5-2016

Use of anti-mullerian hormone to select for fertility in beef heifers

Hannah Newberry
University of Arkansas, Fayetteville

Use of anti-mullerian hormone to select for fertility in beef heifers

Hannah R. Newberry

ABSTRACT

A study was conducted to determine whether concentration of serum Anti-Mullerian Hormone (AMH) at weaning and/or breeding could predict subsequent fertility in beef heifers. Frequency distribution was used to assign serum AMH concentration measured at weaning, breeding, and the change from weaning to breeding, into quartiles. Comparison of heifers based on serum AMH quartiles at weaning failed ($P \geq 0.35$) to detect any effect of AMH on subsequent heifer cyclicity at breeding, estrous response after synchronization, artificial insemination (AI) pregnancy rate, overall breeding season pregnancy rate, or estimated estrous cycle of the breeding season when conception occurred. Based on AMH concentration at breeding, heifers in the lowest quartile (Q1) had a lower ($P = 0.02$) AI pregnancy rate than heifers in other quartiles, and conceived at a later estrous cycle ($P = 0.03$) in the breeding season. Comparison of heifers based on the difference between AMH concentrations at breeding versus weaning revealed that none of the heifers in the lowest quartile (Q1) became pregnant after AI, compared with 80% in the highest quartile (Q4; $P < 0.001$). Heifers in the lowest quartile also conceived at a later estrous cycle in the breeding season than heifers in the other quartiles ($P = 0.01$). Results indicate that either AMH concentration at breeding or the change in AMH from weaning to breeding can identify beef heifers more likely to conceive to AI and to conceive early in the breeding season.

Table of Contents

INTRODUCTION AND LITERATURE REVIEW

Reproduction is the most important factor contributing to the efficiency of beef production. Currently, less than 75% of the cows in beef herds produce a calf each year, with the majority of losses occurring due to pregnancy failure. Improvement in reproductive efficiency could begin with better replacement heifer selection. Replacement heifer development represents a significant cost to beef producers. Heifer development from weaning to breeding age, and then breeding, and maintenance until pregnancy determination represents an expense in excess of 500 dollars per heifer to cattle producers, even when excluding the opportunity costs of not selling a heifer at weaning, (Cleere, 2006). Cattle producers often retain 40 percent or more heifers than anticipated needs, to insure an adequate number of bred herd replacements are available. This practice adds to costs of each herd replacement. Selecting the most fertile heifers for retention in the herd would not only improve production efficiency, but also result in a significant savings in heifer development costs. Likewise, identification of less fertile heifers at a younger age would allow for their marketing at a more optimal time.

Reproductive tract scoring (RTS; Anderson et al., 1991) via ultrasonography, which evaluates reproductive tract development and the presence of ovarian structures, has been used to identify cyclic heifers before breeding but does not provide any information on the animal's potential fertility. Furthermore, ultrasonography for RTS is not possible on heifers at weaning (~ 7 months of age) due to their physical size. Recent research (Ireland et al., 2011) has identified Anti-Mullerian hormone (AMH) as a potential diagnostic marker for fertility in cattle. Measure of AMH in circulation at weaning is a viable option regardless of the heifer's physical size.

Anti-Mullerian hormone is a homodimeric disulfide-linked glycoprotein that was discovered by Alfred Jost in the 1940s (La Marca and Volpe, 2006). In the male, AMH is

involved in sexual differentiation of the male reproductive tract during early fetal development. Expression of Y chromosome-specific genes results in production of testis-determining factor that stimulates development of the testes. Sertoli cells within the developing testes then produce AMH, that in turn causes regression of the mullerian ducts, while leydig cells produce testosterone to develop and maintain the Wolffian ducts (Tuttelmann et al., 2009). Testosterone and AMH produced by the testes of bull calves during early fetal development are responsible for the Freemartin condition in co-twin, heifer calves. As male sexual differentiation occurs during early fetal development, these hormones enter the fetal circulation of the female twin, inhibiting development of the mullerian ducts would give rise to much of the female's reproductive tract.

It might seem counterintuitive that females would produce AMH because of its inhibitory effect on female reproductive tract development. However, AMH is only detectable after 36 weeks gestation, when oogenesis, folliculogenesis and development of the female reproductive tract have already occurred (Rajpert-De Meyts et al., 1999). Anti-Mullerian Hormone (AMH) is produced by granulosa cells of all primordial, primary, secondary follicles, as well as antral follicles up to 4 to 5 mm diameter, and reflects the total number of healthy follicles within the ovaries (Visser et al., 2006). The function of AMH in females is to regulate or limit the recruitment of primordial follicles into folliculogenesis, by reducing the responsiveness to these follicles to follicle stimulating hormone (Visser et al., 2006). Anti-Mullerian hormone production decreases after antral stage follicles reach the 4 to 5 mm, allowing these follicles to regain responsiveness to follicle stimulating hormone and undergo final maturation.

The number of follicles present in the ovaries of heifer calves at birth can range from 10,000 to 350,000 (Erickson, 1966). Heifers with low follicle counts also have smaller ovaries

and fewer morphologically healthy follicles and oocytes, suggesting a link between follicle number and fertility (Ireland et al., 2008). Because AMH in circulation reflects the total follicular reserve of the ovaries and animals with a greater number of follicles, a single measure of AMH in the circulation of breeding age heifers has been used to identify heifers with greater reproductive potential (Ireland et al., 2011). However, the question arises as to how early in development AMH can be measured as an indicator of fertility. Identification of heifers with low or high fertility at birth or weaning would be advantageous to producers for making management decisions. If measure of AMH at weaning could predict subsequent fertility, this would not only reduce replacement heifer costs, but also identify less fertile heifers at an age that would allow their marketing as stocker-feeder cattle at a more optimal time. Therefore, the objective of the present study was to examine the relationship between serum AMH concentration at weaning versus breeding, and to determine it either or both measures could predict subsequent fertility of beef heifers.

MATERIALS AND METHODS

Animal Management

The study utilized 71 beef heifers located at the University of Arkansas Beef Research Unit near Savoy, Arkansas. Prior to the study, all proposed animal procedures were approved by the University of Arkansas Institutional Animal Care and Use Committee (IACUC protocol # 15041). At weaning (~ 7 months of age), a 10-ml blood sample was collected in serum separator tubes, labeled, and the serum was frozen (-20° C) until analysis for Anti-Mullerian Hormone (AMH). The heifers were then developed and maintained on pasture, with access to free-choice mineral, and provided corn gluten feed to meet energy requirements as needed. At breeding age (~ 14 months of age), a second 10-ml blood sample was collected in serum separator tubes,

serum recovered and frozen, to be analyzed for Anti-Mullerian Hormone during the same time interval as the first weaning samples.

Approximately 30 days before the start of the breeding season, transrectal ultrasonography (IBEX Pro with a L6.1 linear array transducer; E.I. Medical Imaging, Loveland, CO) was performed to determine the reproductive tract score (Anderson et al., 1991) of each heifer. At the time of reproductive tract scoring, a scan through the left and right ovary of each heifer was video recorded in order to accurately determine ovary size, the presence or absence of a corpus luteum, and the number and size of the largest follicles present. Based on ovary size and structures present, heifers were categorized as cyclic or non-cyclic.

Estrous Synchronization and Breeding

At the start of the breeding season all heifers received a single 25 mg injection (i.m.) of prostaglandin F2alpha (Lutalyse; Zoetis, Florham Park, NJ) and an estrous detection patch (Estrotect; Rockway Inc., Spring Valley, WI). Heifers were observed 3 or more times daily for onset of estrus, and inseminated approximately 12 hours after detected estrus. Heifers not detected in estrus received a second Lutalyse i.m. injection 7 days after the initial treatment. Estrus detection and insemination continued for 4 days as previously described. Ten days later, the heifers were exposed to fertile bulls for a 45-day breeding season. Bulls were rotated through breeding groups half way through the breeding season. At 50 to 60 days after insemination, transrectal ultrasonography was used to identify pregnant heifers and to confirm conception date, based on fetal crown-to-rump length. At 60 days after bull removal, transrectal ultrasonography was used again to determine pregnancy in heifers conceiving during the breeding season and confirmed a continuing pregnancy in heifers previously identified as pregnant. Based on fetal size at ultrasonography, the estrous cycle after initiation of breeding

when conception occurred was estimated. For comparison, AI pregnancies were considered cycle 0, and pregnancies initiated during the first, second or third 21-day intervals of the breeding season were classified as cycles 1, 2 and 3, respectively.

Anti-Mullerian Hormone Assay

Serum samples were analyzed for AMH, using bovine AMH ELISA kits (Ansh Labs, TX); and following procedures as outlined by the kit. Each assay plate contained a standard curve in duplicate, ranging from 0 to 2.4 ng/ml AMH. Two kits were utilized; one for the serum samples collected at breeding and the other at weaning. The ELISA kit was a 3-step sandwich type immunoassay using 96 well plates, with each well coated with biotinylated AMH antibody. Standards, high and low controls and unknowns (50 µl) were added to appropriate wells, along with 50 µl of assay buffer. Each assay plate was then incubated 2 hours on an orbital plate shaker (Titer Plate Shaker, Lab-Line Instruments, Melrose, IL) at room temperature. Plates were then washed 5 times, using an automated plate washer (ELP-40 Microplate Strip Washer, Bio-Tek Instruments, Winooski, VT).

An AMH antibody-biotin conjugate (100 µl) was added to each well, followed by another incubation on the plate shaker for 1 hour. After washing 5x again, 100 µl of streptavidin-enzyme conjugate was added to each well, followed by incubation on the plate shaker for 30 minutes. Following another 5x plate wash, 100 µl of TMB chromogen was added to each well and the plates placed back on the plate shaker. Visual color change was monitored and after 12 minutes, the plate was removed and 100 µl of stopping solution was added to each well to prevent further color change. Within 15 minutes of addition of stopping solution, the plates were read (0.1 second/well) for absorbance at 450 nm, using a Perkin-Elmer (Waltham, MA) Victor V, Model

1420 Multi-label Counter. Absorbance readings for 'blank" wells were subtracted from all other well readings to correct for plate optical density.

Statistical Analysis

All data analysis was performed using JMP Pro 12.0 statistical software. Regression analysis (bivariate fit) was used to determine the relationship between absorbance readings and standard concentrations of AMH. The resulting regression equations were used to calculate AMH concentration in each unknown sample within the appropriate assay plate. Frequency distribution was also used to assign AMH concentration measured in serum samples at weaning and breeding to quartiles. In addition, quartiles were established for the difference or change in AMH from weaning to breeding (breeding-weaning AMH). Comparisons were then made for heifers in each quartile and the percentage of heifers cyclic at synchronization, expressing estrus after synchronization, conceiving after artificial insemination, pregnant at the end of the breeding season, and the estimated cycle after the initiation of breeding that conception occurred.

RESULTS AND DISCUSSION

The heifers weighed an average of 240.6 ± 2.5 kg at weaning and 358.6 ± 3.7 kg at the start of the breeding season. Transrectal ultrasonography determined that 39/71 (55.0%) of the heifers were cyclic before the start of breeding. After 2 (7 days apart) injections of prostaglandin F2alpha to induce estrus, 48/71 (67.6%) of heifers were detected in estrus and inseminated. Twenty-two of forty-eight (45.8%) of the heifers conceived after artificial insemination. At ultrasonography ~ 60 days after the breeding season 62/71 (87.3%) of the heifers were confirmed to be pregnant.

The inter-assay variation among duplicate samples in the AMH assay performed for serum samples collected at weaning was 2.7%. The regression equation ($R^2 = 0.998$) used to

determine AMH concentration in serum collected at weaning was: AMH ng/ml = -0.034853 + 0.5789461*absorbance. At weaning, serum AMH ranged from 0.04 to 0.99 ng/ml, with a mean of 0.30 ng/ml. The inter-assay variation among duplicate samples in the AMH assay performed for serum samples collected at breeding was 3%. The regression equation ($R^2 = 0.995$) used to determine AMH concentration in serum collected at breeding was: AMH ng/ml = -0.080924 + 0.577811*absorbance. At breeding, serum AMH ranged from 0.04 to 1.73 ng/ml, with a mean of 0.56 ng/ml.

When heifers were compared by quartiles, based on serum AMH at weaning, AMH hormone concentration at that time had no effect ($P \geq 0.35$) on subsequent heifer cyclicity at breeding, response to synchronization, AI pregnancy rate, overall pregnancy rate, or mean cycle of the breeding season when conception occurred (Table 1). Failure to detect an effect of AMH concentration at weaning on subsequent fertility in beef heifers is in contrast with a study conducted with sheep. Lahoz et al. (2012) measured plasma AMH in 76 ewes at 3.6 months of age. The ewes were mated at 10 months of age, with those failing to conceive being mated again 4 months later. Results of that study indicated that fertility of ewes at first mating positively correlated with circulating AMH concentration at 3.6 months of age. The study concluded that a single AMH measurement performed on ewes at an early age was useful for selection of ewes with higher fertility potential at first mating.

Based on AMH concentration at breeding, heifers in the lowest quartile (Q1) had a lower ($P = 0.02$) AI pregnancy rate than heifers in other quartiles and conceived at a later cycle ($P = 0.03$) in the breeding season (Table 2). Studies have shown that heifers conceiving early in their first breeding season will continue to conceive early in subsequent breeding seasons, wean heavier calves, and be more productive throughout their life (Bellows and Staigmiller, 1994).

Recently, Jimenez-Krassel et al. (2015) measured AMH on 11 to 15 month old Holstein heifers before first breeding, and then followed their reproductive performance and productivity through two lactations. Compared to heifers in higher AMH quartiles, heifers in the lowest AMH quartile on average, had a productive herd life that was 196 days shorter, the lowest level of first lactation milk production, the lowest percentage for cows pregnant across all lactations, and the highest culling rate for poor reproduction.

Plasma AMH concentration in bovine females has been reported to remain relatively stable throughout the first year of life (Rota et al., 2002). In the current study, mean AMH in serum increased from 0.30 at weaning to 0.56 ng/ml at breeding. It was also noted that the serum AMH concentration of some individual heifers either did not increase or actually decreased during this time. Therefore, heifers were assigned to quartiles based on the difference between AMH concentration at breeding and weaning (Table 3). None of the heifers became pregnant after AI in the lowest quartile (Q1), compared with 80% in the highest quartile (Q4; $P < 0.001$). Heifers in the lowest quartile also conceived at a later cycle in the breeding season than heifers in the other quartiles ($P = 0.01$).

In the study previously mentioned that was conducted with dairy heifers (Jimenez-Krassel et al., 2015) it was hypothesized that AMH concentration had a positive correlation with high antral follicle counts, fertility, and ovary function. The study confirmed that a single blood sample for AMH from breeding age dairy heifers could be used to select replacements and predict long-term reproductive performance of dairy heifers. Often reproduction is negatively correlated with other desirable traits. However, the results of Jimenez-Krassel et al. (2015) showed that AMH could be used to identify more fertile heifers without compromising milk production.

A study utilizing 1,237 multiparous dairy cows of three different breeds determined if circulating AMH had a direct relationship with fertility during a planned 100 day breeding season (Ribeiro et al., 2014). The cows were synchronized, and either placed in timed insemination protocol or inseminated at estrus. Serum samples were collected on day eight of the estrous cycle for measurement of both AMH and progesterone. Concentrations of AMH were found to vary among the breeds of cows and those at different stages of lactation. Although no relationship was found between AMH levels for dairy cows enrolled in timed insemination, a positive correlation was found between AMH and pregnancy rates with dairy cows bred after detected estrus. In addition, the study reported that pregnancy loss to be greater in cattle with lower AMH.

A study reporting results from both dairy and beef cattle (Baruselli, et al., 2015) confirmed that AMH positively correlates with fertility. The authors reported that ovarian follicle reserves, which are highly variable among females and positively correlate with fertility, are positively correlated with circulating AMH concentration. If follicle numbers are positively correlated with fertility, then it might be assumed that antral follicle counts would be equally effective to AMH measure as an indicator of fertility. However, Baruselli, et al. (2015) reported that the number of antral follicles varies throughout the estrous cycle, whereas AMH remains relative constant, regardless of the stage of the estrous cycle.

Walsh et al., (2014) reported a positive correlation between antral follicle counts between dam-daughter pairs. However, a subsequent study by Batista et al. (2015) suggested that the correlation between antral follicle counts of dam-daughters is low. Epigenetic factors such as negative energy balance throughout the early fetal life can have a large influence on antral

follicle counts (Walsh et al., 2014) and could explain why some studies report a correlation between antral follicle counts of dam-daughters and others do not.

A study in goats reported that AMH could be used as a predictor of in vivo embryo production (Monniaux et al., 2011). Plasma AMH was measured in goats before follicle-stimulating hormone (FSH) treatments were given to stimulate follicular growth at the beginning of the breeding season, at the end of the breeding season, and during the anestrus period. High AMH was positively correlated with higher numbers of corpora lutea and embryo recovery. The study concluded that AMH could help predict the ability of goats to respond to the superovulatory treatment, as well as whether they will produce high numbers of transferable embryos. It was noted that the goats' plasma AMH concentrations gradually decreased after each embryo collection.

Results of the current study and others concur that AMH can be used as a predictor of fertility in replacement animals. 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 fertility. Lahoz et al. (2012) measured AMH in sheep during the prepubertal period and found a correlation between AMH and fertility later in life. In goats, a positive correlation was between AMH and in vivo embryo production after superovulation. In both dairy heifers and mature cows, circulating AMH was shown to be positively correlated with fertility (Jimenez-Krassel et al., 2015, Ribeiro et al., 2014).

ACKNOWLEDGEMENTS

The author would like to thank the University of Arkansas Honors College, the Bumpers College Honors Program, and the University of Arkansas, Division of Agriculture for providing funding for this research. In addition, the author would like to thank the faculty mentor for this

project, Dr. Rick Rorie, and the other two thesis committee members, Dr. Beth Kegley and Dr. Charles Rosenkrans. Recognition should also be given to Toby Lester, who completed the ultrasonography and blood sampling, and Mohan Acharya and Chris Hansen who assisted with the AMH assays.

LITERATURE CITED

Anderson, K.J., D.G. LeFever, J.S. Brinks, and K.G. Odde. 1991. The use of reproductive tract scoring in beef heifers. Agri-Practice 12:19-26.

Baruselli, P.S., E.O.S. Batista, L.M Vieira, and A.H. Souza. 2015. Relationship between follicle population, AMH concentration and fertility in cattle. Anim. Reprod., v.12, n.3, p.487-497.

Batista EOS, Macedo GG, Sala RV, Ortolan M, Sá Filho MF, Del Valle TA, Jesus EF, Lopes R, Rennó, FP, Baruselli PS. 2014. Plasma anti-mullerian hormone as a predictor of ovarian antral follicular population in Bos indicus (Nelore) and Bos taurus (Holstein) heifers. Reprod Domest Anim, 49:448-452.

Bellows, R.A. and R.B. Staigmiller. 1994. Selection for fertility. In: Factors affecting calf crop M.J. Fields and R.S. Sand (Eds). CRC Press, Boca Raton, FL. pp. 197-212.

Capper, J.L. 2011. The environmental impact of beef production in the United States:1977 compared with 2007. J. Anim. Sci. 89:4249-4261.

Cleere, J. 2006. Buying Vs. Raising Replacement Heifers. AgriLife Extension E-371. http://animalscience.tamu.edu/files/2012/04/beef-buying-vs-raising-replacement-heifers.pdf.

Erickson, B.H. 1966. Development and senescence of the postnatal bovine ovary. J. Anim. Sci. 25:800-805.

Ireland, J.L.H., D. Scheetz, F. Jimenez-Krassel, A.P.N. Themmen, F. Ward, P. Lonergan, G.W. Smith, G.I. Perez, A.C.O. Evans, and J.J. Ireland. 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, J.J., G.W. Smith, D. Scheetz, F. Jimenez-Krassel, J.K. Folger, J.L.H. Ireland, F. Mossa, P. Lonergan, and A.C.O. Evans. 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-Mu¨ llerian hormone as a diagnostic marker for fertility and causes of variation in the ovarian reserve in cattle. Reprod. Fertil. Develop. 23:1-14.

Jimenez-Krassel, F. D.M. Scheetz, L.M. Neuder , J.L.H. Ireland, J.R. Pursley, G.W. Smith, and J.J. Ireland. 2015. Concentration of anti-Müllerian hormone in dairy heifers is positively associated with productive herd life. J. Dairy Sci. 98:3036-3045.

Lahoz, B., J.L. Alabar, D. Monniaux, P. Mermillod, and J. Folch. 2012. Anti-Mullerian hormone plasma concentration in prepubertal ewe lambs as a predictor of their fertility at a young age. BMC Vet. Res. 8:118 (http://www.biomedcentral.com/1746-6148/8/118).

La Marca, A., and A. Volpe. 2006. Anti-Mullerian hormone (AMH) in female reproduction: is measurement of circulating AMH a useful tool?. Clin. Endocrin., 64: 603-610.

Monniaux, D., G. Baril, A.L. Laine, P. Jarrier, N. Poulin, J. Cognie, and S. Fabre. 2011. Anti-Mullerian hormone as a predictive endocrine marker for embryo production in the goat. Reprod. 142:845-854.

Rajpert-De Meyts, E., N. Jorgensen, N. Graem, J. Muller, R.L. Cate, and N.E. Skakkebaek. 1999. Anti-Mullerian hormone during normal and pathological gonadal development: Association with differentiation of sertoli and granulosa cells. J. Clinical Endocrin. Metabol. 84:3836-3844.

Ribeiro, E.S., R.S. Bisinotto, F.S. Lima, L.F. Greco, A. Morrison, A. Kumar, W.W. Thatcher, and J.E.P. Santos. 2014. Plasma anti-Mullerian hormone in adult dairy cows and associations with fertility. J. Dairy Sci. 97:6888–6900.

Rota, A., C. Ballarin, B. Vigier, B.Cozzi, and R. Reyd. 2002. Age dependent changes in plasma anti-Mullerian hormone concentrations in the bovine male, female, and freemartin from birth to puberty: relationship between testosterone production and influence on sex differentiation. Gen. Comp. Endocrinol. 129:39-44.

Tuttelmann, F., N. Dykstra, A.P. Themmen, J.A. Visser, E. Nieschlag, and M. Simoni. 2009. Anti-Mullerian hormone in men with normal and reduced sperm concentration and men with maldescended testes. Fertil. Steril. 91:1812-1819.

Visser, J. A., F.H. de Jong, J.S. Laven, and A.P. Themmen. 2006. Anti-Mullerian hormone: a new marker for ovarian function. Reprod. 131: 1-9.

Walsh S, Mossa F, Butler ST, Berry DP, Scheetz D, Jimenez-Krassel F, Tempelman RJ, Carter F, Lonergan P, Evans AC. 2014. Heritability and impact of environmental effects during pregnancy on antral follicle count in cattle. J Dairy Sci, 97:4503-4511.

Table 1. Effect of serum AMH concentration at weaning on cyclicity and pregnancy rate in beef heifers.

Item	Anti-mullerian hormone quartile				P value
	1	2	3	4	
AMH range (ng/ml)	0.04 - 0.15	0.17- 0.24	0.25 - 0.38	0.40 - 0.99	
Cyclic at breeding (%)	8/17(47.1)	11/18(61.1)	8/16(50.0)	9/17(52.9)	0.855
Synchronized estrus (%)	10/17(58.8)	14/18(77.8)	10/18(55.6)	13/17(76.5)	0.354
AI pregnancy rate (%)	4/10(40.0)	8/14(57.1)	5/10(50.0)	5/13(38.5)	0.754
Overall pregnancy rate (%)	15/17(88.2)	16/18(88.9)	17/18(94.4)	13/17(76.5)	0.449
Mean conception cycle	1.33	0.81	1.24	1.00	0.523

Table 2. Effect of serum AMH concentration at breeding on cyclicity and pregnancy rate in beef heifers.

Item	Anti-mullerian hormone quartile				P value
	1	2	3	4	
AMH range (ng/ml)	0.04 - 0.23	0.27 - 0.45	0.50 - 0.77	0.80 - 1.73	
Cyclic at breeding (%)	7/17(41.2)	11/18(61.1)	12/17(70.6)	7/17(41.2)	0.206
Synchronized estrus (%)	10/17(58.8)	13/18(72.2)	14/18(77.8)	11/18(61.1)	0.572
AI preg. rate (%)	1/10(10.0)[a]	7/13(53.9)[b]	6/14(42.9)[b]	8/11(72.7)[b]	0.021
Overall preg. rate (%)	14/17(82.4)	16/18(88.9)	16/18(88.9)	16/18(88.9)	0.919
Mean conception cycle	1.79[a]	1.0[b]	0.75[b]	0.94[b]	0.034

[ab]Within rows, numbers with different superscripts are significantly different ($P < 0.05$).

Table 3. Effect of change in serum AMH concentration from weaning to breeding on cyclicity and pregnancy rate in beef heifers.

	Anti-mullerian hormone quartile				
Item	1	2	3	4	P value
AMH range (ng/ml)	-0.48 - 0.04	0.05 - 0.16	0.17 - 0.44	0.48 - 1.32	
Cyclic at breeding (%)	7/17(41.2)	12/18(66.7)	10/17(58.8)	7/16(43.8)	0.374
Synchronized estrus (%)	10/17(58.8)	14/18(77.8)	13/18(72.2)	10/17(58.8)	0.525
AI preg. rate (%)	0/10(0.0)[a]	7/14(50.0)[b]	7/13(53.9)[b]	8/10(80.0)[b]	>0.001
Overall preg. rate (%)	13/17(76.5)	16/18(88.9)	17/18(94.4)	15/17(88.2)	0.464
Mean conception cycle	1.92[a]	0.93[b]	0.88[b]	0.80[b]	0.014

[ab]Within rows, numbers with different superscripts are significantly different ($P < 0.05$).