aims, a total of 101 gilts (Camborough F1 x PIC 400) were selected from birth litters of ≤ 9 (Small) or ≥ 12 (Large). Approximately 24 hours after birth, male pigs were cross-fostered onto small litters to achieve suckled litter sizes of 12. In this way, a two times two factorial arrangement of treatments was created, as follows: birth litter size ≤ 9, suckled litter size 9 (Small – Small); birth litter size ≤ 9, suckled litter size 12 (Small – Large); birth litter size ≥ 12, suckled litter size 9 (Large – Small); birth litter size ≥ 12, suckled litter size 12 (Large –Large). Gilts were individually identified and their weights recorded at 24 hours of age, at weaning, 20 weeks of age, and slaughter. The P2 backfat depth was measured at 20 weeks of age. Carcass weight and dressing percentage were also recorded. A blood sample was collected at weaning and 20 weeks of age, and assayed to determine levels of anti-mullerian hormone (AMH). From 20 weeks of age gilts received daily exposure to a mature boar for 14 days. Thereafter, gilts were marketed at normal commercial weights. Ovaries were recovered from each gilt, and the following data collected from each animal: the total number of corpora lutea (CL), number of corpora albicantia (CA) and the total number of surface antral follicles greater than 1 mm in diameter.

Results. The total number of surface ovarian follicles was unaffected by treatment. However, being born into a large litter and reared in a large litter resulted in a numerical, but not significant, reduction in total surface follicle number at slaughter. The number of corpora lutea present on the ovaries of pubertal gilts was numerically, but not significantly higher, for gilts born in a Large compared to Small litter and for gilts reared in a Large compared to Small litter. When non-pubertal animals were included in the analysis, there was a tendency ($P=0.1$) for gilts born in Large litters to contain more CL than those born in Small litters (7.8 ± 1.00 versus 3.8 ± 1.56 CL). There was also a tendency ($P < 0.1$) for a higher proportion of gilts born in a Large litter and reared in a Small litter to reach puberty compared to gilts born in a small litter and reared in a small litter.

Gestated litter size did not affect AMH levels. However, gilts reared in a Small compared to a Large litter had significantly higher AMH levels at weaning. The incidence of puberty attainment was higher ($P < 0.2$; 0.62 versus 0.45) for gilts with high (> 8.3 ng/ml) compared to Low (< 8.3 ng/ml). There was an interaction between gestation litter size and AMH levels at weaning and between rearing litter size and AMH levels at weaning with regard to the incidence of puberty attainment. Within the large

gestated litter size treatment, a higher (P < 0.05) proportion of gilts with High weaning AMH levels reached puberty compared to gilts with Low weaning AMH levels (0.74 versus 0.42). Within the Large lactation litter size, the proportion of gilts reaching puberty tended (P < 0.1) to be higher in animals with a High compared to a Low weaning AMH level (0.63 versus 0.38).

It is apparent from the current data that higher concentrations of anti-mullerian hormone (AMH) at weaning are associated with an increased capacity to exhibit a pubertal response to boar exposure at a young age (140 days). Importantly, this relationship is stronger for gilts born into large gestated litter sizes, with 74% of those gilts with high AMH reaching puberty in response to boar stimulation, compared to only 42% of gilts with low AMH levels. Similarly, of those gilts reared in large litters, the incidence of puberty attainment was 63% in the cohort of animals with high AMH compared to 38% of the low AMH cohort. This finding supports previous work from our group indicating that early measures of plasma AMH more accurately predict fertility.

The data from the current study, whilst preliminary, also appears to indicate a higher reproductive potential for gilts born into a Large compared to a Small litter, as evident from the numerical improvements in puberty attainment and ovulation rate for these animals. More importantly, it also appears that the capacity of gilts born into large litters to reach their reproductive potential, in terms of their ability to ovulate in response to early boar stimulation, may be impaired when they are reared in a Large litter. This outcome is evident from the improved pubertal response observed when gilts from Large litters are reared in small litters when compared to gilts born into Small litters. Although further work is required to understand why reared litter size affects reproductive development of piglets with high reproductive potential (i.e. born in large litters), a reduction in nutrient intake, via milk, or indeed colostrum intake could be involved. The impact on uterine development of bioactive compounds in colostrum and milk (lactocrine factors), has become increasingly evident from new research. However, the impact of these bioactives on ovarian development has yet to be investigated. Alternatively, the capacity of reared litter size to affect piglets born in large litters, and therefore with lighter birthweights, may suggest that the reproductive development of these lighter piglets is more sensitive to nutritional intake and availability of bioactives in milk.

Together, the current data provide support for the use of plasma AMH levels at weaning to identify gilts which are more likely to be responsive to boar stimulation, especially when selecting gilts born into, or reared in, large litters. It also appears that reproductive development and fertility of gilts born into large litters may be improved if they are reared in a smaller litter. Further work is required to establish the relationship between AMH levels at weaning and ovarian development, and the relationship between gestated litter size, rearing litter size, AMH and lifetime productivity of the breeding sows. Specifically, the investigators recommend that additional work is conducted to determine the impact on fertility and reproductive longevity of rearing gilts born in large litters in small litters and selecting gilts based on high AMH levels at weaning (or earlier).

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1. Background to Research

Within Australian sow herds, the average sow replacement rate is approximately 55% (Australian Pig Annual, 2010), with reproductive failure documented to be the largest single cause for prematurely culling of sows. This reproductive failure is most evident amongst younger sows, particularly primiparous sows (D'Allaire and Drollet, 2006), with inadequate lactation nutrient intake the primary cause. Since litter sizes are maximal between parities three and six, improved sow retention rates will increase herd average litter size and individual sow lifetime piglet production, as well as providing a positive return on gilt investment costs.

Various strategies involving gilt management may be employed in an attempt to improve sow retention in the early parities; however, premature reproductive failure and, therefore, culling remain a significant issue. An aspect of gilt management which has received little attention is the potential impact of prenatal and neonatal maternal environment on subsequent fertility in the gilt. Understanding the extent to which pre- and neo-natal conditions affect reproductive potential of gilts, and understanding the mechanisms involved, would be a significant benefit to the pig industry. Specifically, the capacity to increase reproductive output and longevity of replacement females using a method as simple as cross-fostering to alter litter sizes would be of enormous benefit to the Australian pig industry. Equally beneficial would be the capacity to use a single plasma measure of circulating AMH to identify, and therefore select, the more fertile gilts.

2. Objectives of the Research Project

The aims of this project are; one, to demonstrate a link between birth and / or suckled litter size and gilt ovarian development; two, to determine the relationship between gilt ovarian development and levels of anti-mullerian hormone.

3. Introductory Technical Information

As previously indicated, current understanding of the impact of pre- and neo-natal conditions on reproductive development, fertility and reproductive longevity is in its infancy, particularly in pigs. There is growing evidence in cattle that alterations in maternal nutrition during gestation affect the reproductive potential of her female progeny through changes in the number and function of ovarian follicles. Early work in pigs (Nelson and Robinson, 1976) in which cross-fostering was used to create large and small litters, demonstrated higher ovulation rates and litter sizes in gilts raised in small litters. More recently, the data of Tummaruk et al. (2001) supported a prenatal affect, with gilts born into a larger as opposed to a smaller litter farrowing more piglets in every parity. A recent study conducted on a large commercial facility in North Carolina examined productivity and longevity of sows originally raised in small or large litters (Flowers 2012). Differences were attained by cross fostering and performance monitored through 6 parities. It was clearly evident that being raised in a small litter provided benefits for piglet production and longevity with 35% retention to parity 6 for sows raised in a small litter vs. 17% of those raised in a large litter. The mechanisms involved in effects of birth litter size or suckled litter size on gilt/sow performance have yet to be determined. However, there is strong evidence in cattle to link nutrient availability in utero with the size of the ovarian follicular reserve and ovarian responsiveness to gonadotrophins. Further, evidence in cattle has demonstrated that levels of anti-mullerian hormone (AMH) are positively correlated with numbers of ovarian antral follicles and also the size of the ovarian follicle reserve. This may impact potential reproductive lifespan. Although speculative, it is possible that pre- or neonatal environments may affect levels of AMH.

4. Research Methodology

A total of 101 gilts (Camborough F1 X PIC 400) were selected from birth litters of ≤ 9 (Small) or ≥ 12 (Large). All animals remained on their birth mothers for at least 12 hours post-partum to establish appropriate passive immunity. Of these, at approximately 24 hours of age, male pigs from large litters were cross-fostered onto small litters to achieve suckled litter sizes of 12. In this way, a 2 x 2 factorial arrangement of treatments was created, as follows:

1) Birth litter size ≤ 9, suckled litter size 9 (Small – Small)
2) Birth litter size ≤ 9, suckled litter size 12 (Small – Large)
3) Birth litter size ≥ 12, suckled litter size 9 (Large – Small)
4) Birth litter size ≥ 12, suckled litter size 12 (Large –Large)

Gilts were individually identified and their weights recorded at 24 hours of age, at weaning, 20 weeks of age, and slaughter. The P2 backfat depth was measured at 20 weeks of age. Carcass weight and dressing percentage were also recorded. A blood sample was collected at weaning and 20 weeks of age, and assayed to determine levels of anti-mullerian hormone (AMH).

From 20 weeks of age gilts received daily exposure to a mature boar for 14 days. Thereafter, gilts were marketed at normal commercial weights (which were recorded). Immediately post-slaughter, ovaries were recovered from each gilt.

4.1 Measures of ovarian development

The following data were collected from each animal: the total number of corpora lutea (CL), number of corpora albicantia (CA) and the total number of surface antral follicles greater than 1 mm in diameter.

4.2 Anti-mullerian hormone assay

Plasma concentrations of AMH were measured using a commercially available Pig anti-mullerian hormone (AMH) ELISA kit (CUSABIO Biotech, Wuhan, China) as per the manufacturer's instructions. All incubations were performed at 37°C. Prior to assaying, plasma samples were thawed, vortexed and centrifuged to remove any interfering cell fragments and all reagents and samples were brought to room temperature prior to use. All AMH concentrations were determined in duplicate. A six point standard curve (0 ng/mL to 50 ng/mL) was included in each assay and tested in duplicate. 50 µl of undiluted sample or standard was added to each well of a 96-well plate followed by 50 µl of Horseradish Peroxidase (HRP) -conjugate and 50 µl of Antibody. A blank well with no HRP-conjugate was also included in each assay. All wells were mixed well using a multichannel pipette and plates were then sealed and incubated for one hour. After incubation, plates were washed three times with wash buffer and then 50 µl of Substrate A and 50 µl Substrate B was added, followed by incubation of sealed plates for 15 minutes. Stop solution (50 µl) was then added to each well. Optical density was determined for each well immediately using a microplate spectrophotometer reader (Benchmark Plus, Bio-Rad Laboratories, Hercules, CA, USA) set to 450 nm.

4.3 Statistical analysis

Data is expressed as mean ± SEM. P values less than 0.05 were accepted as statistically significant, and P values less than 0.1 accepted as a tendency. Treatment effects on all variables were determined using an analysis of variance (ANOVA) model. Piglet birthweight was included in the model, as a co-variate, when treatment effects were examined for body weight, growth and follicle measures. A chi-squared test was used to determine treatment effects on the proportion of gilts possessing CL at slaughter. The presence of CL was taken to indicate that puberty had occurred. The relationship between AMH levels at weaning and 20 weeks, and total follicle number and CL number at slaughter, were determined using a simple linear regression model. A sub-group was created based on the presence or absence of CL to identify between gilts which did or did not attain puberty. The effect of gestation (pre-natal environment) and lactation (neonatal environment) treatment and puberty attainment on AMH levels at weaning and 20 weeks were analysed using an ANOVA. In addition, gilts were allocated to a high or low weaning AMH and a high or low 20 week AMH group, with the cut-off being the median value for the population. The interaction between high or low AMH at weaning or finishing and treatment group on the attainment of puberty was determined using a chi-squared analysis. All statistical analysis was conducted using Genstat 10th Edition (Rothamstead Experimental Station, Harpendon), with the exception of the chi-squared analyses which was conducted using Excel.

5. Results

Ovarian data were collected from a total of 92 gilts: 29 Large-Large gilts, 26 Large-Small gilts, 19 Small-Large gilts and 18 Small-Small gilts. Only data from these gilts is presented in this section.

As expected, gilts born into large litters were 200 g lighter at birth than those born into small litters ($P < 0.05$; Table 1). Interestingly, piglets reared in small litters were 1.51 kg lighter at weaning than those reared in large litters ($P < 0.05$; Table 1). The number of piglets weaned per litter was unaffected by gestated litter size, but was 2.4 piglets higher ($P < 0.05$) in the Large compared to Small rearing litter size treatment (Table 1). Gilt age at slaughter tended ($P = 0.08$) to be higher for those born into a Large compared to Small litter (171.3 ± 2.16 versus 165.4 ± 2.45 days), but was similar ($P = 0.45$) for gilts reared in a Large (170.0 ± 2.28 days) or Small (167.5 ± 2.30 days) litter.

There were no significant treatment effects on the total number of surface follicles present on the ovaries at slaughter. However, being born into a large litter and reared in a large litter resulted in a numerical, but not significant, reduction in total surface follicle number at slaughter. The number of corpora lutea present on the ovaries of pubertal gilts was numerically, but not significantly higher, for gilts born in a Large compared to Small litter and for gilts reared in a Large compared to Small litter (Table 1). When non-pubertal animals were included in the analysis, there was a tendency ($P=0.1$) for gilts born in Large litters to contain more CL than those born in Small litters (7.8 ± 1.00 versus 3.8 ± 1.56 CL). There were no main effects of gestation or rearing litter size on the proportion of gilts attaining puberty. However, there was a tendency ($P < 0.1$) for a higher proportion of gilts born in a Large litter and reared in a Small litter to reach puberty compared to gilts born in a small litter and reared in a small litter (Table 1).

There was no effect of gestation litter size on AMH levels at weaning or 20 weeks of age (Table 2). . However, gilts reared in a Small compared to a Large litter had significantly higher AMH levels at weaning, but not 20 weeks of age (Table 2). The incidence of puberty attainment was higher ($P < 0.2$; 0.62 versus 0.45) for gilts with high (> 8.3 ng/ml) compared to Low (< 8.3ng/ml). There was an interaction between gestation litter size and AMH levels at weaning and between rearing litter size and AMH levels at weaning with regard to the incidence of puberty attainment. Within the large gestated litter size treatment, a higher ($P < 0.05$) proportion of gilts with High weaning AMH levels reached puberty compared to gilts with Low weaning AMH levels (0.74 versus 0.42; Figure 1A). Within the Large lactation litter size, the proportion of gilts reaching puberty tended ($P < 0.1$) to be higher in animals with a High compared to a Low weaning AMH level (0.63 versus 0.38; Figure 1B).

Table 1 Effect of being born into a Small (≤ 9 piglets) or Large (≥12 piglets) litter and being suckled as part of a Small (9 piglets) or Large (12 piglets) litter on piglet growth characteristics, the number of antral follicles and corpora lutea present on the surface of the ovary and the proportion of pubertal gilts

	Gestation Litter size		Lactation Litter size		Individual Treatments (Gest-Lact)			
	Small	Large	Small	Large	Small-Small	Small-Large	Large-Small	Large-Large
No. gilts	37	55	48	44	18	19	26	29
Piglets born alive	7.6 ± 0.24[a]	13.4 ± 0.20[b]	10.9 ± 0.22	11.2 ± 0.21	6.8 ± 0.35[c]	8.4 ± 0.34[d]	13.7 ± 0.29[e]	13.2 ± 0.28[e]
Piglets weaned	9.8 ± 0.11[a]	9.1 ± 0.09[b]	8.1 ± 0.10[a]	10.5 ± 0.09[b]	8.9 ± 0.15[d]	10.5 ± 0.15[e]	7.6 ± 0.13[c]	10.5 ± 0.14[e]
Liveweight, kg								
Birth	1.61 ± 0.03[b]	1.40 ± 0.02[a]	1.49 ± 0.03	1.46 ± 0.03	1.64 ± 0.04	1.57 ± 0.04	1.40 ± 0.03	1.39 ± 0.03
Weaning	5.16 ± 0.25	5.12 ± 0.20	4.35 ± 0.33[a]	5.86 ± 0.31[b]	4.54 ± 0.35	5.72 ± 0.39	4.22 ± 0.46	5.95 ± 0.35
20 weeks old	84.2 ± 1.48	83.3 ± 1.17	85.1 ± 1.22	82.4 ± 1.17	85.4 ± 2.07	83.1 ± 1.91	84.8 ± 1.63	81.9 ± 1.57
Slaughter	102.0 ± 0.90	102.1 ± 0.71	102.9 ± 0.74	101.2 ± 0.71	101.7 ± 1.26	102.2 ± 1.16	103.7 ± 0.99	100.5 ± 0.96
P2 backfat, mm								
20 weeks old	10.3 ± 0.34	10.3 ± 0.27	10.5 ± 0.28	10.1 ± 0.27	10.1 ± 0.48	10.5 ± 0.44	10.8 ± 0.38	9.8 ± 0.36
Carcass Weight, kg	80.0 ± 0.76	80.7 ± 0.60	80.9 ± 0.62	80.0 ± 0.59	79.1 ± 1.06[c]	80.8 ± 0.98[cd]	82.1 ± 0.84[d]	79.4 ± 0.80[cd]
Dressing %	0.78 ± 0.003	0.79 ± 0.003	0.79 ± 0.003	0.79 ± 0.003	0.78 ± 0.005	0.79 ± 0.004	0.79 ± 0.004	0.79 ± 0.004
Ovarian measures								
No. Antral follicles	129.9 ± 10.59	115.3 ± 9.09	126.3 ± 9.00	117.2 ± 8.89	125.6 ± 14.96	134.2 ± 13.72	126.8 ± 12.38	104.1 ± 12.37
No. Corpora lutea[#]	12.0 ± 0.95	13.2 ± 0.67	12.4 ± 0.73	13.2 ± 0.75	11.2 ± 1.38	12.9 ± 1.19	13.1 ± 0.90	13.3 ± 0.98
No. Corpora lutea[¥]	4.8± 1.26[*]	7.8 ± 1.00[*]	6.8 ± 1.04	6.4 ± 0.99	3.8 ± 1.76	5.8 ± 1.63	8.8 ± 1.39	6.83 ± 1.34
Pubertal gilts, %	0.43	0.56	0.48	0.55	0.39[*]	0.47	0.65[*]	0.48

Superscripts within row and main effects indicate differences; [ab]P < 0.05; [*]P=0.1. Superscripts within row and individual treatments indicate differences; [cd]P < 0.05; *P < 0.1. [#]No. Corpora lutea from pubertal animals only. [¥]Includes animals which did not reach puberty

Table 2 Effect of being born into a Small (≤ 9 piglets) or Large (≥12 piglets) litter and being suckled as part of a Small (9 piglets) or Large (12 piglets) litter on plasma anti-mullerian hormone levels at weaning (20 ± 0.2 days of age) and 20 weeks of age

	Gestation Litter size		Lactation Litter size		Individual Treatments (Gest-Lact)			
	Small	Large	Small	Large	Small-Small	Small-Large	Large-Small	Large-Large
AMH ng/ml: weaning	8.10 ± 0.402	8.12 ± 0.324	8.63 ± 0.361[b]	7.61 ± 0.352[a]	8.99 ± 0.578	7.25 ± 0.561	8.40 ± 0.462	7.84 ± 0.453
AMH ng/ml: 20 weeks	6.31 ± 0.285	6.20 ± 0.239	6.12 ± 0.265	6.36 ± 0.254	6.45± 0.426	6.18 ± 0.383	5.89 ± 0.338	6.49 ± 0.338

[ab] Superscripts within row and main effects indicate differences; P < 0.05

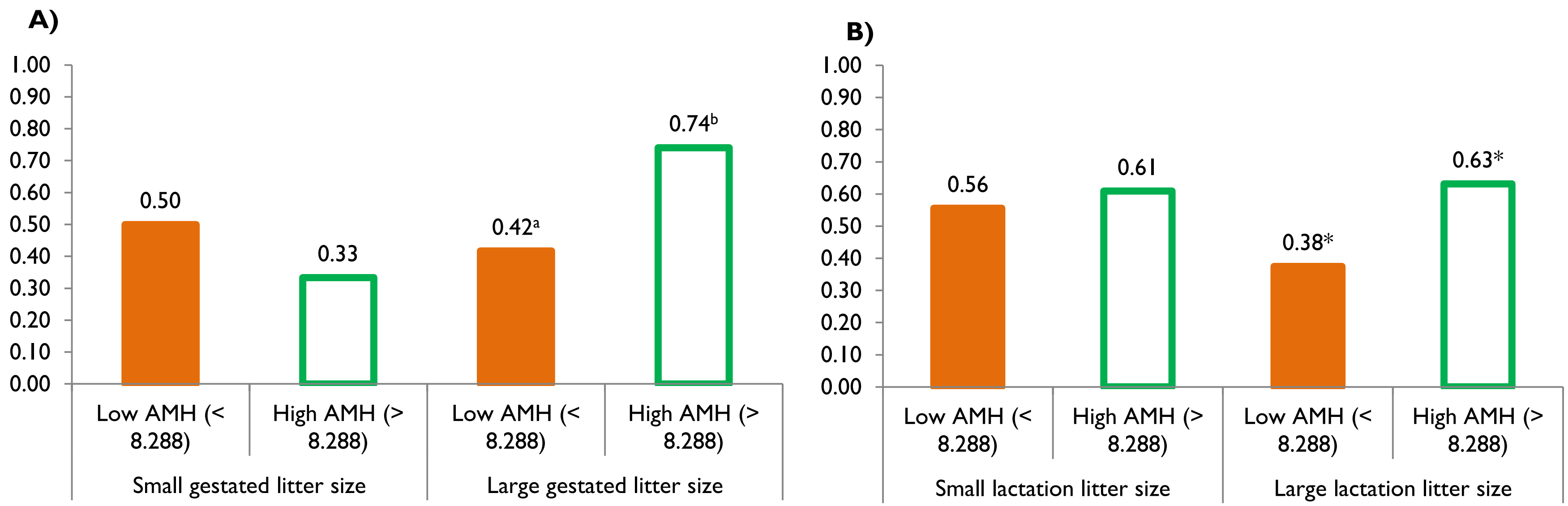

*Figure 1 Effect of Low (< 8.288 ng/ml) and High (> 8.288 ng/ml) anti-mullerian hormone (AMH) levels on the proportion of gilts attaining puberty for animals A) born in a small (< 9 piglets) or large (>12 piglets) litter or B) reared in a small (9 piglets) or Large (12 piglets) litter. Within main effect [ab] P < 0.05 and *P<0.1*

6. Discussion

It is apparent from the current data that higher concentrations of anti-mullerian hormone (AMH) at weaning are associated with an increased capacity to exhibit a pubertal response to boar exposure at a young age (140 days). Importantly, that this relationship is stronger for gilts born into large gestated litter sizes, with 74% of those gilts with high AMH reaching puberty in response to boar stimulation, compared to only 42% of gilts with low AMH levels. Similarly, of those gilts reared in large litters, the incidence of puberty attainment was 63% in the cohort of animals with high AMH compared to 38% of the low AMH cohort. Interestingly, AMH levels at 20 weeks of age were not related to either ovarian characteristics or puberty attainment. This finding supports previous work from our group in gilts (unpublished) and in ewe lambs (Lahoz et al., 2012) indicating that early measures of plasma AMH more accurately predict fertility. An explanation as to why AMH levels are more closely related to puberty attainment in gilts born into or reared in large litters cannot be obtained from the current data, and requires further investigation.

In the gilt, the period from birth to puberty is characterised by specific patterns of ovarian maturation and gonadotrophin release. Antral follicles first appear on the surface of the ovary at approximately 70 days of life, with numbers increasing gradually up until puberty (Dyck and Swierstra, 1983). Prior to puberty attainment there are dynamic, and often rapid, changes in the number of small (1-3mm) and large (> 6 mm) surface follicles present on the surface of the ovary, leading to the conclusion that ovarian follicle growth occurs in waves during the pre-pubertal period (Bolamba et al., 1994). Although data is lacking in pigs, data in cattle demonstrate that the expression of AMH is restricted to small, growing follicles, suggesting that levels of AMH in the peripheral circulation will vary with the development of each ovarian follicle wave (Ireland et al., 2011). In support of this, AMH levels do vary during the oestrous cycle in cows; however, at the same stage of the oestrous cycle differences between high and low AMH animals are still evident (Ireland et al., 2011). It is, therefore, suggested that as gilts mature, AMH levels will reflect, and vary with, the growth of each successive wave of follicle growth, making relationships between AMH levels and fertility harder to obtain by a singular blood sample. The lack of waves of antral follicle growth in younger (< 70 day old) gilts may explain why AMH levels in young animals are more closely related to fertility measures in later life. However, further work is required to establish the relationship between AMH levels at these early time points and ovarian development. Regardless of this, it is evident that animals with high AMH levels at weaning, are more responsive to boar-component stimuli, which supports evidence from cattle that high AMH levels reflect an ovarian follicle pool which is more responsive to exogenous gonadotrophins (Ireland et al., 2011).

Comparisons between anti-mullerian hormone and surface antral follicle counts should also be viewed with some caution, as the accuracy with which surface follicle numbers reflect the size and maturity of the animals ovarian reserve remains to be fully established. In support of this, our previous work (unpublished) indicates that plasma AMH levels are more closely related with the total number of antral follicles present on the ovary (as determined by histological analysis) than the surface follicle pool.

The data from the current study, whilst preliminary, also appears to indicate a higher reproductive potential for gilts born into a Large compared to a Small litter, as evident from the numerical improvements in puberty attainment and ovulation rate for these animals. More importantly, it also appears that the capacity of gilts born into large litters to reach their reproductive potential, in terms of their ability to ovulate in response to early boar stimulation, may be impaired when they are reared in a Large litter. This outcome is evident from the improved pubertal response observed when gilts from Large litters are reared in small litters when compared to gilts born into Small litters. Although further work is required to understand why reared litter size affects reproductive development of piglets with high reproductive potential (i.e. born in large litters), a reduction in nutrient intake, via milk, or indeed colostrum intake could be involved. The impact on uterine development of bioactive compounds in colostrum and milk (lactocrine factors), has become increasingly evident from new research (eg Bartol et al., 2013). However, the impact of these bioactives on ovarian development has yet to be investigated. Alternatively, the capacity of reared litter size to affect piglets born in large litters, and therefore with lighter birthweights, may suggest that the reproductive development of these lighter piglets is more sensitive to nutritional intake and availability of bioactives in milk. Certainly, negative effects of low birthweight on age at puberty, ovulation rate and embryonic survival are evident from preliminary work (Flowers, 2012). Further, the impact of low birthweight on reproductive longevity is exacerbated when gilts are reared in large litters (> 10 piglets).

Together, the current data provide support for the use of plasma AMH levels at weaning to identify gilts which are more likely to be responsive to boar stimulation, especially when selecting gilts born into, or reared in, large litters. It also appears that reproductive development and fertility of gilts born into large litters may be improved if they are reared in a smaller litter. Further work is required to establish the relationship between AMH levels at weaning and ovarian development, and the relationship between gestated litter size, rearing litter size, AMH and lifetime productivity of the breeding sows.

7. Implications & Recommendations

The current data indicate that the fertility and reproductive development of gilts born into large litters will be improved if they are reared in a small litter. Accepting that these gilts are likely to be born from more prolific sows, and with potentially greater genetic merit, it is logical to assume that taking better care of these animals will improve the reproductive efficiency of the breeding herd. However, additional work is required, and should focus on, the impacts of rearing gilts born into large litters in small litters on their lifetime reproductive performance.

The current data also demonstrated that early measures of plasma AMH are indicative of gilt fertility, with further work required to determine whether selection of gilts with high AMH levels at weaning is a viable strategy to increase breeding herd productivity. A single measure of AMH will cost between $18 and $35, and needs to be offset against the loss of profit resulting from select gilts failing to attain puberty and sows failing to remain in the herd past their first lactation. It also needs to be offset against the increase in profit obtained per sow for every additional parity and piglet she produces.

Overall, based on the current data the investigators recommend that additional work is conducted to determine the impact on fertility and reproductive longevity of:

- Rearing gilts born in large litters in small litters
- Selecting gilts based on high AMH levels at weaning (or earlier)

8. Intellectual Property

A detailed description of intellectual property arising from the research, eg. commercially significant developments, patents applied for or granted, licences, etc is required. If necessary this may be provided in a confidential attachment.

The development of an AMH assay for the Australian Pork Industry would be a potential commercialisation opportunity. Based on the current data, it appears that animals with high AMH levels are more fertile, in terms of responsiveness to boar stimulation. Setting up this assay in a relevant lab (i.e. The School of Animal and Veterinary Sciences) may be a viable option. However, further, large scale commercial data is needed before considering this option. Following on from this, if the data demonstrated a link between AMH and sow fertility and longevity, would be market research to determine the interest from commercial entities.

9. Literature cited

Bartol, F. F., Wiley, A. A., Miller, D. J., Silva, A. J., Roberts, K. E., Davolt, M. L., Chen, J. C., Frankshun, A. L., Camp, M. E., Rahman, K. M., Vallet, J. L., Bagnell, C. A., (2013). Lactation biology symposium; lactocrine signaling and developmental competence. *Journal of Animal Science*, 696 – 705.

Bolamba, D., P. Matton, R. Estrada, and J. J. Dufour. 1994. Ovarian follicular dynamics and relationship between ovarian types and serum concentrations of sex steroids and gonadotrophin in prepubertal gilts. *Animal Reproduction Science,* 291-304.

D'Allaire, S., and Drolet, R. (2006). Longevity in breeding animals. In Diseases of Swine, eds B.E. Straw, D. E. Zimmerman, S. D'Allaire and D. J. Taylor. Blackwell Publishing, Ames, IA, p. 1011-1025.

Dyck, G., and Swierstra, E. (1982). Growth of the reproductive tract of the gilt from birth to puberty. *Canadian Journal of Animal Science*, 81 - 87.

Flowers, W. L. (2012). Possible physiological benchmarks for sow longevity prior to puberty. Allen D. Leman Swine Conference, 113 – 117.

Ireland, J. J., Smith, G. W., Scheeyz, D., Jiminez-Krassel, R., Folger, J. K., Ireland, J. L., Mossa, F., Lonergan, P., Evans, A. C. (2011). Does size matter in females? An overview of the impact of the high variation in the ovarian reserve on ovarian function and fertility, utility of anti-Mullerian hormone as a diagnostic marker for fertility and cause of variation in the ovarian reserve in cattle. *Reproduction, Fertility, and Development*, 1 – 14.

Lahoz, B., Alabart, J. L., Monniaux, D., Mermillod, P., and Folch, J. (2012). Anti-Mullerian hormone plasma concentration in prepubertal ewe lambs as a predictor of their fertility at a young age. *Veterinary Research*, 118 – 127.

Nelson, R. E. and Robinson, O. W. (1976). Effects of post-natal maternal environment on reproduction in gilts. *Journal of Animal Science*, 71.

Tummaruk, P., Lundeheim, N., Einarsson S., and Dalin, A.M. (2001). Effect of birth litter size, birth parity number, growth rate, backfat thickness and age at first mating of gilts on their reproductive performance as sows. *Animal Reproduction Science*, 225 – 237.

10. Publications Arising

A paper will be submitted to an international journal for publication.

We are also considering submitted a one page paper to APSA