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RESEARCH ARTICLE

Serum Anti-Mullerian Hormone Profile from 10 Days of Age to Puberty and Its Relationship with Serum Testosterone and Estradiol Concentrations in Beetal Goat Kids

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ABSTRACT

The current study aimed to define the serum profile of anti-mullerian hormone (AMH), testosterone and estradiol (E2) concentrations in Beetal goat kids from 10 days of age to puberty (175±12.6 days). The study was conducted from July 2018 to January 2019. Blood samples were collected from male kids (n=26) at 10 days interval from 10 days of age to puberty for measurement of AMH, testosterone and E2 concentrations. The body weight of all kids was measured at 10 days of age and then on monthly basis till puberty. The results showed that the mean body weight at 10 d after birth was 7.04±0.86 kg, and significantly increased till the onset of puberty (36.4±4.49 kg). The minimal plasma AMH concentration was seen at 10 d, gradually increased to its peak level ($P<0.05$) at 40 d and 100 d and then declined steadily till puberty. The serum testosterone level increased non-significantly from day 10 to day 50 and then gradually increased to its peak level at 170 and 180d ($P<0.05$). The serum E2 concentration was low at 10 days of age (5.76±0.78 pg/ml), increased to its peak level at 60d (74.3±6.26 pg/ml; $P<0.05$ compared with 10d), then declined till puberty. The serum AMH at early pre-pubertal age (40, 50, 70 and 80d) was positively correlated with pubertal age around 180d (r=0.99, 0.95, 0.99 and 0.94), whereas testosterone concentration was negatively correlated with serum AMH level at 60d, while positively correlated at 150 and 180d. In conclusion, goat kids exhibit a characteristic serum AMH profile along with testosterone and E2 during pre-pubertal life, such that serum AMH at an early pre-pubertal age could be used as a biomarker for predicting initiation of sexual behavior and onset of puberty.

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INTRODUCTION

Anti-mullerian hormone (AMH) is a homodimeric (140-kDa) glycoprotein, which belongs to transforming growth factor β (TGFβ) family secreted from gonads (Josso and Clemente, 2003). In female, it is secreted from granulosa cells of growing antral follicles in the ovary (Gobikrushanth *et al.*, 2018), while in male, it is secreted from Sertoli cells at the time of fetal sexual differentiation (embryonic stage) till puberty (Claes *et al.*, 2013). The physiological actions of AMH in female include regulation of early follicular growth, controlling recruitment of number of follicles for growing groups (Newberry *et al.*, 2016) and preventing premature depletion of follicular reserve in adult female ovary (Jimenez-Krassel *et al.*, 2015). In male, AMH plays very important function in the sexual differentiation and regression of mullerian duct during embryonic development (Claes and Ball, 2016). However, studies on the functions of AMH during post-natal life in males are scanty.

AMH concentration changes from birth to puberty and adulthood in several species like in beef bulls (Kitahara *et al.*, 2016), cross and zebu bulls (Rajak *et al.*, 2017). Its level significantly increases during 1st and 2nd month of age and then gradually declines till puberty. The level of this hormone is reported as an endocrine marker for many physiological and pathological conditions, for example in dogs, AMH concentration is a significant biomarker for diagnosis of immature Sertoli cells and testicular atrophy (Ano *et al.*, 2014) and in Holstein Friesian, AMH is a biomarker for diagnosis of granulosa-theca cell tumor (Ali *et al.*, 2013). Although these reports indicate the importance of this hormone in identifying specific conditions, it would be interesting to find out its physiological role in males during pre-pubertal period. In this regard, a previous study indicated that AMH at an early pubertal age could be a potential candidate for predicting age of puberty onset and future fertility in Holstein cattle (Ali *et al.*, 2017).

Circulating testosterone is the most important reproductive hormone in males. Serum AMH concentration was inversely correlated with testosterone concentration in humans (Rey *et al.*, 1993), horses (Claes *et al.*, 2013) and cattle (Rajak *et al.*, 2017) but not in sheep (Cazorla *et al.*, 1998) and Asian/African elephants (Dow *et al.*, 2011). Based on these reports, we intended to investigate the relationship among serum AMH, testosterone and E2 concentrations during pre-pubertal life and hypothesized that AMH level at this stage would be a potential biomarker for prediction of puberty onset in goat kids. Therefore, aims of the present study were to: (a) define the serum AMH profile from 10 days of age to puberty, (b) monitor serum testosterone and E2 profile during pre-pubertal period until puberty, and (c) investigate correlation among serum AMH, testosterone and E2 levels in Beetal goat kids.

MATERIALS AND METHODS

Experimental animals: The experiments were approved under guidelines of ethical committee of University of Veterinary and Animal Sciences, Lahore. This experiment was performed at a private goat farm (Al-Haiwan Sires, Sahiwal, Pakistan) from July 2018 to January 2019. Twenty six (n=26) newly born Beetal male goat kids were enrolled in the current experiment. All kids were allowed to suck natural milk directly from their mothers, weaned at 3 months of age and offered equal quantities of green fodder (Alfalfa), wheat straw and had free access to fresh water. In addition, 200-250 g/day supplementary concentrate (92.8% organic matter, 15.3% crude protein and 18.5% crude fiber) was fed to each kid from weaning till onset of puberty.

Experimental design: All kids were weighted at 10 days after birth and then on monthly basis till puberty onset. Moreover, to investigate serum profile of AMH, testosterone and estradiol (E2) during pre-pubertal period, blood samples were collected by jugular venipuncture at 10 days intervals from10 days of age to puberty. Each blood sample was allowed to clot for 1 h at room temperature, centrifuged at 2800G for 15 min and serum samples were stored at -20ºC until assays performed. Attempts for semen collection from each kid were started at 120d of age (due to peak mounting behavior at that time) and continued at 10 days intervals until successful semen collection. Puberty onset was defined as the age at which kids ejaculated first time 50 million sperm/ml, with 10% motile sperm in semen (Longpre *et al.*, 2016).

Hormone assays: Serum AMH concentrations were measured through enzyme-linked immunosorbent assay (ELISA) by using diagnostic kit (Goat AMH ELISA kit # MBS267219; MyBiosource-USA) following manufacturer's instructions. Sensitivity of assay was 0.06 ng/ml, while intra- and inter-assay coefficients of variation were 8 and 12%, respectively.

Testosterone concentrations were measured through RIA by using diagnostic kit (RIA testosterone kit # IM246303; Beckman Coulter, California- USA). The sensitivity of assay was 0.04 ng/ml and intra- and inter-assay coefficients of variation were 8.9 and 16.2%, respectively. Similarly, serum estradiol concentrations were measured through RIA by using diagnostic kit (RIA Estradiol kit # A21854; Beckman Coulter, California-USA). The sensitivity of assay was 9.58 pg/ml, and intra- and inter-assay coefficients of variation were 14.4 and 14.5%, respectively.

Statistical analysis: Normality of data was investigated by Shapiro Wilk test. The data were analyzed through statistical software SPSS version 20. Analysis of variance (ANOVA) and Turkey's post hoc test was applied to analyze the 10d change in serum AMH, testosterone and E2 concentrations. Pearson's correlation was used to analyze the relationship between different variables.

RESULTS

Beetal kids reached puberty at the age of 175±12.6 days (range 165-185 days). The live body weight of kids at 10 days of age was 7.04±0.86 kg. It gradually increased from 10 days of age to puberty (Fig. 1) and reached 36.4±4.49 kg at the age of puberty. However, no significance difference was found in body weight of kids between 5th and 6th month of age (33.81±3.25 and 36.4±4.49 kg; respectively).

Serum concentrations of AMH, testosterone and E2: Serum concentrations of AMH exhibited characteristics profile during pre-pubertal age in goat kids. Serum AMH concentration was low at 10d (11.51±0.17ng/ml) and gradually increased to peak levels at 40d and 100d (43.98±0.62 and 42.17±1.92 ng/ml, respectively; $P<0.001$) before declined steadily till puberty; at puberty, its level was 10.44±0.47 ng/ml (Fig. 2). Serum testosterone concentration differed non-significantly from 10 days to 50 days of age (0.24± 0.05 to 1.27±0.15 ng/ml, respectively), then gradually increased in a steady pattern till puberty (5.67± 0.09 ng/ml; $P<0.001$ compared with 10 d), as shown in Fig. 3.

The serum E2 concentration was at basal level at 10d of age (5.76±0.78 pg/ml) and then gradually increased to its peak level at the age of 60d (74.3±6.26 pg/ml; $P<0.001$ compared to 10d). Then serum E2 concentration declined in a steady pattern till puberty (39.23±3.45 to 10.81±0.44 pg/ml), as shown in Fig. 4.

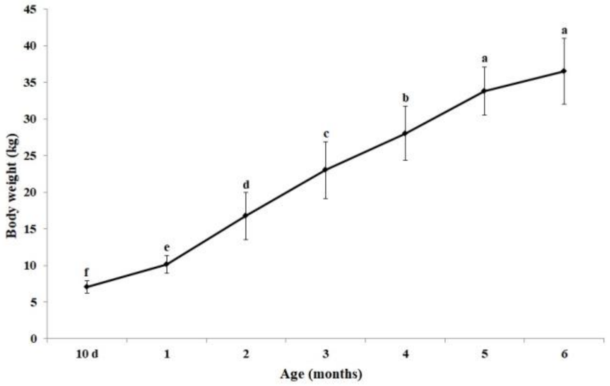

Fig. 1: Changes in monthly body weight from birth to pubertal onset in Beetal kids (n=26). Different superscripts (a, b, c, d, e and f) indicate significant differences (P<0.05) among different months.

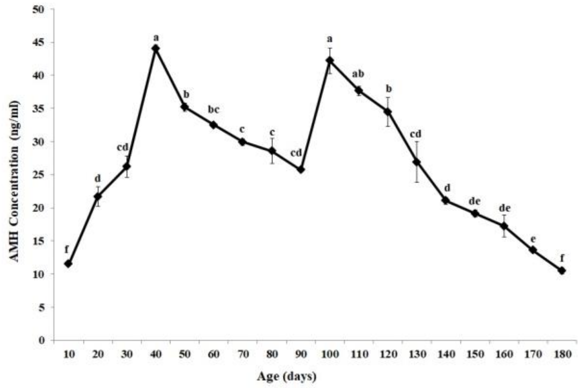

Fig. 2: Changes in serum AMH concentrations on the basis of 10 days interval from birth (10 days old) to puberty in Beetal kids (n=26). Different superscripts (a, b, c, d, e and f) indicate significant differences (P<0.05) among different days.

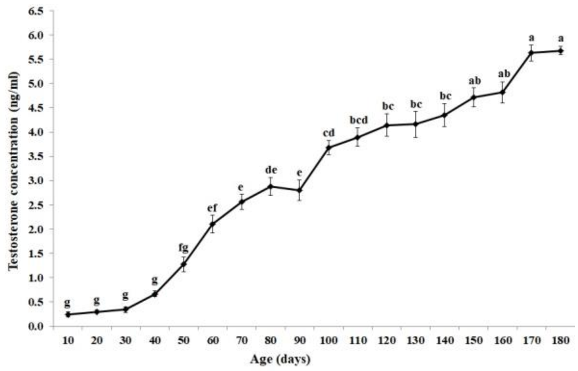

Fig. 3: Changes in serum testosterone concentrations on the basis of 10 days interval from birth (10 days old) to puberty in Beetal kids (n=26). Different superscripts (a, b, c, d, e, f and g) indicate significant differences (P<0.05) among different days.

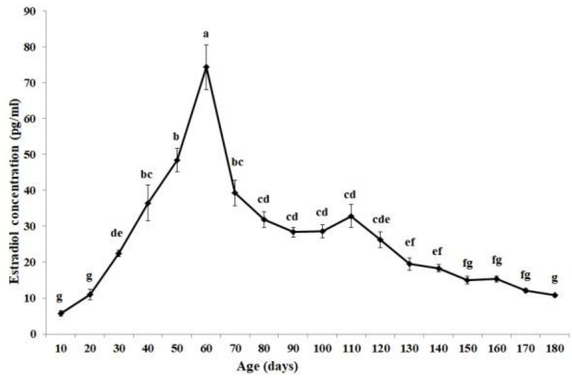

Fig. 4: Changes in serum estradiol concentrations on the basis of 10 days interval from birth (10 days old) to puberty in Beetal kids (n=26). Different superscripts (a, b, c, d, e, f and g) indicate significant differences (*P*<0.05) among different days.

Table 1: Pearson's correlation coefficients between serum AMH concentrations at 10 days interval during pre-pubertal period in kids

	20d	30d	40d	50d	60d	70d	80d	90d	100d	110d	120d	130d	140d	150d	160d	170d	180d
10d	-0.976	0.151	-0.423	-0.666	-0.623	-0.41	-0.59	-0.763	-0.84	-0.62	-0.978	-0.687	.992*	-0.98	0.096	-0.629	-0.463
20d		-0.732	-0.816	-0.95	-0.184	-0.95	-0.919	0.84	0.452	-0.41	0.909	0.828	-.996*	0.915	-0.918	0.935	-0.867
30d			0.508	0.53	0.676	0.619	0.64	0.036	0.204	0.326	-0.949	-0.224	0.792	-0.94	.951*	-0.833	0.547
40d				.921*	0.767	.989**	.905*	-0.026	0.851	.968*	-0.502	-0.962**	0.759	-0.52	0.72	-0.316	.996**
50d					.940*	.944*	.990**	0.363	.938*	.940*	-0.733	-0.962	0.916	-0.74	0.658	-0.141	.950*
60d						0.837	.967*	0.548	0.814	0.768	-0.576	0.398	0.275	-0.56	0.701	-0.206	0.822
70d							.946*	0.044	0.833	.941*	-0.732	-0.962	0.916	-0.74	0.795	-0.385	.996**
80d								0.358	0.882	0.896	-0.956**	-0.54	0.952	-0.929**	0.742	-0.25	.940*
90d									0.416	0.128	0.538	0.914**	-0.786	0.551	-0.12	0.501	0.055
100d										.948*	0.782	-0.125	-0.533	0.773	0.369	0.183	0.87
110d											0.011	-0.848	0.32	-0	0.543	-0.067	.967*
120d												0.52	-0.944	0.947**	-0.958**	.998*	-0.581
130d													-0.773	0.533	-0.538	0.576	-.997*
140d														-0.95	0.951	-0.964	0.817
150d															-0.948**	.999*	-0.593
160d																-0.834	0.739
170d																	-0.31

Asterisks indicate a significant correlation *(*P*< 0.05) and **(P<0.01). "d" indicates days.

Correlation between AMH, testosterone and E2: Serum AMH at 180d showed significantly (P<0.05) positive correlations with 40d, 50d, 70d and 80d (r=0.99, 0.95, 0.99 and 0.94, respectively). Moreover, serum AMH level at 180d showed significantly (P<0.05) negatively correlated with 130d (r= -0.97), as shown in Table 1.

Serum AMH was negatively correlated with testosterone concentration at 60d (r= -0.87, *P*=0.02) and positively correlated at 150 and 180d (r=0.92 and 0.84, *P*=0.01; respectively). However, no relationship was found between AMH and testosterone concentration on other days (Table 2). There was positive correlation between serum E2 and AMH concentration at day 60 and 90 (r=0.96 and 0.97; P<0.001 respectively). However, serum E2 was positively correlated with testosterone concentration at 20d (r=0.93; P<0.02) and negatively correlated with serum testosterone concentration at160 and 170d (r=-0.84 and -0.87; P<0.05). Moreover, serum E2 concentration at early age (10, 30, 40, 50 and 60d) showed negative but non-significant relationship with testosterone concentration (Table 2).

Table 2: Pearson's correlation coefficients between AMH and testosterone, AMH and E2, and E2 and testosterone concentrations in kids during pre-pubertal period

Days	Correlation between AMH and testosterone		Correlation between AMH and E2		Correlation between testosterone and E2	
	r value	P value	r value	P value	r value	P value
10	0.58	0.2	-0.26	0.6	-0.43	0.3
20	-0.46	0.4	-0.25	0.6	0.93*	0.02
30	-0.67	0.2	0.57	0.3	-0.73	0.2
40	0.28	0.6	-0.04	0.9	-0.56	0.08
50	0.73	0.07	-0.48	0.3	-0.69	0.09
60	-0.92*	0.04	0.96*	0.002	-0.73	0.4
70	-0.41	0.3	-0.37	0.4	0.08	0.9
80	0.13	0.5	-0.18	0.7	0.42	0.8
90	0.14	0.7	0.97*	0.002	-0.05	0.8
100	-0.71	0.1	0.11	0.8	0.11	0.2
110	0.79	0.07	0.22	0.6	0.16	0.9
120	0.21	0.6	0.23	0.6	0.57	0.8
130	-0.08	0.7	-0.45	0.4	0.15	0.6
140	-0.07	0.7	-0.42	0.5	-0.18	0.1
150	0.96*	0.02	-0.66	0.3	0.28	0.6
160	0.58	0.1	-0.11	0.8	- 0.84*	0.04
170	0.04	0.8	-0.13	0.6	- 0.87*	0.03
180	0.91*	0.02	0.63	0.2	0.61	0.2

Asterisks indicate a significant correlation *(P<0.05). "r values" show Pearson's correlation coefficients "E2" indicates estradiol and "AMH" indicate anti-mullerian hormone.

DISCUSSION

Many studies reported serum AMH profile during pre-pubertal life in domestic animals. However, not a single study is present on AMH profile in male goats. Therefore, to the best of our knowledge, the present study is the first of its kind in which longitudinal plasma AMH profile was reported in goat kids based on 10d interval blood sampling till puberty.

The result of the current study showed that mean body weight of kids significantly increased from 10d to puberty. The data showed that live body weight of kids increased fivefold from 10d to puberty, as previously reported in buffalo bulls (Ahmad *et al.*, 1989), cattle bulls (Evans *et al.*, 1996), black Japanase cattle (Ali *et al.*, 2017) and Sannen goats (Longpre *et al.*, 2016). The kids attained sexual maturity when maximum adult body weight was gained. Interestingly, non-significant difference in body weight was found between 5th and 6th months, possibly due to harsh environmental conditions at that time, such as very low temperature.

The AMH is released from immature Sertoli cells and thus its peripheral level depends on immature Sertoli cells count, as reported in bulls (Kitahara *et al.*, 2016), stallion (Claes and Ball, 2016) and alpaces (Ciccarelli *et al.*, 2018). As immature Sertoli cells mitotically divide in the seminiferous tubules, peripheral AMH concentration gradually increases until Sertoli cells differentiation (Rajak *et al.*, 2017). In the current study, serum AMH was low at 10d and 180d than at any other age during pre-pubertal period. After 10d, serum AMH level gradually increased to its peak at 40d. Furthermore, serum AMH peak was followed by a significance rise in serum testosterone concentration after 50d and gradually increased till puberty. Interestingly, AMH concentration was negatively correlated with that of testosterone at 60d and this rapid increase in testosterone might be the indicative of initiation of sexual behavior in kids. Our findings are supported by those of a previous study on Tokora goats that earlier sexual behavior starts at 2nd month of age (Nishimura *et al.*, 2000). Interestingly, AMH increase in our study was biphasic till 100d, after which its level continuously declined. High level of AMH at 40d might be the indicative of initiation of mitosis in immature Sertoli cells. A decline in serum AMH level around this time can be explained by the arrest of Sertoli cells proliferation and start of spermatogenesis, as reported in bulls (Rota *et al.*, 2002), stallions (Claes *et al.*, 2013) and rams (Cazorla *et al.*, 1998). Furthermore, histological studies on goat kid testes revealed an increased count of Sertoli cells and their maturation at 1-3 months of age (Júnior *et al.*, 2012).

Results of the present study showed that serum AMH concentration at puberty (180d) was positively correlated with pre-pubertal ages (40, 50, 70 and 80d). These results indicate that plasma AMH at these days could be predictive of pubertal AMH levels. Thus, in this scenario, level of AMH around 40d can serve as criteria for puberty and selection of future breeder bucks before sexual maturation, which would allow farmers to make early decisions for less fertile bucks. Serum AMH inhibits the production of androgen from Leydig cells and aromatase activity of FSH during pre-pubertal age until Sertoli cells mature (Dutertre *et al.*, 1997; Rouiller-Fabre *et al.*, 1998). These findings suggest that AMH level at early pre-pubertal age (40d) could be useful in estimating pubertal age. However, additional experiments are required to verify cut off values of plasma AMH as biomarker for prediction of early puberty under different nutritional regimes.

In the current study, serum E2 concentration was low at birth, significantly increased at the age of 60d and declined gradually till onset of puberty. Our result showed positive correlation at 20d between E2 and testosterone. A previous study indicated that declined E2 concentration was associated with rise of testosterone concentration and attainment of puberty in rams (Cazorla *et al.*, 1998). Moreover, declined E2 concentration might indicate activation of hypothalamic-pituitary axis during this period, which leads to the initiations of sexual behavior, as previously reported in buffalo bulls (Ahmad *et al.*, 1989) and mice (Dutertre *et al.*, 1997).

Conclusions: In conclusion, kids exhibit a characteristic serum AMH profile along with testosterone and estradiol during pre-pubertal life, such that serum AMH concentration at an early age (40-80d) could be a useful biomarker for the predication of initiation of sexual behavior and onset of puberty in goat kids.

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Authors contribution: HR and MA conceived, designed, analyzed and wrote the manuscript. MA executed the study and statistically analyzed the data. MA and MU performed the hormone analysis. MRY, NA, KJ, AR and other authors critically reviewed the manuscript.

REFERENCES

Ahmad N, Shahab M, Khurshid S, *et al.*, 1989. Pubertal development in the male buffalo: longitudinal analysis of body growth, testicular size and serum profiles of testosterone and oestradiol. Anim Reprod Sci 19:161-70.

Ali HES, Kitahara G, Nibe K, *et al.*, 2013. Plasma anti-müllerian hormone as a biomarker for bovine granulosa-theca cell tumors: comparison with immunoreactive inhibin and ovarian steroid concentrations. Theriogenology 80:940-9.

Ali EH, Kitahara G, Takahashi T, *et al.*, 2017. Plasma anti-Müllerian hormone profile in heifers from birth through puberty and relationship with puberty onset. Biol Reprod 97:153-61.

Ano H, Hidaka Y and Katamoto H, 2014. Evaluation of anti-mullerian hormone in a dog with a Sertoli cell tumour. Vet Dermatol 25:142-e141.

Cazorla O, Seck M, Pisselet C, *et al.*, 1998. Anti-Müllerian hormone (AMH) secretion in prepubertal and adult rams. Reproduction 112:259-66.

Ciccarelli M, Tibary A, Campbell AJ, *et al.*, 2018. Effect of age and castration on serum anti-Müllerian hormone concentration in male alpacas. Theriogenology 105:174-7.

Claes AN and Ball BA, 2016. Biological functions and clinical applications of anti-Müllerian hormone in stallions and mares. Vet Clin Equine Pract 32:451-64.

Claes A, Ball BA, Almeida J, *et al.*, 2013. Serum anti-Müllerian hormone concentrations in stallions: developmental changes, seasonal variation, and differences between intact stallions, cryptorchid stallions, and geldings. Theriogenology 79:1229-35.

Dow T, Roudebush W, Parker F, *et al.*, 2011. Influence of age and gender on secretion of anti-Müllerian hormone in Asian (*Elephas maximus*) and African (*Loxodonta africana*) elephants. Theriogenology 75:620-7.

Dutertre M, Rey R, Porteu A, *et al.*, 1997. A mouse Sertoli cell line expressing anti-Müllerian hormone and its type II receptor. Mol Cell Endocrinol 136:57-65.

Evans A, Pierson R, Garcia A, *et al.*, 1996. Changes in circulating hormone concentrations, testes histology and testes ultrasonography during sexual maturation in beef bulls. Theriogenology 46:345-57.

Gobikrushanth M, Purfield DC, Colazo M, *et al.*, 2018. The relationship between serum anti-Müllerian hormone concentrations and fertility, and genome-wide associations for anti-Müllerian hormone in Holstein cows. J Dairy Sci 101:7563-74.

Jimenez-Krassel F, Scheetz D, Neuder L, *et al.*, 2015. Concentration of anti-Müllerian hormone in dairy heifers is positively associated with productive herd life. J Dairy Sci 98:3036-45.

Josso N and Clemente N, 2003. Transduction pathway of anti-Müllerian hormone, a sex-specific member of the TGF-β family. Trends Endocrinol Metab 14:91-7.

Júnior AM, Oliveira L, Neto AA, *et al.*, 2012. Spermatogenesis in goats with and without scrotum bipartition. Anim Reprod Sci 130:42-50.

Kitahara G, Kamata R, Sasaki Y, *et al.*, 2016. Changes in peripheral anti-Müllerian hormone concentration and their relationship with testicular structure in beef bull calves. Domest Anim Endocrinol 57:127-32.

Longpre KM, Guterl JN and Katz LS, 2016. Proximity to females alters circulating testosterone concentrations and body weight in male goats. Small Rumin Res 144:334-40.

Newberry H, Kegley B, Rosenkrans C, *et al.*, 2016. Use of anti-mullerian hormone to select for fertility in beef heifers. Proc "17th Discovery, The Student Journal of Dale Bumpers College of Agricultural, Food and Life Sciences", University of Arkansas, Fayetteville-USA pp:79-84.

Nishimura S, Okano K, Yasukouchi K, *et al.*, 2000. Testis developments and puberty in the male Tokara (Japanese native) goat. Anim Reprod Sci 64:127-31.

Rajak S, Kumaresan A, Attupuram N, *et al.*, 2017. Age-related changes in transcriptional abundance and circulating levels of anti-Mullerian hormone and Sertoli cell count in crossbred and Zebu bovine males. Theriogenology 89:1-8.

Rey R, Lordereau-Richard I, Carel JC, *et al.*, 1993. Anti-müllerian hormone and testosterone serum levels are inversely during normal and precocious pubertal development. J Clin Endocrinol Metab 77:1220-6.

Rota A, Ballarin C, Vigier B, *et al.*, 2002. Age dependent changes in plasma anti-Müllerian 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.

Rouiller-Fabre V, Carmona S, Merhi RA, *et al.*, 1998. Effect of anti-mullerian hormone on Sertoli and Leydig cell functions in fetal and immature rats. Endocrinology 139:1213-20.