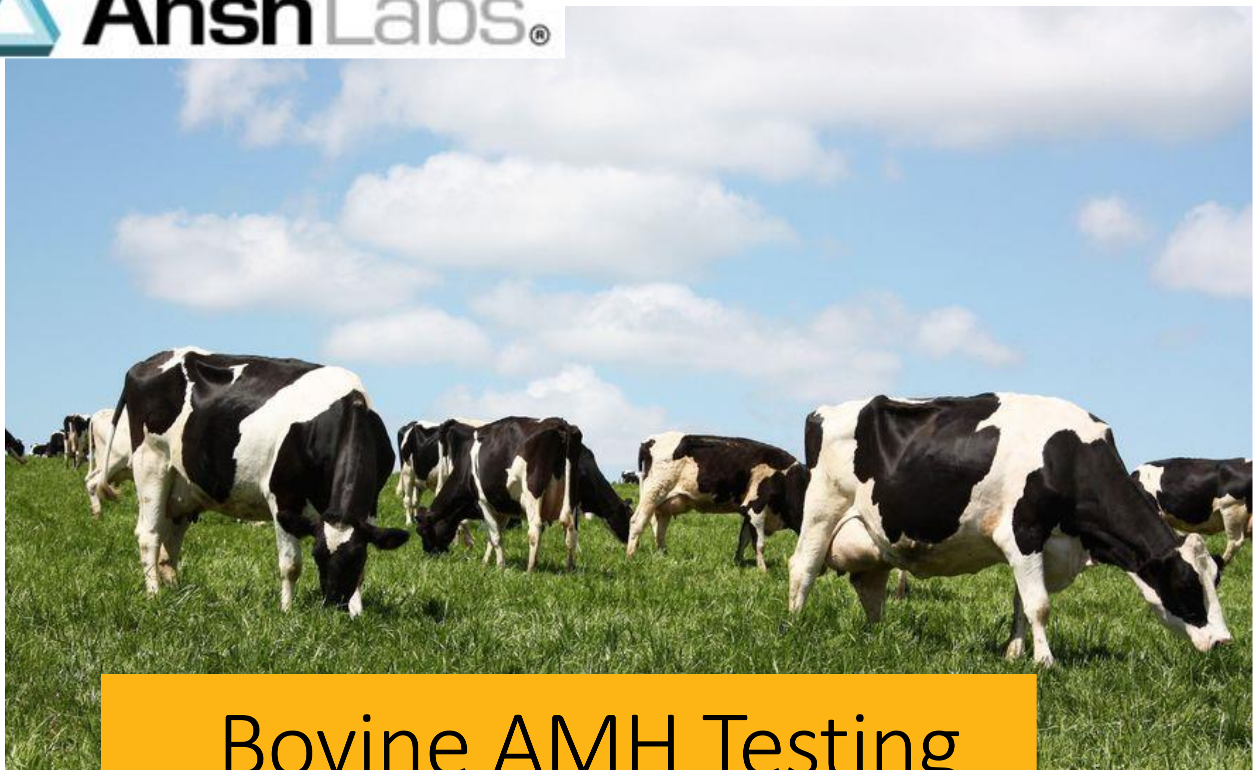
Ansh Labs



Bovine AMH Testing

Ansh Labs Bovine Anti-Müllerian Hormone Test: A biomarker selection tool for replacement heifer management for dairy and beef producers

Summary

Anti-Müllerian Hormone (AMH) is a protein hormone produced by granulosa cells in growing ovarian follicles. AMH can be measured any time in the estrous cycle and is a direct measure of ovarian reserve and has a high correlation to Antral Follicle Count (AFC). Declining AMH levels have been correlated to decline in fertility/fecundity in several species. In human IVF clinics, AMH levels are used to predict the size of a woman's ovarian reserve and her likelihood of producing viable eggs upon ovarian stimulation. AMH testing has been used very successfully in human IVF clinics. Similarly, bovine AMH levels can be used to select a dairy or beef cattle herd with greater fertility/fecundity.

In dairy and beef cattle, measuring AMH will:

- Enable the selection of the best heifers that will produce the highest number of transferable embryos
- Identify high producing embryo transfer donors for AI programs
- Identify cows with higher AI pregnancy rates
- Monitor the reproductive performance of cows
- Identify cows with higher first-conception service rates and fewer service requirements

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The Ansh Labs Bovine AMH test has been validated in the following breeds:

Bos taurus	*Bos indicus*	Mixed
- Jersey	- Brahman	- Beefmaster
- Hereford	- Gyr	- Brangus
- Holstein	- Nelore	- Holstein-Jersey
- Angus		- Braford
- Red Angus	*Bubalus bubalis*	- Bonsmara
- Black Angus	- Murrah	- Wagyu

The Ansh Labs Bovine AMH test can be used to monitor and manage the optimal reproductive performance of beef and dairy herds and thereby maximize profitability for producers.

Introduction

In most dairy or beef operations, animals are selected based on milk or meat quality, respectively. However, this practice may inadvertently de-select for reproductive performance. A production animal with good meat or milk quality may not necessarily be more fertile/fecund. What if you were able to select your desired trait, great milk quality and excellent meat quality, and also optimal reproductive performance? Decline in reproductive performance can have long-lasting economic consequences for any animal production operation. Selecting for fertility/fecundity is a major challenge in cow-calf or other food animal production operations. Breeding challenges are influenced by many factors. Some of these factors are determined by the needs of the market and those decisions should be made by the owners/managers of that operation[1]. Finding superior reproductively efficient cows are essential to establish an effective breeding program.

In small farms, selection of animals based on a fertility value for breeding are limited because nearly all animals will be used as breeding stock for the next generation. On the other hand, in large farms, it is costly to keep all the heifers as replacements. Replacement of your cow herd with approximately 20% per year of heifers is a good goal for most producers. A breeding program with the relevant traits, collection of performance data, data analysis, and selection of genetically superior animals are some of the main components of a structured breeding program. Breeding objectives can be simple or complex. In the case of commercial cow-calf program, reproductive performance is an important criterion. A high calving rate which amounts to a high weaning weight would be ideal. Including a reproductive biomarker, like AMH, along with other important genetic markers such as milk production, ribeye measurements, udder development, insect/disease resistance, temperament will increase productivity and profitability.

Bovine Anti-Müllerian Hormone Test – A Biomarker of Reproductive Performance

Anti-Müllerian Hormone (AMH) is a member of Transforming Growth Factor Beta family of proteins synthesized by growing ovarian follicles. AMH plays a major role in the recruitment of primordial follicles during ovarian follicle development. AMH is a robust endocrine marker of the population of gonadotropin-responsive small antral follicles in the cow and has been shown in other farm animal species as well**.** Serum or plasma concentration of AMH is highly correlated with Antral Follicle Count (AFC) and ovarian reserve in many mammalian species studied to date[2]. Ovarian reserve is associated with fertility and fecundity in mammals. AMH concentration can be readily measured by immunoassays and is highly reliable and reproducible[3].

Biomarker-assisted selection is an indirect selection process where trait(s) of interest are selected based on markers (genetic) linked to specific phenotypic outcomes (productivity, disease resistance, pregnancy rate). Biomarkers are useful to select for traits that are difficult or expensive to measure (e.g. DNA marker for a pregnancy rate), exhibit low heritability (reproductive rate) and/or are expressed late in development (udder size/teat size)[1].

Genetic information is widely used for selecting replacement heifers in large commercial farms. However, selection based on genetic information alone does not account for environmental or epigenetic effects on the animal. A robust selection process also takes into account the reproductive performance of the herd (i.e. high conception rates at service/second service and fecundity).

Prediction of the reproductive rate of replacement females/heifers would be an extremely valuable selection and monitoring tool for livestock production practices. AMH has emerged as a robust biomarker for assessment of reproductive performance (first service conception rate, reproductive longevity) in several production animals[2]. Bovine AMH testing in serum/plasma can be implemented alongside other testing parameters to select and monitor animals with greater fertility/fecundity, thereby, enhancing the reproductive performance of the herd.

Cost Benefit Analysis of Introducing AMH test in a Beef Heifer Breeding Program

Culling and replacement rates are the two factors that will impact the cow numbers in a herd. Based on the study by Hughes et al, six steps were used to project the cost of developing pregnancy checked replacement heifers on the demonstration herd in eastern Wyoming and western Nebraska[8] (Table 6).

Table 6: Cost benefit analysis model of introducing AMH test in replacement heifer selection

	Without AMH test		**With AMH test**	
	$/ heifer	**$/ herd (250)**	**$/ heifer**	**$/ herd (250)**
Period 1: Conception to weaning	1097	274,250	1097	274,250
AMH test @ $25/test			25	6,250
Period 2: Weaning to breeding	247	61,750	247	61,750
AMH test @ $25/test			25	6,250
Period 3: Breeding to Pregnancy check	211	52,750	211	52,750
Subtotal	1555	388,750	1555	388,750
Adjust for heifer conception rate 85% [(100X1555)/85]	1830	457,500		
Adjust for heifer conception rate 95% [(100X1555)/95] (with AMH test)*			1646	411,500
Cost benefit of introducing AMH in replacement heifer selection			**$194 per heifer**	**$46,000 per herd**

Case Studies

Application of Bovine AMH Test in Animal Production

Case Study 1: Use of AMH testing to select beef heifers with higher AI pregnancy rates

This study was conducted to determine the relationship between the concentration of serum AMH at weaning and/or breeding with fertility in beef heifers[4]. Seventy-one Angus heifers, (7 and 14 months of age) were used in the study. Based on AMH concentration at breeding, heifers in the lowest quartile (Q1) had a lower artificial insemination (AI) pregnancy rate than heifers in other quartiles in the breeding season (Table 1). The heifers in the lowest quartile (Q1) were not pregnant after AI, compared with 80% in the highest quartile (Q4) (Table 2). AMH concentration at breeding or the change in AMH from weaning to breeding can identify beef heifers more likely to conceive via AI and to conceive early in the breeding season. This study concluded that AMH concentration at breeding and/or the change in AMH from weaning to breeding showed a positive correlation between AMH and reproductive performance in beef heifers[4].

Table 1: Correlation of serum AMH levels at breeding with cyclicity and pregnancy rate in beef heifers

	Anti-Müllerian Hormone - Quartile			
	Q1	Q2	Q3	Q4
AMH range (ng/ml)	0.04 - 0.23	0.27 - 0.45	0.50 - 0.77	0.80 - 1.73
Cycling at breeding (%)	7/17 (41.2)	11/18 (61.1)	12/17 (70.6)	7/17 (41.2)
Synchronized estrus (%)	10/17 (58.8)	13/18 (72.2)	14/18 (77.8)	11/18 (61.1)
AI pregnancy rate (%)	1/10 (10.0)	7/13 (53.9)	6/14 (42.9)	8/11 (72.7)

Table 2: Correlation of change in serum AMH levels from weaning to breeding on cyclicity and pregnancy rate in beef heifers.

	Anti-Müllerian Hormone - Quartile			
	Q1	Q2	Q3	Q4
AMH range (ng/ml)	-0.48 - 0.04	0.05 - 0.16	0.17 - 0.44	0.48 - 1.32
Cycling at breeding (%)	7/17 (41.2)	12/18 (66.7)	10/17 (58.8)	7/16 (43.8)
Synchronized estrus (%)	10/17 (58.8)	14/18(77.8)	13/18 (72.2)	10/17 (58.8)
AI pregnancy rate (%)	0/10 (0.0)	7/14 (50.0)	7/13 (53.9)	8/10 (80)

Case Study 2: Use of AMH to select for dairy cows with higher first-service conception rate
In this study, serum concentrations of AMH within a population of dairy cows were compared with AFC, first service conception rate, number of services and days open[5]. One hundred lactating Holstein cows were used in the study. Cows were ranked in a descending order based on serum AMH concentrations. The top and bottom thirds were categorized into HIGH-AMH (n = 33) and LOW-AMH (n = 33), respectively (Table 3). Mean AFC was significantly greater in HIGH-AMH than those in LOW-AMH category cows. Serum AMH concentrations were highly correlated with AFC. Cows in the HIGH-AMH category tended to have higher first service conception rate, fewer number of services, and fewer days open than those in LOW-AMH category[5].

Table 3: Association of serum AMH concentration with Antral Follicle Count, conception rate and days open in dairy cows

	Low AMH	High AMH
No of animals	33	33
Serum AMH (pg/ml)	95.8 ± 15.2	386.2 ± 14.9
Antral Follicle Count (No)	16.7 ± 1.4	28.1 ± 1.4
1st Service conception rate (%)	24.2 ± 0.4	45.4 ± 0.4
Number of services	2.7 ± 0.2	2.1 ± 0.2
Days open	162.3 ± 11.7	133.6 ± 11.5

Case study 3: Use of AMH to select high embryo yield donor cows for *in vitro* transfer

The collection of high numbers of oocytes for *in vitro* embryo production in the cow depends on the number of antral follicles available for oocyte pick-up. The number of antral follicles varies significantly among cows and can be estimated by ovarian ultrasonography and serum/plasma concentrations of AMH. Antral Follicle Count is highly correlated to serum AMH in cows[6]. In this study, serum AMH levels were strongly correlated to number of *in vitro* embryos produced from Holstein (Bos taurus) and Nellore (Bos indicus) donors (Table 4, 5). More cumulus-oocyte complexes were aspirated from cows with high serum AMH levels. In addition, donor cows with high serum AMH level a greater ratio of good quality embryos produced to oocytes picked-up, compared to donor cows with low AMH levels (Table 4,5). Therefore, serum/plasma AMH levels can be a robust endocrine biomarker to determine *in vitro* embryo yield from donor cows in AI programs[6,7].

Table 4: Positive correlation of serum AMH concentration with *in vitro* embryo production in Holstein (B. taurus)

	Low AMH	High AMH
No of animals	32	27
Plasma AMH (ng/ml)	0.2 ± 0.01	0.4 ± 0.02
Visible aspirated follicles (No)	13.6 ± 0.9	20.9 ± 1.5
Cumulus-oocyte complexes (No)	9.0 ± 0.9	17.3 ± 1.5
Blastocyst production rate (%)	19.8% ± 4.2%	20.6% ± 4.0%
Embryos (No)	1.2 ± 0.3	3.0 ± 0.7

Table 5: Positive correlation of serum AMH concentration with in vitro embryo production in Nellore (B. indicus)

	Low AMH	High AMH
No of animals	18	16
Plasma AMH (ng/ml)	0.5 ± 0.005	2.0 ± 0.3
Visible aspirated follicles (No)	18.6 ± 2.1	54.3 ± 6.1
Cumulus-oocyte complexes (No)	13.4 ± 1.7	45.3 ± 6.4
Blastocyst production rate (%)	27.4% ± 5.5%	33.7% ± 6.5%
Embryos (No)	2.2 ± 0.5	7.0 ± 1.7

Study 1: Use of Bovine AMH to select for high reproductive potential in beef heifers (Rorie et al, 2016)

	Without AMH test		With AMH test	
	$/ heifer	**$/ herd (68)**	**$/ heifer**	**$/ herd (68)**
Adjust for heifer conception rate 33%	4712	320,416		
Adjust for heifer conception rate 43% (with AMH test)*			3709	252,212
Cost benefit of introducing AMH in replacement heifer selection			**$833 per heifer**	**$68,204 per herd**

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