final report

Project code: B.BSC.0115

Prepared by: S. Lee, F. Brien, W. Van Wettere
and W. Pitchford

The University of Adelaide &
South Australian Research and
Development Institute

Date published: September 2012

PUBLISHED BY
Meat & Livestock Australia Limited
Locked Bag 991
NORTH SYDNEY NSW 2059

Determining the value of Plasma Anti-Mullerian Hormone as a phenotypic and genetic marker for reproductive rate

Meat & Livestock Australia acknowledges the matching funds provided by the Australian Government to support the research and development detailed in this publication.

Abstract

Anti-mullerian hormone (AMH) is a glycoprotein belonging to the transforming growth factor – ß family and can be measured in plasma. This project modelled the potential value of plasma AMH as a phenotypic and genetic marker for reproductive rate in sheep and beef cattle. Plasma AMH concentration can be readily measured in mammals, is repeatable, and is favourably related to some measures of fertility reported in human and animal species. Potential genetic gain in cow weaning rate for Angus and Brahman and number of lambs weaned in sheep from use of AMH as a marker is low. The major limitation to AMH improving genetic gain is that recording of males provides no predictive value for female reproduction rate. Potential phenotypic gain was also predicted to be low because of the low repeatability of weaning rate and low selection intensity of females for self replacing herds/flocks. However, the potential to use AMH as a screening tool to identify females that are more likely to yield high numbers of transferrable embryos is moderate. This potential increase represents a substantial improvement relative to current practice and appears of significant economic value to the artificial breeding sector for livestock, particularly cattle, in Australia.

Executive summary

There is significant potential to increase reproductive rate in Australian sheep and beef industries leading to improved productivity and economic benefits. Therefore, the ability to predict the genetic merit and/or likely reproductive rate of young replacement females would be an extremely valuable selection and monitoring tool for livestock producers.

This project evaluated the potential value of anti-mullerian hormone (AMH) measured in plasma as a phenotypic and genetic marker for reproductive rate in sheep and beef through modelling. This involved estimating the production benefit and genetic gain for a range of correlations between AMH and reproductive rate, defined as annual number of lambs weaned (NLW) in sheep and annual cow weaning rate in cattle, the common measures used in Australian genetic evaluations for sheep and beef respectively. The outcome of this project is an informed understanding of the likely value of AMH as a predictor of reproductive rate (weaning rate) in sheep and cattle. This report details the predicted value of AMH for use as a phenotypic indicator and for genetic selection in Australian beef and sheep enterprises. The report provides a basis to evaluate investment in AMH based on the potential returns to industry under various scenarios.

Plasma AMH levels can be readily measured in mammals, is considered to be repeatable and reported to have strong correlations to antral follicle count and size of the ovarian reserve in humans, rodents and bovine. Favourable relationships between antral follicle count and size of the ovarian reserve with some measures of fertility have been reported. However, no estimates of correlations between antral follicle count and size of the ovarian reserve with weaning rate have been reported. This study considered strengths of genetic correlations between plasma AMH and weaning rate from 0.00 to 0.99.

Potential genetic gain in cow weaning rate for southern beef and Brahman from use of AMH as a marker was low. Specifically, at an assumed genetic correlation of 0.50, the response per year was 0.4% and 0.5% (percentage point increase) above the base scenario (described in section 3.1) for Angus and Brahman respectively. In sheep, the potential genetic gain in number of lambs weaned was low to moderate. The response per year was 1.9% higher than the base scenario (6.0% vs. 4.1%) at a genetic correlation of 0.50. The major limitation to AMH improving genetic gain is that recording of males appears to provide no predictive value for female reproduction rate.

The use of AMH as a phenotypic marker for reproductive rate was also considered. For each system (southern beef, Brahman and a sheep enterprise) benefits for use as a current generation selection tool were assessed for correlations from 0.1 to 0.5 between AMH and weaning rate with repeatability for weaning rate of 0.1, 0.2 or 0.3 considered. Overall, potential phenotypic gain was also predicted to be low because of the low repeatability of weaning rate and low selection intensity of females in both cattle and sheep for self replacing herds/flocks. Under the scenario of phenotypic repeatability of weaning rate being 0.2 and a correlation between AMH and weaning rate of 0.3, the estimated improvement in cohort weaning was 1.3%, 2.0% and 1.8% for southern beef, Brahman and sheep respectively.

The potential to use AMH as a screening tool to identify animals that are more likely to yield high numbers of transferrable embryos is moderate. At published estimates for dairy cattle, gains in number of transferrable embryos would be 13%. This potential increase represents a substantial improvement and is of economic value to the artificial breeding sector for beef cattle in Australia. The lower value of sheep per unit may limit the value in advanced sheep breeding enterprises. It is recommended that a project is developed to determine the application of using Plasma AMH as a screening tool prior to entry into an embryo production program for both southern beef and Brahman type cattle.

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1 Background

Improving agricultural productivity is an Australian rural research and development priority. In the context of red meat production, improving reproductive rate through current generation identification and selection for animals with increased merit for overall reproductive rate is important.

Anti-mullerian hormone (AMH) is a glycoprotein belonging to the transforming growth factor – ß family and can be measured in plasma. Ireland *et al.* (2011) demonstrated that plasma AMH concentration can be readily measured in dairy cattle, is repeatable, and is favourably related to some measures of fertility. Recent studies in cattle, humans and rodents, suggest that circulating levels of AMH can be used as a reliable and accurate marker of antral follicle numbers, the number of healthy oocytes present in the ovary, and the size of the ovarian reserve. La Marca *et al.* (2009) reviewed the role of AMH in components of fertility in women and concluded that

1. AMH levels can be measured in serum;
1. Is proportional to the number of small antral follicles; and
2. Has great promise as a marker of ovarian reserve.

The main role of AMH for males is in sex differentiation during the early stages of gestation. The presence of AMH induces the regression of the Mullerian ducts which otherwise develop into the female reproductive tract (Munsterberg and Lovell-Badge, 1991). Thus, there is no expected relationship between AMH levels in males and reproductive performance of females.

AMH has emerged as an important marker of ovarian reserve in human medicine (Hehenkamp *et al.* 2006) and also in cattle (Ireland *et al.* 2011). In humans, lower AMH is associated with lower natural conception rates in women (Steiner *et al.* 2011). Nelson *et al.* (2012) cited numerous examples of AMH being related to oocyte yield and thus prediction of live birth in humans and potentially success in artificial breeding settings.

Some contention exists over the within individual repeatability of AMH during the stages of the menstrual cycle. Hagen *et al.* (2012) reported that AMH in pre-pubertal and pubertal girls exhibits only minor fluctuations during childhood and adolescence, and a random AMH measurement seems representative for a given girl. In dairy cattle, Ireland *et al.* (2008) reported that AMH concentration in serum is a viable marker for relative size of the ovarian reserve and antral follicle count in dairy heifers. In their study, 88% of the variance in antral follicle count in dairy heifers was accounted for by a series of 8 AMH measurements recorded on a daily basis in the 8 day period prior to oestrus. Importantly, repeatability over the 8 measurements was very high (0.97). In addition, Ireland *et al.* (2011) reported a correlation of 0.97 between first AMH sample collected at an unknown time during the oestrus cycle and average AMH over three samples during an oestrus cycle in 23 dairy heifers. These very high repeatability estimates contrast with some literature for humans. For example, Overbeek *et al.* (2012) reanalysed the complete data set first reported on by Hehenkamp *et al.* (2006) and found considerably different results. Overbeek *et al.* (2012) concluded that AMH levels have substantial fluctuation intra-women during the menstrual cycle thus suggesting that in women, a single sample may not yield as much informative power as has been reported for dairy cattle.

Given published results, further consideration of AMH as a valuable indicator of reproductive rate in beef cattle and sheep is warranted. In particular it is important to estimate the potential genetic and phenotypic gain that could be achieved through measurement of AMH for use as an indirect selection criteria.

2 Project Objectives

- Develop a model which will inform understanding of the likely value of AMH as a predictor of reproductive rate (weaning rate) in sheep and cattle.
- Final report comprising summary of likely range in heritability, repeatability, variance, strength of phenotypic and genetic correlations and value for use as a phenotypic indicator and for genetic selection in Australian beef and sheep enterprises.

3 Methodology

Three separate modelling exercises were undertaken for evaluation of AMH as a genetic improvement tool, phenotypic improvement tool and for use as a screening tool for potential donor females in an embryo production campaign.

3.1 Genetic improvement tool

Modelling was undertaken for three production systems, southern beef, Brahman and dual purpose Merino using an Excel based multi-trait selection index tool developed by Prof Julius van der Werf, University of New England, NSW Australia. All 3 systems were self replacing herds/flocks with 400 breeding cows/ewes and used age optimisation for culling to maintain breeding numbers at 400 each year. Age optimisation is focused on maximising response to selection generally by reducing generation interval, but there are trade-offs with selection intensity. For each system, a representative farm was formed (Table 1) based on:

- Number of breeding females
- Number of males used each year
- Mating ratio
- Weaning rate
- Age at first progeny born
- Maximum age of breeding cows/ewes still in herd

Table 1: structure of each enterprise modelled

Enterprise	**Angus**	**Brahman**	**Merino**
Number of breeding females	**400**	**400**	**400**
Mating ratio (females/male)	**50**	**50**	**50**
Number of males used per year	**8**	**8**	**8**
Weaning rate	**85%**	**65%**	**100%**
Number progeny born per year	**340**	**260**	**400**
Mortality rate % (from pregnancy scan)	**10**	**10**	**5**
Earliest possible age of first drop males	**2**	**3**	**2**
Earliest possible age of first drop females	**2**	**3**	**2**
Max age class (years)	**10**	**10**	**10***

*98% of ewes were 6 years or younger

A dearth of information about heritability and the relationship of AMH to total lifetime reproductive rate in either the human or bovine exists making it difficult to identify the likely heritability and strength of correlations. To overcome this issue, correlations between AMH and lifetime reproductive performance have been modelled from 0.00 to 0.99 at intervals of 0.10.

Conflicting results from the human literature also make it hard to determine the repeatability of AMH and association with antral follicle count and size of ovarian reserve. In cattle, Ireland *et al*. (2008) and Ireland *et al*. (2011) reported significant relationships between AMH and antral follicle count, size of ovarian reserve and number of viable embryos harvested in a multiple embryo transfer program. In this study, for modelling of the value of AMH as a genetic selection tool, a single conservative value compared with the literature for repeatability was modelled at 0.35. This is far more conservative than the 0.97 reported above because generally technical error on ELISA assays would be greater than 3% so the 0.97 seems too high. Data has been collected recently in Adelaide and is being processed currently so sources of variation will be able to be estimated. This value was also used for heritability of AMH and is similar in strength to that of weight for age traits in both cattle (Koots *et al*. 1994) and sheep (Brien *et al.* 2011). For cattle, genetic and phenotypic parameters used in this study were informed by S. Barwick, J. van der Werf and align with those currently used for genetic evaluation in industry. For sheep, genetic parameters were based on those reported by Sheep Genetics (http://www.sheepgenetics.org.au/Home) as used by Brien *et al.* (2011) in modelling genetic gain in number of lambs weaned for sheep enterprises. For both species, genetic parameters and variances assumed reflect those currently in place in genetic evaluation and thus the base scenarios should be representative of current industry performance.

In all scenarios modelled, 100% of selection pressure was placed on cow weaning rate and NLW for cattle and sheep respectively effectively resulting in single trait selection. This approach was adopted given the economic value of weaning rate / NLW is known for each of the production systems modelled making it possible to consider the result for currently used industry based breeding objectives.

Level of performance recording for genetic evaluation was assumed to be comparatively high. For southern beef, traits recorded on cows were calving ease (direct), birth weight, 200 day growth, 400 & 600 day weight, mature cow weight (MCWt), ultrasound scan traits for P8 fat depth (P8), loin eye muscle area (EMA) and intramuscular fat (IMF), 200 day maternal effect (200M) and days to calving (DTC) and AMH. In bulls, sex limited traits (DTC, MCWt, 200M) were not recorded but scrotal size (SS) was. As addressed in the background (section 1), AMH in males is unlikely to be an indicator of sister/daughter fertility and thus was assumed not to be recorded on males.

For Brahman, performance recording on females was assumed to be 200 day growth, 600 day weight, DTC, MCWt and AMH. In bulls, recording of 200 day growth, 600 day weight and SS was assumed. Recording of AMH was assumed to take place prior to first use at 14mo in Angus and 26mo in Brahman and at 12mo in Merino sheep.

The sheep enterprise modelled did not mate ewe lambs. Parents were assumed to be recorded for the same set of traits along with siblings in management groups (size 20 including individual). The animal of interest was assumed to have no progeny at time of recording and first selection. Similar to cattle enterprises, extensive recording of the sheep enterprise was assumed. Table 2 reports the traits assumed to be measured in the sheep enterprise.

Assumed performance traits recorded for animal genetic evaluation

Trait	Dam	Sire	Individual
Lambing ease - direct	✓	✓	✓
Lambing ease - maternal	✓		
Yearling clean fleece weight	✓	✓	✓
Yearling mean fibre diameter	✓	✓	✓
Yearling CV fibre diameter	✓	✓	✓
Yearling staple strength	✓	✓	
Number of lambs weaned	✓		
Birth weight	✓	✓	✓
Weaning weight	✓	✓	✓
Post weaning weight	✓	✓	✓
yearling weight	✓	✓	✓
Yearling eye muscle depth	✓	✓	✓
Yearling fat depth	✓	✓	✓
Lamb survival	✓	✓	
Yearling scrotal circumference		✓	
AMH	✓		✓

3.2 Phenotypic improvement tool

Phenotypic modelling was conducted using the same herd structure and phenotypic variance as for the genetic modelling exercise. In these scenarios, the only parameter that was considered was AMH. The phenotypic response in cow weaning rate and NLW was assessed at various strengths of phenotypic correlation between AMH and the target trait (NLW or cow weaning rate) from 0.1 to 0.5 in 0.1 increments and various repeatabilities of cow weaning rate and NLW from 0.1 to 0.3 in 0.1 increments. AMH measured prior to first joining was assumed to be the only available information for selection. This reflects a commercial production system with no other performance recording. All selection pressure applied in the female herd was assumed to be on reproductive rate (i.e. single trait selection). Repeatability of AMH was assumed to be 0.99. Using a high assumed repeatability of AMH ensured maximum opportunity to yield a substantial improvement in weaning rate.

3.3 Evaluation of AMH as a tool for screening potential donor females

Plasma AMH has been reported to be associated with the number of transferable embryos in dairy cows (Monniaux *et al.* 2010). This exercise sought to determine the potential benefit of using AMH as a screening tool for potential donor cows prior to entry into an embryo production program.

Mean rates of embryo production and associated variation were obtained from literature and personal communication with commercial practitioners. In modelling the potential to use AMH as a pre screen tool, 4 levels of repeatability from 0.1 to 0.4 in 0.1 increments for number of transferable embryos were considered, a mean number of transferable embryos of 5 and an SD of 4,5,6 or 7. These assumptions cover the range of published estimates for repeatability, mean rates and SD of transferable embryos from a flush. Similar to the phenotypic response in reproductive rate exercise, repeatability of AMH was assumed to be 0.99. Response was modelled for various correlations between AMH and number of transferable embryos (0.1, 0.3, 0.5 and 0.7).

The system modelled focused on the selection of 18 donor females to enter a program based on screening of 30, thus generating a selection intensity of 0.64. Results are reported as the predicted increase in the number of transferable embryos per super-ovulation.

4 Results from modelling

4.1 Genetic gain southern beef

Potential for genetic gain in cow weaning rate for southern beef from using AMH as an indirect selection criteria is low. Under the scenario modelled, annual response in cow weaning rate through currently available measurement (i.e. not including AMH) for seedstock producers was 0.8% (Figure 1) with an index EBV accuracy of 32% (Table 3). At a 0.50 genetic correlation between plasma AMH and cow weaning rate, the predicted annual response was 1.2% per year with an index EBV accuracy of 48%. Under the (upper) realistic scenario of 0.50 genetic correlation, the gain in weaning rate was only 0.4% above the base scenario, albeit a 50% increase because it started from such a low value (1.2/0.8). Young bull (14mo) EBV accuracy was 27.4%. At a genetic correlation of 0.90, annual gain was predicted at 1.6% per year with young bull accuracy of 40.6% for cow weaning rate (Table 3).

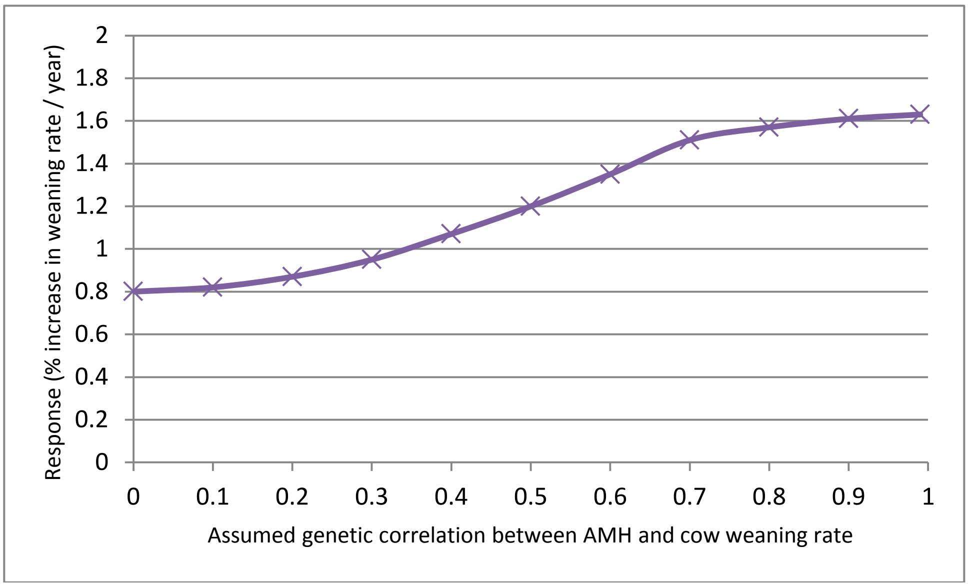

Figure 1: Response in cow weaning rate if AMH measured on replacement females at 14mo with assumed genetic correlations from 0.00 to 0.99 between AMH and cow weaning rate

Table 3: increase in EBV accuracy for bulls and heifers at 14mo and genetic gain in cow weaning rate at various assumed genetic correlations between AMH and cow weaning rate for Angus

Assumed genetic correlation	$Index EBV acc*	Young bull EBV acc (14mo)	Young heifers EBV acc (14mo)	Response**
0	0.32	0.12	0.12	0.80
0.1	0.33	0.13	0.14	0.82
0.2	0.36	0.15	0.18	0.87
0.3	0.39	0.19	0.24	0.95
0.4	0.43	0.23	0.3	1.07
0.5	0.48	0.27	0.36	1.2
0.6	0.53	0.32	0.43	1.35
0.7	0.59	0.37	0.49	1.51
0.8	0.62	0.39	0.53	1.57
0.9	0.63	0.41	0.55	1.61
0.99	0.63	0.42	0.57	1.63

*averaged over all ages and sexes
**(% increase in weaning rate / year)

For the Angus breed, industry applied breeding objectives for self replacing herds have an average profit driver weighting on cow weaning rate of 9% (range 5-13%) (see: http://www.angusaustralia.com.au/breedplan/understanding/205.html). Therefore, the 0.4% greater response per year observed at a 0.50 genetic correlation reduces to an average 0.04% per year for a balanced industry breeding objective with a self replacing component on average across the three self replacing breeding objectives.

Even with a higher AMH heritability estimate of 0.50 compared with 0.35, predicted genetic gain in cow weaning rate was low. For example, at a genetic correlation of 0.99, and a heritability of 0.50 the predicted response rate only increased to 1.7% per year compared with a predicted gain of 1.6% per year with a heritability of 0.35.

4.2 Genetic gain Brahman

Similar to southern beef, potential additional genetic gain in Brahman was low. Under a scenario with 65% weaning rate across the herd and 100% selection pressure on cow weaning rate, predicted annual genetic gains were 0.9% per year with a index EBV accuracy of 32.2% (Table 4). At a 0.50 genetic correlation between plasma AMH and cow weaning rate, predicted annual gain was 1.4% per year with an index EBV accuracy of 48%. Young bull (26mo) accuracy was 27.4%. At a genetic correlation of 0.90, annual gain was predicted was 1.9% with a young bull accuracy of 40.9%. Under the (upper) realistic scenario of 0.50 genetic correlation between plasma AMH and Brahman cow weaning rate, there was only a predicted increase in cow weaning rate of 0.5% relative to the base scenario. For Brahman, there are two breeding objectives that are used in industry that include a self replacing herd component, the Jap Ox Index and Live Export Index. Both indexes have a 47% profit driver for cow weaning rate. Therefore, the 0.5% greater response observed at 0.50 genetic correlation per year relative to the base scenario reduces to an average of 0.24% genetic gain per year for the industry breeding objectives. This means over a 10 year period, the predicted genetic improvement over the base scenario in weaning rate would be 2.4%. However, to achieve this rate of gain would require testing all heifer calves each year. Depending on the amount of variation accounted for, gene marker tests are likely to be better since they can be used in young bulls whereas AMH can only be measured in females.

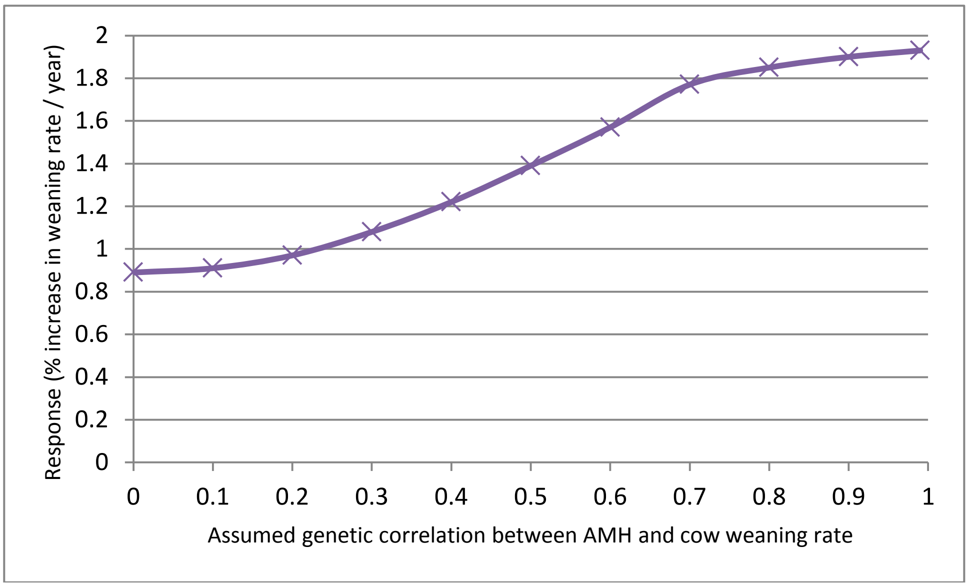

Figure 2: Response in cow weaning rate if AMH measured on replacement females at 26mo with assumed genetic correlations from 0.00 to 0.99 between AMH and cow weaning rate for Brahman

Table 4: increase in EBV accuracy for bull and heifers at 26mo and genetic gain in cow weaning rate at various assumed genetic correlations between AMH and cow weaning rate for Brahman

Assumed genetic correlation	$Index EBV acc*	Young bull EBV acc (26mo)	Young heifers EBV acc (26mo)	Response**
0	0.32	0.11	0.11	0.89
0.1	0.33	0.12	0.13	0.91
0.2	0.35	0.15	0.18	0.97
0.3	0.39	0.19	0.24	1.08
0.4	0.43	0.23	0.30	1.22
0.5	0.48	0.27	0.36	1.39
0.6	0.54	0.32	0.43	1.57
0.7	0.6	0.37	0.49	1.77
0.8	0.63	0.39	0.53	1.85
0.9	0.64	0.41	0.56	1.90
0.99	0.65	0.42	0.58	1.93

*averaged over all ages and sexes
**(% increase in weaning rate / year)

4.3 Genetic gain in sheep

For sheep, at a base scenario modelled of 100% weaning rate, i.e. 100 lambs weaned for 100 ewes mated or NLW of 1, when 100% of selection pressure was placed on NLW, there was a 4.1% increase in NLW per year with an index accuracy of 26% (Table 5). At a 0.50 genetic correlation between plasma AMH and NLW, genetic gain in NLW was predicted to be 6.0% with an index accuracy of 37%. This represents a 1.9% increase in annual rate of genetic gain for NLW over the base scenario. However, if the strength of the genetic correlation was 0.30 (same as yearling scrotal circumference in sheep), then genetic gain in NLW was predicted to be 4.8% or only 0.7% greater rate of gain in NLW over the base scenario.

For the Merino Dual Purpose 7% Index, 14% of the economic gain occurs from NLW (see: http://www.sheepgenetics.org.au/MERINOSELECT/InformationItem.aspx?ITEM=140#Index_Explanations). Therefore, the 1.9% predicted genetic gain over the base scenario at a 0.50 genetic correlation between AMH and NLW reduces to 0.27% per year greater predicted gain when the balanced breeding objective is used. Over a 10 year period this equates to a 2.7% improvement in NLW, but similar to the cattle assumptions does require testing of AMH for all ewe lambs each year.

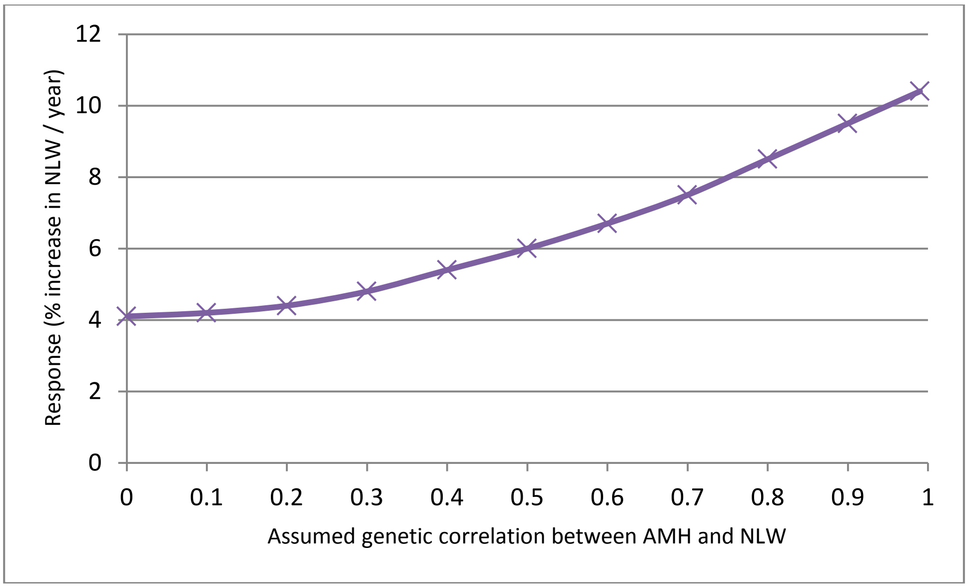

Figure 3: Response in NLW if AMH measured on replacement maiden ewes with assumed genetic correlations from 0.00 to 0.99 between AMH and NLW for Merinos.

Table 5: Increase in EBV accuracy for rams at ewes prior to selection and genetic gain in NLW at various assumed genetic correlations between AMH and NLW for Merino

Assumed genetic correlation	$Index EBV acc*	Young ram EBV acc	Young ewe EBV acc	Response**
0	0.26	0.25	0.25	4.1
0.1	0.26	0.25	0.26	4.2
0.2	0.28	0.26	0.28	4.4
0.3	0.30	0.27	0.31	4.8
0.4	0.33	0.28	0.35	5.4
0.5	0.37	0.31	0.39	6.0
0.6	0.41	0.34	0.44	6.7
0.7	0.46	0.38	0.50	7.5
0.8	0.52	0.43	0.56	8.5
0.9	0.57	0.48	0.61	9.5
0.99	0.59	0.51	0.64	10.4

*averaged over all ages and sexes
**(% increase in NLW / year)

5 Phenotypic response in weaning rate using AMH as a current generation selection tool

Predicted increase in phenotypic cow weaning rate or NLW through use of AMH as a current generation selection tool was low. In southern beef under a scenario of cow weaning rate repeatability of 0.2, and phenotypic correlation of 0.3 between cow weaning rate and AMH, predicted percentage increase in cohort weaning rate was 1.30% (Figure 4). When cow weaning rate repeatability was assumed to be 0.1 then maximum predicted phenotypic response was 1.09%.

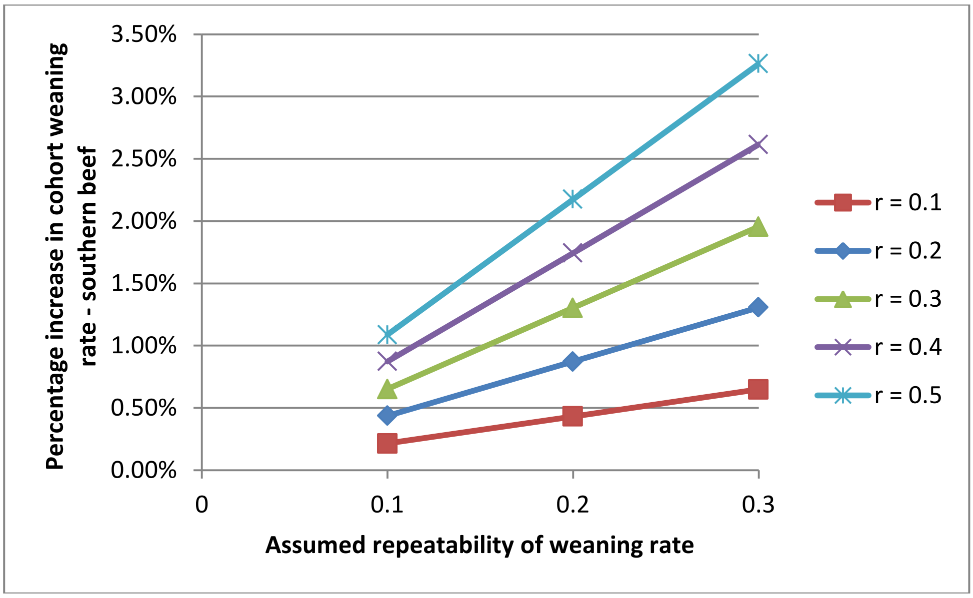

Figure 4: Percentage increase in cohort weaning rate for southern beef at various levels of repeatability for weaning rate (0.1 to 0.3) and various strength phenotypic correlation between AMH and cow weaning rate (r = 0.1 to 0.5).

Predicted phenotypic response was higher in Brahman because of the higher phenotypic variance in cow weaning rate. Under the scenario of 0.2 repeatability of annual cow weaning rate and a 0.3 phenotypic correlation to AMH, the predicted response was 2.02% (Figure 5). When the phenotypic repeatability of cow weaning rate was assumed to be 0.1, predicted maximum response was 1.69%. A 2.02% increase (from 1 to 3% per year) in weaning rate would only be achieved where all potential replacement females are screened and selected solely on weaning rate via information from AMH.

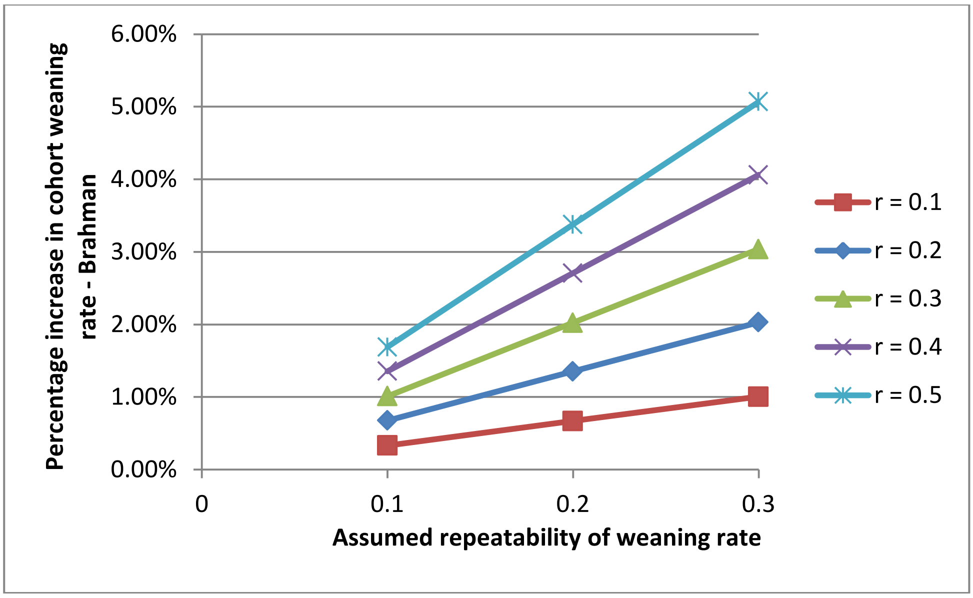

Figure 5: Percentage increase in cohort weaning rate for Brahman at various levels of repeatability for weaning rate (0.1 to 0.3) and various strength phenotypic correlation between AMH and cow weaning rate (0.1 to 0.5).

The predicted increase in cohort NLW was modest. In Australian sheep genetic evaluations, current repeatability of NLW is assumed to be 0.06. At a repeatability of 0.1 and phenotypic correlation between NLW and AMH of 0.5, the increase in cohort NLW was predicted to be 1.5% (Figure 6). To achieve this gain in a commercial setting would require screening all ewe lambs for AMH.

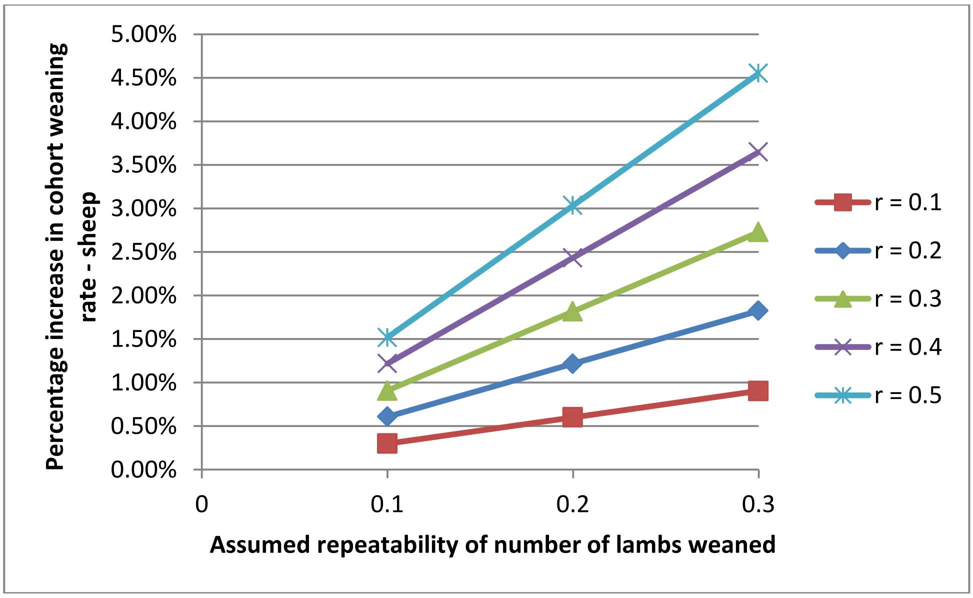

Figure 6: Percentage increase in cohort NLW at various levels of repeatability for weaning rate (0.1 to 0.3) and various strength phenotypic correlation between AMH and NLW (0.1 to 0.5).

6 Using AMH as a screening tool prior to females entering an embryo production campaign

The use of AMH as a screening tool prior to females entering an embryo production program has considerable potential. The average rate of transferable embryos in Australia beef cattle is approximately 5 with considerable variation in number of transferable embryos observed. The cost of achieving a transferable embryo is approximately $200 to $250. Predicted percentage increase in number of transferable depended on assumed embryo production repeatability (0.1, 0.2, 0.3 or 0.4), assumed correlation between AMH and number of transferable embryos (0.1, 0.3, 0.5, 0.7) and standard in mean number of transferable embryos. Predicted responses ranged from 0.03 embryos per flush (repeatability of 0.1, correlation 0.1, SD 4) to 1.25 embryos (repeatability 0.4, correlation 0.7, SD 7) (Figure 7). At a mean of 5 embryos per flush, these response rates range equate to an increase of between 0.6% to 24.9%.

An increase of 13.3% transferable embryos is predicted when using the parameters reported by Monniaux *et al*. (2010) for dairy cattle (0.36 repeatability, 0.58 correlation between AMH and number of transferable embryos). At this rate, over a 5 flush embryo production program an extra 3.3 transferable embryos per cow entering the program would be achieved (where 30 cows are screened and highest 18 based on AMH selected to enter the program) compared with simply selecting 18 from 30 cows at random.

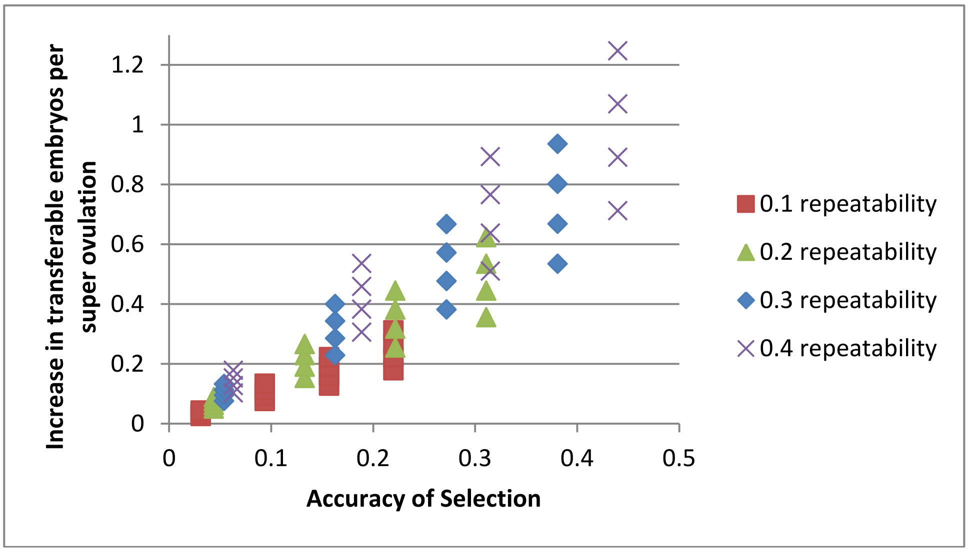

Figure 7: Predicted increase in number of transferable embryos per flush for various scenarios where AMH is used as a pre-screening tool.

7 Discussion

In human focused research, use of AMH is typically applied to identify women who have reduced probability of pregnancy, both through natural mating (Steiner *et al*. 2011) and assisted breeding programs as reviewed by Nelson *et al.* (2012). In both settings and in the work of Ireland *et al.* (2011) in dairy cattle, minimum threshold levels are proposed for AMH to achieve high levels of antral follicles, ovarian size etc. The non-linear relationship indicates that AMH may not have high utility in screening for high genetic merit for reproductive rate, but rather only screen out cows that are unlikely to have large ovarian reserves and high antral follicle number.

7.1 Facilitating genetic gain in reproductive rate

The potential for the use of plasma AMH concentration as a biological marker for selection to improve reproductive rate genetically for Australian beef cattle and sheep production systems appears low.

Cow weaning rate and NLW account for only a small proportion of the overall breeding objective for southern beef and Merino sheep respectively. Therefore, the small predicted improvement in cow weaning rate and NLW at 100% selection pressure reported in section 4.1 and 4.3 becomes negligible when a balanced breeding objective is applied. The outcome from this section of the project provides evidence that value of investment in research on AMH as a genetic indicator or female reproductive rate is limited.

For Brahman, the predicted genetic improvement in cow weaning rate was higher than that observed in southern beef at the equivalent genetic correlation. This is a function of the higher variance in cow weaning rate in Brahman compared with Angus (2116 vs. 900 calves weaned[2]). However, even with the higher predicted response rate, at a genetic correlation of 0.50 the 10 year genetic gain in cow weaning rate was only 2.4% higher than the base scenario when a balanced breeding objective was applied.

The major limitation in facilitating genetic gain through AMH is the inability to use the male AMH level as an indicator of cow weaning rate. As an example, if males could be measured for AMH, the response for Brahman increases by 0.1%/year to 1.5% (compared with 1.4%) and the accuracy of cow weaning rate EBV for bull at first use at 26mo increases from 27% to 37% (when assuming the same proportions of animals selected as in the scenarios modelled, and a 0.5 genetic correlation between male AMH and cow weaning rate). This observation is further supported by recent Beef CRC results on male indicator traits where it was shown that a male indicator trait for female Brahman reproductive rate could increase cow weaning rate by 6.0% over 10 years, or 2.9% higher than the base scenario which included using cow reproductive records (*pers. comm*. D. Johnston, 13.6.12).

7.2 Facilitating phenotypic improvement in weaning rate

Based on the results presented, AMH appears to have low utility for use as a current generation selection tool to improve weaning rate in a commercial production system. With reproductive as the sole focus of selection, an optimistic repeatability of 0.3 and a phenotypic correlation between AMH and weaning rate of 0.5, current generation increases in weaning rate were only predicted to be 3.26%, 5.07% and 4.55% for southern beef, Brahman and sheep respectively. Much lower increases were predicted with lower repeatability and / or correlation between plasma AMH and weaning rate.

The major limitations to achieving a greater phenotypic response in weaning rate were the low repeatability of weaning rate and the low selection intensity of females in a self replacing herd. Key components of weaning rate have been widely reported to have low repeatability. For Australian cattle, published days to calving repeatability estimates range from 0.10 to 0.22 (Meyer et al. 1990, Meyer et al. 1991, Johnston et al. 1996, Meyer et al. 2003). Internationally, Oyama *et al.* (2002) reported a repeatability of calving interval of 0.09 for Japanese Black. It is also low in sheep. In a review of sheep genetic parameters Safari *et al.* (2005) reported a repeatability of NLW of 0.07 based on 5 studies.

Selection intensity is a function of the proportion of animals selected to form the next generation. For the southern beef scenario, selection intensity was 0.73 with a selection intensity of 0.47 for sheep. The lower selection intensity in sheep is due to shorter productive life of the ewe compared with a cow even though weaning rate per breeding female is higher. In practice, in northern Australia, cattle breeders are limited in the ability to cull dry cows and maintain herd size. This results in low selection intensity for reproduction for females in a self replacing herd. In this study, the same selection intensity as in southern beef was used. However, to achieve that selection intensity would require an increase in weaning rate from 65% to 85% for northern cattle breeders to then be in a position to select on a proportion of heifer calves for mating.

Repeatability estimates of cow weaning rate from the Beef CRC northern and southern research programs have not yet been published. Should the estimates of repeatability for cow weaning rate or similar traits be higher than 0.3, it is recommended that this section of work is reviewed. However, it is unlikely that repeatability of any comparable trait will exceed 0.3.

7.3 The potential of AMH to provide act as an screening tool prior to entry in an embryo transfer program

There is considerable potential benefit in using AMH as a pre-entry screening tool for embryo production campaigns. Monniaux *et al.* (2010) reported that a single sample of AMH on females provides extensive information about probability of being a successful donor cow for multiple ovulation embryo transfer programs in dairy cows. Predicted increase in number of transferable embryos ranged from 1-25% in this modelling. Published estimates of repeatability of number of transferable embryos are variable. For example, both Bari *et al*. (2001) Monniaux *et al*. (2010) reported a repeatability of 0.36, whereas Hahn *et al*. (1992) reported a repeatability of only 0.15.

At $50 per test, screening of 30 potential donor cows for AMH would cost $1500. To recover this outlay, at a cost of $250 per transferable embryo an additional 6 embryos would have to be recovered for transfer. Over a 5 flush embryo production campaign this requires a response of 0.066 embryos per cow per flush. Based on modelling this would occur even when either the repeatability (0.1) or correlation between AMH and number of transferable embryos are low (0.1). Importantly, when using the parameters reported by Monniaux *et al*. (2010), it is predicted an extra 0.67 embryos per flush could be achieved, some 10 times higher than the breakeven point of 0.066 embryos per flush.

The benefit of this technology is considerable. Better screening of potential donor cows would allow more greater rates of genetic gain through identification of cows that are likely to yield high numbers of transferable embryo’s, thereby creating higher selection intensity on females in seedstock herds than is currently the case. This benefit can be conservatively valued at $180,600 per year cumulative benefit for the nations beef industry. This estimate assumes no increase in the number of cows entering donor programs and is based on an assumption that 9% of seedstock calves are ET.

This provides considerable opportunity for measurement of AMH in cattle and sheep to determine if comparable correlations exist and whether AMH screening at anytime during the production cycle provides a cost effective method of improving the success (number of transferable embryos) of embryo transfer programs. It is recommended any further work in this area seeks to quantify repeatability (& variation) of embryo production in cows for both southern and northern systems. This can be easily conducted with a small number of industry collaborators.

A United States patent application titled, ‘Method to select animals with a high capacity of embryo production’ has been lodged by Monniaux *et al.* (2011). Initial advice suggests this patent is not in effect in Australia. However, if there is a patent in Australia this needs to be taken into account for commercialisation.

8 Conclusions

Tests are available to measure AMH as a current cost of $56 per duplicate sample. This price could feasibly reduce if large numbers of animals were tested such that some automation of the assay could be warranted. AMH is reported to be repeatable but appears of limited value for either genetic selection because of minimal capacity to measure/screen on males, or phenotypic selection because of low repeatability of weaning rate. The modelling reported provides justification for ongoing work in the area of investigating the value of AMH as a screening tool for cows prior to entry into an embryo production campaign.

8.1 Next steps/required work

It is recommended that

a) A project be developed to establish the repeatability and variation in of number of transferable embryos and the correlation with AMH measured at different times both before and during an embryo production campaign.
b) It is important to ensure repeatability of cow weaning rate for Australian production systems has been appropriately modelled. As such, it is recommended that soon to be published estimates of cow weaning rate are considered.

9 References

Bari F, Khalid M, Wolf B, Haresign W, Murray A and Merrell B (2001) The repeatability of superovulatory response and embryo recovery in sheep. Theriogenology 56,147-155.

Brien FD, Hinch GN, van der Werf JHJ, Brown DJ and Swan AA (2011) Selection strategies for the genetic improvement of reproductive performance in sheep. Proceedings of AAABG 19, 151-158

Hagen CP, Aksglaede L, Sørensen K, Mouritsen A, Andersson A, Petersen JH, Main KM and Juul A (2012) Individual serum levels of anti-Mullerian hormone in healthy girls persist through childhood and adolescence: a longitudinal cohort study. Human Reproduction 27, 861-866.

Hahn J (1992) Attempts to explain and reduce variability of superovulation. Theriogenology 38:269-275.

Hehenkamp WJK, Looman CWN, Themmen APN, de Jong FH, te Velde ER and Broekmans FJM (2006) Anti-Mullerian Hormone Levels in the Spontaneous Menstrual Cycle Do Not Show Substantial Fluctuation. J Clin. Endocrinol. Metab. 91(10):4057-4063.

Ireland JLH, Scheetz D, Jimenez-Krassel F, Themmen APN, Ward F, Lonergan P, Smith GW, Perez G I, Evans ACO and Ireland JJ (2008) Antral follicle count reliably predicts number of morphologically healthy oocytes and follicles in ovaries of young adult cattle. Biol. Reprod. 79, 1219-1225.

Ireland JJ, Smith GW, Scheetz D, Jimenez-Krassel F, Folger JF, Ireland JLH, MossaC F, LonerganC P and Evans ACO (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 causes of variation in the ovarian reserve in cattle. Reproduction, Fertility and Development, 23, 1-14.

Johnston DJ and Bunter KL (1996) Days to calving in Angus cattle: Genetic and environmental effects, and covariances with other traits. Livestock Production Science 45, 13-22.

Koots KR, Gibson JP, Smith C and Wilton JW (1994) Analyses of published genetic parameter estimates for beef production traits. 1. Heritability. Animal Breeding Abstracts 62, 309-338.

La Marca A, Broekmans FJ, Volpe A, Fauser BC, and Macklon NS (2009) Anti-Mullerian hormone (AMH): what do we still need to know? Human Reproduction, 24, 2264-2275.

Meyer K, Hammond K, Parnell PF, Mackinnon MJ and Sivarajasingam S (1990) Estimates of heritability and repeatability for reproductive traits in Australian beef-cattle. Livestock Production Science 25, 15-30.

Meyer K, Hammond K, Mackinnon MJ and Parnell PF (1991) Estimates of covariances between reproduction and growth in Australian beef cattle. Journal of Animal Science 69, 3533-3543.

Meyer K and Johnston DJ (2003) Estimates of genetic correlations between live ultrasound scan traits and days to calving in Hereford cattle. Proc. 15th Association for the Advancement of Animal Breeding and Genetics, 387-390.

Monniaux D, Barbey S, Rico C, Fabre S, Gallard Y and Larroque H (2010) Anti-Mullerian hormone: a predictive marker of embryo production in cattle? Reproduction, Fertility and Development 22, 1083–1091.

Monniaux D, Fabre S and Dalbies R. US Patent application publication Nov 10th 2011. Method to select animals with a high capacity of embryo production. Pub No: US 2011/0275886 A1.

Munsterberg A and Lovell-Badge R (1991) Expression of the mouse anti-Mullerian hormone gene suggests a role in both male and female sexual differentiation. Development 113, 613-624.

Nelson SM, Anderson RA, Broekmans FJ, Raine-Fenning N, Fleming R and La Marca A (2012) Anti-Mullerian hormone: clairvoyance or crystal clear? Human Reproduction 27, 631-636.

Overbeek A, Broekmans FJ, Hehenkamp WJ, Wijdeveld ME, van Disseldorp J, van Dulmen-den Broeder E and Lambalk CB (2012) Intra-cycle fluctuations of anti-Mu¨llerian hormone in normal women with a regular cycle: a re-analysis. Reproductive BioMedican Science Online 24, 664-669.

Oyama K, Katsuta T, Anada K, and Mukai F (2002) Heritability and repeatability estimates for reproductive traits of Japanese Black cows. Asian-Australasian Journal of Animal Sciences 15, 1680-1685.

Safari E, Fogarty NM and Gilmour AR (2005) A review of genetic parameter estimates for wool, growth, meat and reproduction traits in sheep. Livestock Production Science 92, 271-289.

Steiner AZ, Herring AH, Kesner JS, Meadows JW, Stanczyk FZ, Hoberman S and Baird DD (2011) Antimullerian Hormone as a Predictor of Natural Fecundability in Women Aged 30-42 years. Obstetrics and Gynaecology, 117, 798-804.