Serum anti-Müllerian hormone concentrations in female alpacas: variations during the reproductive cycle and correlation with ovarian superstimulation response

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Abstract

Earlier, we validated an anti-Müllerian hormone (AMH) enzyme-linked immunosorbent assay kit for male alpacas. First, we compared the validation data with another kit. There was a high correlation ($R^2 = 0.94$) between these 2 kits. Second, we used the latter kit to determine serum AMH concentrations during follicular and luteal phases of the reproductive cycle in female alpacas. There were no differences ($p = 0.39$) in serum AMH concentrations in alpacas (n = 11) between peak follicular and luteal phases (mean ± SEM, 1.33 ± 0.35 versus 1.18 ± 0.34 ng/ml, respectively). Third, we treated female alpacas (n = 13; 5 - 11 years) after 14-day treatment with decreasing doses of porcine follicle-stimulating hormone. There was no effect ($p > 0.05$) of day of treatment on serum AMH concentrations. Number of follicles (7 - 10 mm; mean ± SD [as determined via transrectal ultrasonography]) at end of treatment (12.69 ± 5.25; range: 6 - 24) was positively correlated ($R^2 = 0.7$; $p < 0.01$) with serum AMH concentrations. To conclude, the kit tested is usable for female alpacas; serum AMH concentrations were not affected by the cycle stage nor by ovarian superstimulation treatment. Furthermore, a significant correlation between serum AMH serum concentrations and response to superstimulation suggested that estimation of serum AMH concentrations may be valuable in determining ovarian follicular reserve.

Keywords: Camelid, anti-Müllerian hormone, enzyme-linked immunosorbent assay, follicular reserve

Introduction

Anti-Müllerian hormone (AMH), also known as Müllerian inhibiting substance, is a homodimeric disulfide-linked glycoprotein member of the transforming growth factor ß superfamily.[1] In male mammals, AMH is produced by Sertoli cells during fetal development.[2,3] In contrast, in females, AMH is expressed for the first time in the neonatal ovary exclusively by healthy granulosa cells of small primary growing follicles after recruitment from the primordial follicle pool.[4,5] Anti-Müllerian hormone binds to specific receptors to activate intracellular signal transduction mechanisms.[6-8] In females, AMH has 2 functions: it inhibits primordial follicular growth (to prevent premature exhaustion of ovarian follicular reserve) and reduces FSH responses of preantral and small antral follicles (to suppress the development of nondominant follicles).[9] Serum concentrations of AMH were determined in cows,[10,11] ewes,[12,13] mares,[14] and women.[9,15,16] Serum concentrations increased a few months after birth, declined until the onset of puberty, then increased again and remained relatively stable throughout the cycle until ovarian senescence/menopause.[11,17,18]

In cattle,[19-22] women,[7,9,23] mice,[17] and small ruminants,[10,24] serum AMH concentrations remained constant during cycles and were positively correlated with the number of antral follicles in women.[9] Serum AMH serum concentrations are considered an indirect measure of the number of morphologically healthy ovarian follicles. It is speculated that a single measurement of AMH can predict follicular reserve. There was a good correlation between AMH serum concentrations and quality of embryos produced after ovarian superstimulation in cattle,[19,25-27] goats,[28] and sheep.[11,12,29] Additionally, in cattle, serum AMH concentrations were highly correlated with pregnancy rate and early embryo survival.[22,30] There was a positive correlation between AMH concentrations (measured just before FSH treatment) and the number of gonadotropin-responsive follicles that developed to the preovulatory stage in several ruminant species (cattle,[20,25-27] goats,[28] and sheep [11,12,29]).

Although assisted reproductive technologies in camels[31] and south American camelids[32,33] have increased since the 1990s, studies on AMH serum concentrations in female camelids are lacking. Female camels present overlapping follicular waves in anovulation that makes ovarian superstimulation more difficult (reviewed by[34]). Ovarian superstimulation protocol for came-

lids has been extrapolated from other species, despite many differences in reproductive physiology. In camelids, however, embryo yield after superstimulation is highly variable and often poor due to lack of response, overstimulation, or poor fertilization.[31-34] Studies on AMH in female camelids may shed some light on reasons for this variability in response to superstimulation and ovarian follicular dynamic abnormalities. Furthermore, like several mammalian species, estimation of serum AMH concentrations in camelids may be a good indicator of ovarian follicular reserve, fertility, and ovarian response to superstimulation.

Most studies used either human or species-specific enzyme-linked immunosorbent assay (ELISA) commercial kits for AMH serum concentration determination. Validated ELISA kits are available for cattle, dogs, horses, humans, mice, and sheep.[35] Earlier, we validated an AMH enzyme-linked immunosorbent assay kit for male alpacas[36] and determined that serum AMH concentrations remained relatively stable in prepubertal and post pubertal males, but declined in adult males after castration.[36]

Our aims were to validate a commercial ELISA kit for female alpacas, characterize serum concentrations of AMH in cycling alpacas, and to determine whether there was a correlation between AMH serum concentrations and number of antral follicles after FSH ovarian superstimulation.

Materials and methods

Animals and sample collection

Healthy, multiparous, and reproductively sound Huacaya female alpacas (5 - 11 years) were used. Animals were housed and evaluated at the Washington State University Veterinary Teaching Hospital. Animals were fed alfalfa and grass hay at a maintenance level to sustain a body condition score between 2.5 and 3 out of 5 (1 = emaciated, 5 = obese). Water and mineral salt were provided ad libitum. Animals were on regular internal and external parasite control and vaccination program. Animals were not subjected to prior ovarian superstimulation. Procedures were approved by the institutional animal care and use committee of Washington State University.

Validation of anti-Müllerian hormone enzyme-linked immunosorbent assay commercial kit

Unavailability of a validated[36] commercial AMH assay (AMH ELISA test Kit Cat # KAMH-01; Preventia Diagnositcs, Siasconset, MA) prompted us to validate an alternate commercial kit (AMH ELISA AL-113 kit [Ansh Labs, Webster, TX AL-113]). Sera collected from 5 male alpacas (before and after castration)[36] were used.

Serum anti-Müllerian hormone concentrations during cycle

Female alpacas (n = 11) were evaluated via transrectal ultrasonography (Exago®, Universal Imaging, New York) using a 7.5 MHz linear array transducer mounted on a polyvinyl chloride extension handle. Alpacas were monitored every other day until they developed a mature follicle (7 - 10 mm in diameter) and had uterine edema. Ovulation was induced with intramuscular GnRH (50 µg/animal, [Cystorelin®, Merial, Duluth, GA]) and verified via ultrasonography (corpus luteum) and serum progesterone concentrations. Blood samples were collected via jugular venipuncture at ovulation induction (day 0) and during the luteal phase (7 days after ovulation induction). Blood samples were allowed to clot, centrifuged (1,500 g for 10 minutes), and harvested sera were stored at – 20°C until analysis.

Ovarian superstimulation and serum anti-Müllerian hormone concentrations

Females (n = 13) were synchronized with intramuscular GnRH (50 µg/animal [Cystorelin®) and progesterone vaginal implants (controlled internal drug release [CIDR, Easy breed®, 0.3 mg progesterone, Zoetis, Kalamazoo, MI]), were inserted. Ovaries were superstimulated with intramuscular porcine FSH (Folltropin®, Vetoquinol Inc, Fort Worth, TX), twice daily at 12-hour intervals (day 12 - 16) in decreasing doses (30, 25, 20, 15, and 10 mg). Vaginal implant was removed on day 14, and animals were given intramuscular cloprostenol (250 µg). Transrectal ultrasonography was performed on days 0, 7, 12, 17, and 24; ovarian follicular status was determined on days of examination and mature follicles (7 - 10 mm) were counted at the end of porcine follicle-stimulating hormone (pFSH) treatment. Blood samples were collected via jugular venipuncture for AMH and progesterone assays on days 0, 3, 7, 12, 17, and 24 (Figure 1). Ovulation was induced with GnRH when most follicles reached 8 mm diameter. Blood samples were allowed to clot, centrifuged (1,500 g for 10 minutes), and harvested sera were stored at – 20°C until essayed.

Hormone analyses

Serum AMH concentrations were determined from 50 µl duplicate aliquots. Monoclonal antibody pairs bind to the noncovalent AMH complex that do not detect other related members of the TGF-ß superfamily. Samples from each female were included in the same analytical run. Intra-assay coefficients of variation for serum pools with high (13.5 ng/ml, n = 14), medium (6.2 ng/ml, n = 14), and low concentrations (2.3 ng/ml, n = 14) were 2.6, 5.5, and 4.1%, respectively. Lowest calibrator was 0.41 ng/ml and values below were estimated by extrapolation.

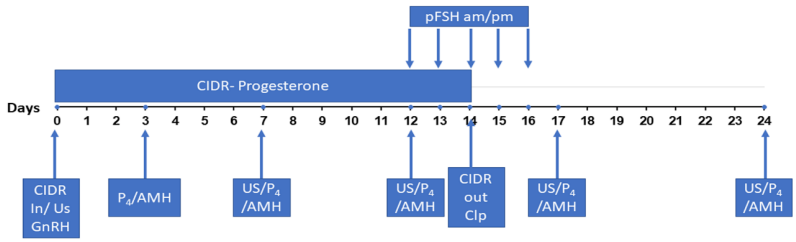

Figure 1. Synchronization, ovarian superstimulation, and sampling protocol; porcine follicle-stimulating hormone (pFSH), controlled internal drug release (CIDR), transrectal ultrasonography (US), progesterone (P_4) anti-Müllerian hormone (AMH), and cloprostenol (Clp)

Progesterone concentrations were determined using a competitive enzyme immunoassay.[37] Briefly, 100 µl of serum was extracted with 2 ml of petroleum ether, the solvent was decanted and dried. Extracts were reconstituted with 150 µl of horseradish peroxidase-conjugated progesterone in buffer, and 50 µl aliquots (in duplicate) were transferred to 96-well plates precoated with antisera raised against 11α-hemi-succinate-conjugated progesterone. Progesterone analysis was completed in 2 analytical runs. The average intra-assay coefficient of variation of low (0.5 ng/ml) and high (4.9 ng/ml) pools of samples included in each run was 11.4%, with an assay sensitivity of 5 pg/well.[37]

Data analysis

Serum AMH concentrations obtained using Preventia® kit were compared to those obtained with Ansh (AL-113) using Pearson's correlation coefficient. Serum AMH concentration at ovulation induction (day 0) and luteal phase (day 7) was compared by paired Student's t-test. Effect of superstimulation on AMH serum concentrations was evaluated using repeated measurement analysis. Correlation between the number of antral follicles in response to FSH treatment and serum AMH concentrations were determined by linear regression analysis. Statistical analysis was performed using commercial software (Statistix 10®, Analytical Software, Tallahassee, FL). Significance was set at $p < 0.05$.

Results

Validation of anti-Müllerian hormone enzyme-linked immunosorbent assay commercial kit

Serum concentrations of AMH from 5 male alpacas, before and after castration, as measured by the Ansh assay, were closely associated with the previously published results using the Preventia kit ($r = 0.94$, $n = 10$, $p < 0.01$) (Figure 2).

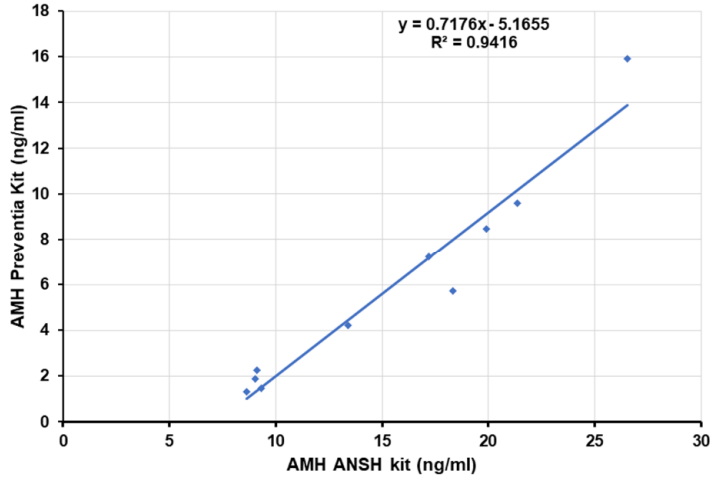

Figure 2. Correlation between serum anti-Müllerian hormone concentrations measured via 2 kits in samples from male (n = 5) alpacas (before and after castration)

Serum anti-Müllerian hormone concentrations during cycle

Serum AMH concentrations (mean ± SEM) on the day of ovulation induction (day 0) and during luteal phase (day 7) were 1.33 ± 0.35 ng/ml and 1.14 ± 0.34 ng/ml, respectively (Figure 3). Luteal phase (occurrence of ovulation in response to GnRH treatment) was verified on day 7 by visible corpus luteum via transrectal ultrasonography and by serum progesterone concentrations (samples had > 1.5 ng/ml; 3.26 ± 1.26 ng/ml [mean ± SEM]). There was no difference ($p = 0.39$) in serum AMH concentrations between estrus and diestrus. However, there was a significant variation in serum AMH concentrations among females. Minimum and maximum serum AMH concentrations were 0.027 and 4.28 ng/ml on day 0 and 0.01 and 3.72 ng/ml on day 7, respectively.

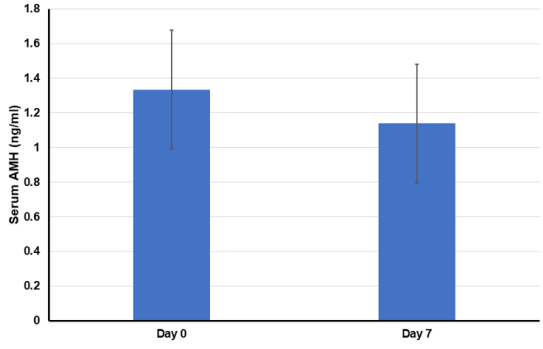

Figure 3. Serum anti-Müllerian hormone concentrations on day 0 (estrus) and day 7 after ovulation induction in alpacas (n = 11)

Ovarian superstimulation and serum anti-Müllerian hormone concentrations

Number of antral follicles (> 7 mm) after FSH treatment (day 17) was variable (12.69 ± 5.25 [mean ± SD]; range 6 - 24). There were no differences ($p > 0.005$) in serum AMH concentrations within animals across sampling dates (Table). However, there was a difference ($p > 0.005$) among female alpacas. Correlation between mean AMH serum concentration for each female alpaca and the number of follicles at the end of superstimulation treatment (day 17) was determined. There was a correlation ($r = 0.46$, $p < 0.01$) between the number of follicles after FSH treatment and serum AMH concentrations (Figure 4). Further analysis after excluding 1 outlier had higher positive correlation ($r = 0.7$, $p < 0.001$; [Figure 5]). Correlation between AMH serum concentrations and the number of corpora lutea could not be assessed as there was ovulation failure in several animals. Serum progesterone concentrations increased in animals immediately after CIDR insertion and then dropped; however, they remained above 1.5 ng/ml until CIDR removal (Figure 6).

Table. Serum anti-Müllerian hormone concentration (ng/ml) on various days of ovarian superstimulation

Female	Day 3	Day 7	Day 12	Day 17	Day 24	Mean (SEM)
1	0.525	0.612	0.564	0.564	0.653	0.58 (0.022)
2	1.337	1.298	0.791	0.736	0.887	1.01 (0.12)
3	3.193	3.123	3.698	2.497	3.917	3.29 (0.24)
4	3.193	3.123	3.698	2.497	3.917	3.29 (0.25)
5	3.213	2.529	2.771	2.04	2.404	2.59 (0.19)
6	4.282	3.719	4.314	7.719	4.582	4.92 (0.71)
7	0.741	0.972	0.802	0.828	0.775	0.82 (0.04)
8	2.961	2.785	2.498	2.131	3.429	2.76 (0.22)
9	0.491	0.491	0.491	1.769	2.286	1.11 (0.39)
10	1.627	4.785	2.454	1.576	2.506	2.59 (0.58)
11	1.292	1.535	1.439	2.052	1.708	1.61 (0.13)
12	0.027	0.01	0.01	0.01	0.061	0.02 (0.01)
13	0.541	0.32	0.313	0.357	0.515	0.41 (0.05)

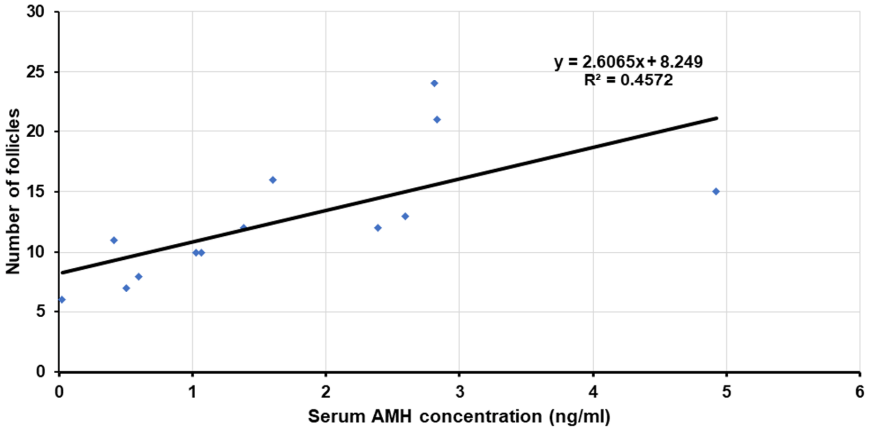

Figure 4. Correlation between serum anti-Müllerian hormone concentrations and number of follicles after FSH treatment in alpacas (n = 13)

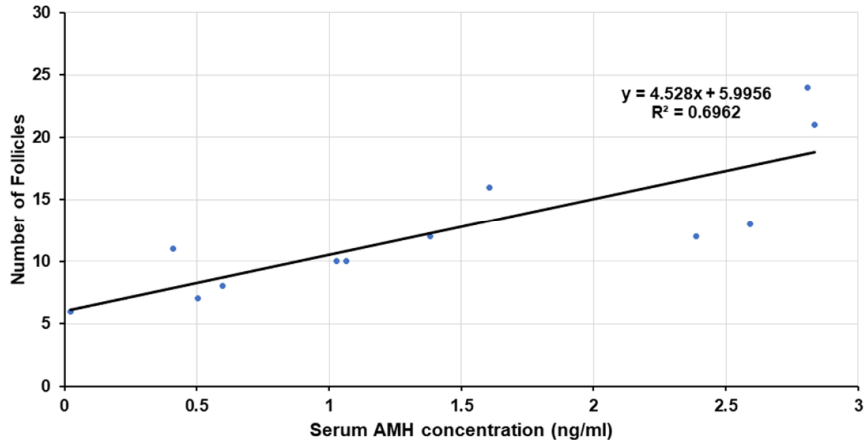

Figure 5. Correlation between serum anti-Müllerian hormone concentrations and number of follicles after FSH treatment in alpacas (n = 12) after removal of an outlier

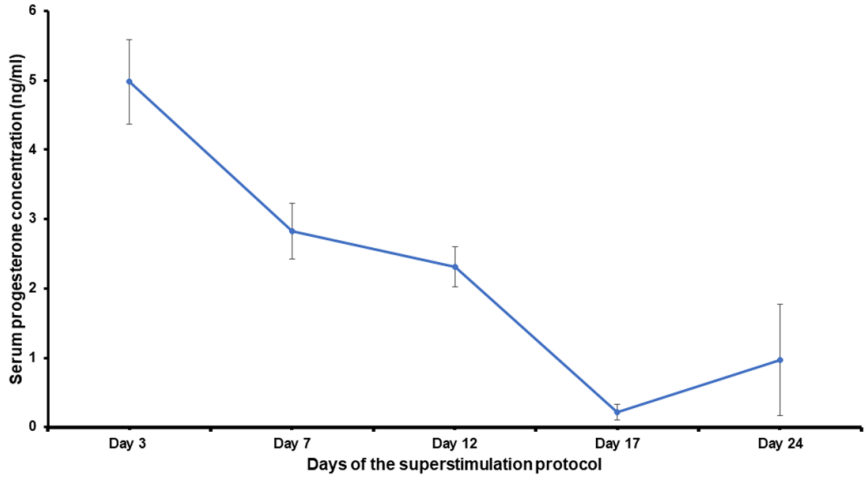

Figure 6. Serum progesterone concentrations (mean ± SEM) on days 3, 7, and 12 after CIDR insertion (day 0)

Discussion

Anti-Müllerian hormone is a well-conserved glycoprotein among mammalian species. Commercial AMH assays kits are available for humans, cattle, small ruminants, horses, dogs, and rodents.[35] Recently, we validated[36] a commercial test kit (AMH ELISA test Kit Cat # KAMH-01; Preventia Diagnostics) for serum AMH in male alpacas. However, the unavailability of this kit necessitated validation of an alternate kit. We used male alpaca serum collected earlier[36] to validate the alternate commercial kit (AMH ELISA, AL-113®, Ansh lab). There was a high correlation among data obtained with these 2 commercial assay kits. Both kits utilized monoclonal antibody pairs in a sandwich assay format.

Determination of the effect of cycle stage on AMH serum concentration has diagnostic applications. If the cycle stage had no effect, a single random test could be used for prognostication of ovarian reserves. Serum AMH concentrations (with repeatability across multiple estrous cycles) were not influenced by cycle stage (estrus versus diestrus) in cows,[11,21,38] goats,[24] and mares.[39,40] These patterns are expected since AMH is expressed only in follicles that have been recruited from primordial follicle pool and not selected for dominance. Neither dominant follicles nor corpora lutea are believed to contribute to circulating AMH concentrations in these species.[7,41,42] Only in pigs is AMH is expressed in preovulatory follicles and corpora lutea.[43]

Serum AMH concentrations during peak follicular activity (follicle diameter > 8 with uterine edema) and luteal phase were not different in our study, similar to other species (cattle,[11,44] goats,[24] and humans[7,42]). Hence a random determination of serum AMH concentration can be used (as in other species) to evaluate ovarian follicular reserve in alpacas.

The potential for AMH serum concentrations as a biomarker for fertility was established for cattle.[11,21,30,44] Lower AMH serum concentrations reflected suboptimal fertility, lower pregnancy rate, and higher incidence of pregnancy loss compared to higher AMH serum concentrations. Poor reproductive efficiency, a major problem in the camelid industry, is primarily

due to 'repeat breeding' (75.6%) and recurrent early pregnancy loss (18.3%).[45] Determination of AMH concentrations could help to define the reproductive potential of a female alpaca, prevent excessive mating, and delay diagnosing follicular dynamics disorders. Multiple ovulation and embryo transfer programs' are important tools for genetic improvement in camelids. Ovarian superstimulation treatments have often been extrapolated from ruminant studies. Such treatments (e.g. pFSH and eCG alone or in combination) were used during the luteal phase and follicular wave emergence with variable results. Initiation of treatment in relation to follicular dynamics, dose, protocol, and individual variation was considered for individual variability.[31-34] A better understanding of camelids' ovarian follicular dynamics and physiology of ovulation is needed to develop species-specific protocols.

We used pFSH daily in decreasing doses over 5 days after a 14-day course of progesterone.[34,46,47] Despite a good response (number of follicles), number of ovulations (as estimated by corpora lutea) was very low, a common problem in alpacas.[34,47,48]

Response to hormonal ovarian stimulation treatments in buffalo,[49] cattle,[19,21,22,25,26] goats,[24,28] sheep,[12,29] and woman[41] was predicted using AMH blood concentrations. In our study, serum AMH concentrations were highly correlated with the number of follicles developed after ovarian superstimulation treatment. However, further studies using a larger number of females are warranted in order to determine whether alpacas with higher circulating AMH concentrations will consistently develop a higher number of follicles in response to FSH ovarian superstimulation. Consistent with research in cattle,[19,20,25-27] in our study, there were no differences in serum AMH concentrations before and after FSH treatment.

Conclusion

We determined serum AMH concentrations in male and female alpacas using a commercial assay kit. Consistent with other species, serum AMH concentrations in female alpacas remained constant during peak follicular and luteal phases. Hence a single sample to estimate female ovarian follicular reserve and potentially predict their reproductive performance. Serum AMH concentrations in female alpacas were positively correlated with the number of dominant follicles that developed following ovarian superstimulation. As demonstrated in ruminants, future experiments should investigate whether there are correlations among AMH serum concentrations, oocyte quality, and embryo survival in alpacas.

Funding

Partial funding from Northwest Camelid Research Foundation.

Conflict of interest

The authors do not have any conflict of interest to declare.

References

1. Cate RL, Mattaliano RJ, Hession C, et al: Isolation of the bovine and human genes for Müllerian inhibiting substance and expression of the human gene in animal cells. Cell 1986;45:685-698.
2. Josso N, Clemente N: Transduction pathway of anti-Müllerian hormone, a sex-specific member of the TGF-beta family. Trends Endocrinol Metab 2003;14:91-97.
3. Jost A. Hormonal factors in the development of the fetus. Cold Spring Harb Symp Quant Biol 1954;19:167-181.
4. Durlinger AL, Gruijters MJ, Kramer P, et al: Anti-Müllerian hormone attenuates the effects of FSH on follicle development in the mouse ovary. Endocrinology 2001;142:4891-4899.
5. Rajpert-De Meyts E, Jørgensen N, Graem N, et al: Expression of anti-Müllerian hormone during normal and pathological gonadal development: association with differentiation of Sertoli and granulosa cells. J Clin Endocrinol Metab 1999;84:3836-3844.
6. Umer S, Zhao SJ, Sammad A, et al. AMH: Could it be used as a biomarker for fertility and superovulation in domestic animals? Genes (Basel) 2019;10. https://doi.org/10.3390/genes10121009
7. La Marca A, Volpe A: Anti-Müllerian hormone (AMH) in female reproduction: is measurement of circulating AMH a useful tool? Clin Endocrinol 2006;64:603-610.
8. Baarends WM, van Helmond MJ, Post M, et al: A novel member of the transmembrane serine/threonine kinase receptor family is specifically expressed in the gonads and in mesenchymal cells adjacent to the Müllerian duct. Development 1994;120:189-197.
9. Dewailly D, Andersen CY, Balen A, et al: The physiology and clinical utility of anti-Mullerian hormone in women. Hum Reprod Update 2014;20:370-385.
10. Monniaux D, Drouilhet L, Rico C, et al: Regulation of anti-Müllerian hormone production in domestic animals. Reprod Fertil Dev 2012;25:1-16.
11. Mossa F, Jimenez-Krassel F, Scheetz D, et al: Anti-Müllerian Hormone (AMH) and
fertility management in agricultural species. Reproduction 2017;154:R1-R11.
12. Lahoz B, Alabart JL, Cocero MJ, et al: Anti-Müllerian hormone concentration in sheep and its dependence of age and independence of BMP15 genotype: an endocrine predictor to select the best donors for embryo biotechnologies. Theriogenology 2014;81:347-357.
13. Lahoz B, Alabart JL, Monniaux D, et al: Anti-Müllerian hormone plasma concentration in prepubertal ewe lambs as a predictor of their fertility at a young age. BMC Vet Res. 2012;8:118.
14. Scarlet D, Wulf M, Kuhl J, et al: Anti-Müllerian hormone profiling in prepubertal horses and its relationship with gonadal function. Theriogenology 2018;117:72-77.
15. Cui L, Qin Y, Gao X, et al. Antimüllerian hormone: correlation with age and androgenic and metabolic factors in women from birth to postmenopause. Fertil Steril 2016;105:481-485.e481.
16. Lashen H, Dunger DB, Ness A, et al: Peripubertal changes in circulating antimüllerian hormone levels in girls. Fertil Steril 2013;99:2071-2075.

17. Kevenaar ME, Meerasahib MF, Kramer P, et al: Serum anti-mullerian hormone levels reflect the size of the primordial follicle pool in mice. Endocrinology 2006;147:3228-3234.
18. Uliani RC, Conley AJ, Corbin CJ, et al: Anti-Müllerian hormone and ovarian aging in mares. J Endocrinol 2019;240:147-156.
19. Rico C, Médigue C, Fabre S, et al: Regulation of anti-Müllerian hormone production in the cow: a multiscale study at endocrine, ovarian, follicular, and granulosa cell levels. Biol Reprod 2011;84:560-571.
20. Rico C, Fabre S, Médigue C, et al: Anti-mullerian hormone is an endocrine marker of ovarian gonadotropin-responsive follicles and can help to predict superovulatory responses in the cow. Biol Reprod 2009;80:50-59.
21. Ireland JJ, Smith GW, Scheetz D, et al: 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-Müllerian hormone as a diagnostic marker for fertility and causes of variation in the ovarian reserve in cattle. Reprod Fertil Dev 2011;23:1-14.
22. Ireland JL, Scheetz D, Jimenez-Krassel F, et al: Antral follicle count reliably predicts number of morphologically healthy oocytes and follicles in ovaries of young adult cattle. Biol Reprod 2008;79:1219-1225.
23. Dewailly D, Laven J: AMH as the primary marker for fertility. Eur J Endocrinol 2019;181:D45-D51.
24. Monniaux D, Baril G, Laine AL, et al: Anti-Mullerian hormone as a predictive endocrine marker for embryo production in the goat. Reproduction 2011;142:845-854.
25. Souza AH, Carvalho PD, Rozner AE, et al: Relationship between circulating anti-Müllerian hormone (AMH) and superovulatory response of high-producing dairy cows. J Dairy Sci 2015;98:169-178.
26. Monniaux D, Rico C, Larroque H, et al: L'hormone antimüllérienne, prédicteur endocrinien de la réponse à une stimulation ovarienne chez les bovins. Gynecol Obstet Fertil 2010;38:465-470.
27. Hirayama H, Naito A, Fukuda S, et al. Long-term changes in plasma anti-Müllerian hormone concentration and the relationship with superovulatory response in Japanese Black cattle. J Reprod Dev 2017;63:95-100.
28. Karakas Alkan K, Alkan H, Kaymaz M. The effect of anti-müllerian hormone and progesterone concentrations on superovulation response and embryo yield in goats. Theriogenology 2020;143:1-9.
29. Torres-Rovira L, Gonzalez-Bulnes A, Succu S, et al. Predictive value of antral follicle count and anti-Müllerian hormone for follicle and oocyte developmental competence during the early prepubertal period in a sheep model. Reprod Fertil Dev 2014;26:1094-1106.
30. Ribeiro ES, Bisinotto RS, Lima FS, et al. Plasma anti-Müllerian hormone in adult dairy cows and associations with fertility. J Dairy Sci 2014;97:6888-6900.
31. Anouassi A, Tibary A: Development of a large commercial camel embryo transfer program: 20 years of scientific research. Anim Reprod Sci 2013;136:211-221.
32. Vaughan J, Mihm M, Wittek T: Factors influencing embryo transfer success in alpacas: a retrospective study. Anim Reprod Sci 2013;136:194-204.
33. Campbell AJ, Tibary A: Embryo transfer in camelids. Clinical Theriogenology 2017;9:321-327.
34. Tibary A: Monitoring and controlling follicular activity in camelids. Theriogenology. 2018;109:22-30.
35. Kumar A, Kalra B, Patel A, et al: New sensitive anti-Müllerian hormone (AMH) ELISAs for non-human primate, rodent, equine, bovine, canine and other species. Poster, American Association for Clinical Chemistry; 2013; Houston, TX.
36. Ciccarelli M, Tibary A, Campbell AJ, et al: Effect of age and castration on serum anti-Müllerian hormone concentration in male alpacas. Theriogenology 2018;105:174-177.
37. Munro C, Stabenfeldt G: Development of a microtitre plate enzyme immunoassay for the determination of progesterone. J Endocrinol 1984;101:41-49.
38. Pfeiffer KE, Jury LJ, Larson JE: Determination of anti-Müllerian hormone at estrus during a synchronized and a natural bovine estrous cycle. Domest Anim Endocrinol 2014;46:58-64.
39. Ball BA, El-Sheikh Ali H, Scoggin KE, et al: Relationship between anti-Müllerian hormone and fertility in the mare. Theriogenology 2019;125:335-341.
40. Almeida J, Ball BA, Conley AJ, et al. Biological and clinical significance of anti-Müllerian hormone determination in blood serum of the mare. Theriogenology 2011;76:1393-1403.
41. Hehenkamp WJ, Looman CW, Themmen AP, et al. Anti-Müllerian hormone levels in the spontaneous menstrual cycle do not show substantial fluctuation. J Clin Endocrinol Metab 2006;91:4057-4063.
42. Tsepelidis S, Devreker F, Demeestere I, et al: Stable serum levels of anti-Müllerian hormone during the menstrual cycle: a prospective study in normo-ovulatory women. Hum Reprod 2007;22:1837-1840.
43. Almeida F, Costermans NGJ, Soede NM, et al. Presence of anti-Müllerian hormone (AMH) during follicular development in the porcine ovary. PLoS One. 2018;13:e0197894.
44. Burns DS, Jimenez-Krassel F, Ireland JL, et al: Numbers of antral follicles during follicular waves in cattle: evidence for high variation among animals, very high repeatability in individuals, and an inverse association with serum follicle-stimulating hormone concentrations. Biol Reprod 2005;73:54-62.
45. Tibary A, Campbell A, Rodriguez JS, et al: Investigation of male and female infertility in llamas and alpacas. Reprod Fertil Dev 2021;33:20-30.
46. Correa JE, Ratto MH, Gatica R: Superovulation in llamas (*Lama glama*) with pFSH and equine chorionic gonadotrophin used individually or in combination. Anim Reprod Sci 1997;46:289-296.
47. Ratto MH, Silva ME, Huanca W, et al: Induction of superovulation in South American camelids. Anim Reprod Sci 2013;136:164-169.
48. Ratto MH, Gatica R, Correa JE: Timing of mating and ovarian response in llamas (*Lama glama*) treated with pFSH. Anim Reprod Sci 1997;48:325-330.
49. Liang A, Salzano A, D'Esposito M, et al: Anti-Mullerian hormone (AMH) concentration in follicular fluid and mRNA expression of AMH receptor type II and LH receptor in granulosa cells as predictive markers of good buffalo (*Bubalus bubalis*) donors. Theriogenology 2016;86:963-970.