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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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.

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-Krasse 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 from 10 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 -20°C 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 (33.8±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±0.17 ng/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 (7.3±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.
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 at 160 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

<table>
<thead>
<tr>
<th>Days</th>
<th>Correlation between AMH and testosterone</th>
<th>Correlation between AMH and E2</th>
<th>Correlation between testosterone and E2</th>
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<tr>
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<td>r value</td>
<td>P value</td>
<td>r value</td>
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<tr>
<td>10</td>
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<tr>
<td>20</td>
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<tr>
<td>50</td>
<td>0.73</td>
<td>0.07</td>
<td>-0.48</td>
</tr>
<tr>
<td>60</td>
<td>-0.92*</td>
<td>0.04</td>
<td>0.96*</td>
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<tr>
<td>70</td>
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<tr>
<td>110</td>
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<tr>
<td>150</td>
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<td>-0.66</td>
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<tr>
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<td>0.04</td>
<td>0.8</td>
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<tr>
<td>180</td>
<td>0.91*</td>
<td>0.02</td>
<td>0.63</td>
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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 Japanese 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 alpacas (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 prediction 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.

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