Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2021.023

RESEARCH ARTICLE

# Effects of Different Nutritional Regimens during Pre-Weaning Period on Body and Testis Growth, Sexual Behavior, Hormonal Profile and Genes Expression in Male Beetal Goat Kids

Muhammad Ashraf[1], Ijaz Farid[2], Wasim Shehzad[3], Muhammad Rizwan Yousaf[1], Nasim Ahmad[1], Awais Ihsan[2], Khalid Javed[4], Hasan Riaz[2*] and Amjad Riaz[1*]

[1]Department of Theriogenology; [3]Institute of Biochemistry and Biothecnology; [4]Department of Animal Breeding and Genetics, University of Veterinary and Animal Sciences, Lahore, Pakistan; [2]Department of Biosciences, COMSATS University Islamabad, Sahiwal Campus, Sahiwal, Pakistan
*Corresponding author: dramjadriaz@uvas.edu.pk and hasan@cuisahiwal.edu.pk

ARTICLE HISTORY (20-557)

Received: October 28, 2020
Revised: January 23, 2021
Accepted: January 25, 2021
Published online: February 17, 2021

**Key words:**
Beetal goat kids
Genes expression
Hormonal profile
Milk replacer
Natural milk
Puberty onset
Sexual behavior

**ABSTRACT**

From birth till puberty, body weight (BW), scrotal circumference (SC), serum hormonal profile, genes expression pattern and pubertal onset were longitudinally studied in male Beetal goat kids reared on two nutritional regimens during pre-weaning period. Kids were reared either on suckling (NR; n=26) or milk replacer (AR; n=26) from day-10 to 12 weeks of age. Body weight and SC showed linear increase till puberty, although pubertal onset was earlier ($P<0.05$) in NR than AR kids (175.00±12.00 vs 235.00±8.00 days). Serum testosterone and TSH concentrations remained higher ($P<0.05$) in NR than AR kids from 2 months of age till puberty. Interestingly, kids of both groups started showing sexual behavior activities when serum testosterone achieved a threshold level (1.36 ng/ml for NR and 1.34 ng/ml for AR kids). There were significant differences in anti-mullerian hormone levels at 2-6 months of age between two groups. The mRNA level of spermatogenic genes (ODF2, PRM1, STRA8) was higher at 4-8 months than 1-3 months of age in both groups. However, the expression pattern of Sertoli cells specific genes (SOX9, FSHR) was higher at 0-1 month of age in NR and 2-4 months of age in AR kids. The androgenic gene (CYP19A1) expression increased ($P<0.001$) at 4-6 month of age in NR and 6-8 months of age in AR kids. In conclusion, sexual behavior activities started in both groups of Beetal goat kids when testosterone level achieved cutoff value around 1.3 ng/ml. Kids reared on suckling attained puberty earlier than those reared on milk replacer during pre-weaning period. Relationship of age with BW ($r^2$=0.9) and SC ($r^2$=0.9) may be used as selection indices for these kids.



**To Cite This Article:** Ashraf M, Farid I, Shehzad W, Yousaf MR, Ahmad N, Ihsan A, Javed K, Riaz H and Riaz A, 2021. Effects of different nutritional regimens during pre-weaning period on body and testis growth, sexual behavior, hormonal profile and genes expression in male beetal goat kids. Pak Vet J, 41(2): 242-248. http://dx.doi.org/10.29261/pakvetj/2021.023

## INTRODUCTION

Goat is gaining popularity as an important livestock species due to its low cost of feeding and easy rearing with lower management requirements in the tropical and subtropical regions. It is a source of many products like meat, milk, hair, and hides (Murtaza *et al.*, 2019). The growing demand for mutton and goat milk requires efforts to enhance its production through measuring reproductive indices of the herd. One aspect is the establishment of selection criteria for young males for the future breeding programs. For this purpose, understanding regarding the reproductive physiology of males from birth to the onset of puberty is crucial.

Onset of puberty is a complex process that requires a synchronized increase in body weight, testis size, and levels of certain hormones at an early age (Arangasamy *et al.*, 2018). The increased levels of gonadotrophic hormones and testosterone are essential for testicular development and initiation of sexual behavior (Ramukhithi *et al.*, 2017). Circulating testosterone is related to sexual behavior, spermatogenesis, and other secondary sex characteristics of the male (Nishimura *et al.*, 2000). Moreover, common pre-pubertal sexual behavior events reported for different animals include sexual interest, mounting, penile protrusion and penile erection in buffalo bulls (Ahmad *et al.*, 1991) and South African indigenous bucks (Ramukhithi *et al.*, 2017). In this context, it was found that circulatory testosterone concentration and sexual behavior scores are the best indices for selection criteria in buffalo bulls (Ahmad *et al.*, 1991). Moreover, anti-mullerian hormone (AMH) is an important hormone, used as a biomarker in early age for the predication of puberty in goat kids (Ashraf *et al.*, 2020).

In newborn goat kids, initial dam milk feeding is essential because milk is the only source of energy at that age. However, this practice decreases the availability of marketable milk. Therefore, early weaning and artificial rearing of kids with cow and buffalo milk or milk replacer is practiced to increase the availability of sellable milk (Delgado-Pertíñez *et al.*, 2009). However, this practice can compromise the growth rate of kids, as many studies have found significantly higher growth rate of naturally suckled kids than those reared on milk replacer (De Palo *et al.*, 2015). Moreover, the indigenous rearing system pays more attention to the growth of adult goats than young kids (Bopape *et al.*, 2015). Data are available on the effect of artificial rearing of kids on body growth parameters, meat quality, and milk yield (Argüello *et al.*, 2004). However, studies on the effects of such rearing practices on reproductive indices such as initiation of sexual behavior and pubertal onset are scanty.

Therefore, the present study was designed to characterize the longitudinal pre-pubertal changes in growth parameters, testicular measurements, molecular events, and sexual behavior along with hormonal profile in goat kids raised through feeding on natural milk or milk replacer up to the weaning age, and then under similar nutrition and management until onset of puberty. The study aimed to measure body weight (BW), scrotal circumference (SC), endocrine profile, genes expression pattern, and sexual behavioral activities from birth to puberty in natural reared (NR) vs artificial reared (AR) Beetal goat kids.

## MATERIALS AND METHODS

**Animals and management:** This study was conducted at the Al-Haiwan Sires, Sahiwal, Pakistan (30.6682°N, 73.1114°E) from July 2018 to March 2019. The experiments were performed under the guidelines of the Ethical Committee, University of Veterinary and Animal Sciences, Lahore, Pakistan. Fifty-two newly born Beetal goat kids were assigned into two groups. In natural rearing group (NR; n=26), kids were allowed to suckle their mothers up to 12 weeks of age. In artificial rearing group (AR; n=26), after colostrum feeding kids were separated from mothers and fed on milk replacer at (Table 1, Elvor Kids, SOFIVO, Condesur-Vire, France), warmed at 37ºC and offered twice daily for 12 weeks (17% of body weight). Then kids of both groups were weaned at three months of age, offered equal quantities of green fodder (lucerne) and wheat straw. Moreover, feeding of 150 -200 gm supplementary concentrate (92.8% organic matter, 15.3% crude protein, and 18.5% crude fiber) was started before weaning in both groups till pubertal onset.

**Body weight and scrotal circumference:** Body weight of each kid was taken through an electronic weighing balance. For recording SC, testicles were grasped with one hand and the widest area was measured by a flexible measuring tape. Recording of both parameters was started at 10 days of age and continued on monthly intervals till pubertal onset (7-9 observations for each kid).

**Blood collection and hormones analysis:** Starting from 10 days of age, blood samples were collected from each kid by jugular venipuncture at monthly intervals till pubertal onset (NR=6, AR=8 months of age). Serum was separated through centrifugation (2800 G for 15 min) and stored at -20ºC. Serum testosterone concentrations were measured through RIA using a commercial diagnostic kit (IM246303; Beckman coulter, California- USA). The sensitivity of the assay was 0.04 ng/ml and the intra- and inter-assay coefficients of variation were 8.9 and 16.2%, respectively. Concentrations of thyroid stimulating hormone (TSH) and AMH were measured through goat specific ELISA kits (Cusa-Bio, TX, USA and MBS267219; MyBiosource-USA; respectively). The sensitivity for TSH was 0.25 µIU/ml and intra- and inter-assay coefficients of variation were 10.4 and 14.7%, respectively, while sensitivity for AMH was 0.06 ng/ml and the intra- and inter-assay coefficients of variation were 8.0 and 12.0%, respectively.

**Evaluation of sexual behavior:** Starting from two months of age till pubertal onset, sexual behavior of each kid was accessed by visual observation. Every two-week interval, kids were exposed to an estrus teaser doe for 5-10 minutes. Sexual behavioral events were scored as previously describe by Anzar *et al.* (1993) and shown in Table 2. The pubertal onset was declared when an ejaculate with sperm concentration of 50 million/ml and 10% sperm motility was obtained for the first time (Longpre *et al.*, 2016).

**RNA isolation and Real time PCR:** To establish the longitudinal sexual developmental pattern, right from 10 days of age till puberty, three goat kids from each feeding regimen were slaughtered on monthly basis for testes collection. From 80-100 mg of the testicular tissue, total RNA was extracted through RNA extraction kit (QIAGEN Shanghai Co Ltd, China), as described previously (Faucette *et al.*, 2014). The RNA was reverse transcribed with oligo-dT by using cDNA kit (Thermo Fisher Scientific, UK), and was stored at -40°C till further analysis. The cycles profile was; 95°C for 15s, followed by 40 cycles of 95°C for 10s and 58-60°C for 30s. Relative gene expression quantification was done through $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). Primers were designed by using integrated DNA technologies (Table 3).

**Statistical analysis:** The data were analyzed using statistical software SPSS, version 20. Sexual behavior activities were analyzed by the chi-square test. The general linear model (GLM) was used to compare the significance of testosterone, TSH, AMH, BW, SC and genes expression pattern between the groups. P<0.05 was considered as level of significance.

## RESULTS

Early nutrition effects (P<0.05) on BW, SC along with the concentrations of testosterone, AMH and TSH, and genes expression pattern in kids fed on their dam milk (NR) or milk replacer (AR) were compared. The results showed that through 1 to 6 months of age, WB was significantly (P<0.05 to P<0.001) higher in NR goat kids as compared to AR group (Fig. 1A). However, irrespective of age, BW at the time of pubertal onset in NR and AR kids averaged 36.42±4.49 and 31.01±2.26 kg, respectively. The SC was significantly higher (P<0.05-P<0.001) in NR kids than AR group from 2 months of age till end of the study (Fig. 1B). The age and BW appeared to be strongly associated with SC in both groups, as the correlation coefficient for both parameters was always above $r^2$=0.9 (P<0.01; Fig. 1C&D). Pubertal onset was significantly (P<0.0001) earlier in NR than AR goat kids (175.00±12.00 vs 235.00±8.00 days; Fig. 2A). Age had a profound effect on blood concentrations of testosterone and TSH in both groups, as serum concentrations of both these hormones gradually increased to peak levels at 6 months of age in NR and 8 months of age in AR goat kids (Fig. 2A&B). However, concentrations of both these hormones were significantly higher in NR than AR goat kids from 2 months of age till end of the study (P<0.01-P<0.0001).

**Changes in serum anti-mullerian hormone levels:** Serum AMH concentrations were low at 10 d of age in NR and AR goat kids (11.51±0.79 and 11.13± 1.14 ng/ml, respectively) and gradually increased to peak levels at 4 months of age (34.45±2.17 vs 44.12±1.58 ng/ml, respectively), then declined till puberty onset in kids of both groups. However, from 2 months of age till end of the study, there was significant (P<0.01-P<0.0001) difference in AMH level between NR and AR kids, being higher in AR group except at the age of 2 months when it was higher in NR kids (Fig. 2C).

**Sexual behavior and serum testosterone levels:** Sexual behavior activities for NR vs AR kids are presented in Figure 3. No sexual behavior activity was observed during the first month of age, when serum testosterone concentration was <1.0 ng/ml in kids of both groups. Sexual behavior started at the age of around 2 months in NR kids, when serum testosterone concentration was 1.36±0.58 ng/ml (Fig. 2A), and kids showed sexual behavior score of 21%. Concurrently, sexual behavior activities in kids of AR group started at the age of around 4 months, when serum testosterone concentration was 1.34±0.26 ng/ml (Fig. 2A). However, sexual behavior score was significantly (P<0.05) higher in NR goat kids than AR during pre-pubertal age. Moreover, a sharp increase in the sexual behavior score was observed at pubertal onset in kids of both groups (Fig. 3). Similarly, the peak level of testosterone was at pubertal onset in both NR and AR goat kids (5.52±0.32 ng/ml vs 3.04±0.21 ng/ml, P<0.0001), as shown in Fig. 2A**.**

**Changes in genes expression pattern:** The spermatogenic genes (ODF2, PRM1 and STRA8) mRNA level was higher (P<0.05-P<0.001) at advance age (4-8 months) as compared to early age (1-3 months) in kids of both groups (Fig. 4A-C). The expression of Sertoli cells specific genes (SOX9 and FSHR) was significantly (P<0.001) higher in early ages (0-1 month) in NR goat kids and 2-4 months in AR goat kids, with lower expression at advance age (Fig. 4E&F). The steriodogenic gene (CYP19A1) mRNA level increased (P<0.001) from 4 to 6 month of age in NR and 6-8 months of age in AR kids (Fig. 4G). The HSPA8 gene expression was maintained throughout the study period in kids of both groups. However, its expression was significantly higher (P<0.01) at 1-2 months of age in NR kids and 4-5 months (P<0.05-P<0.01) in AR kids (Fig. 4D).

## DISCUSSION

Multiple studies have reported a strong positive correlation of SC with semen quality and fertility, and being a heritable trait, it is often recommended for the selection of breeding sires (Ahmad *et al.*, 1991). In the

**Table 1:** Composition of milk replacer and natural milk fed to the experimental kids

| Ingredients | Milk replacer | Natural milk |
|---|---|---|
| Dry matter (%) | 96.0 | 13.7 |
| Crude protein (%) | 23.7 | 3.4 |
| Fat (%) | 25 | 4.83 |
| Ash (%) | 6.7 | |
| Crude cellulose (%) | 0.3 | |
| Vitamin A (UI/kg) | 25,000 | |
| Vitamin $D_3$ (UI/kg) | 4,000 | |
| Vitamin E (mg/kg) | 50 | |
| Vitamin $B_1$ (mg/kg) | 15 | |
| Vitamin C (mg/kg) | 1,000 | |
| Fe (mg/kg) | 10 | |
| Metabolizable energy (Mj/kg DM) | 11.93 | 15.64 |

Composition of milk replacer was the same as used by Delgado-Pertinez *et al.* (2009).

**Table 2:** Scoring system for the evaluation of sexual behaviors in pre-pubertal and pubertal goat kids

| Reaction time and sexual aggressiveness during approach toward teaser animal | | | |
|---|---|---|---|
| Sexual aggressiveness (SA) | Score | Reaction time (RT) (sec) | Score |
| Shy | 1 | 10-20 | 6 |
| Slow | 2 | 21-30 | 5 |
| Active | 3 | 31-60 | 4 |
| Aggressive | 4 | 61-120 | 3 |
| | | 121-240 | 2 |
| | | 241-360 | 1 |
| | | >360 | 0 |
| Mating ability score based on various behaviors | | | |
| Sexual behavior events | | Score | |
| Mounting only (MO) | | 1 | |
| Penile erection (PE) | | 2 | |
| Complete | 2 | | |
| Partial | 1 | | |
| Absent | 0 | | |
| Penile protrusion (Pp) | | 1 | |
| Ejaculation thrust (Et) | | 2 | |
| Ejaculation (E) | | 2 | |
| Concavity to back line (Bl) | | 1 | |
| Penile movement to locate AV (Pm) | | 1 | |

Tactile stimulation "TS" (Nudging, Licking, Nosing, Bleating and Flehmen). For sexual aggressiveness score (%) = [{(RT score+SA score) - 0.2 per TS}÷ 10]×100. Futile attempt "Ft" (when kid mount and did not ejaculate) score = 1. For mating ability score (%)=[{(MO+PE+Pp+Et+E+Bl+Pm) – Futile attempts}÷ 10]×100. For sexual behavior score = (sexual aggressiveness score + mating ability score) ÷2 Scoring of sexual behavior was the same as previously describe by Anzar *et al.* (1993).

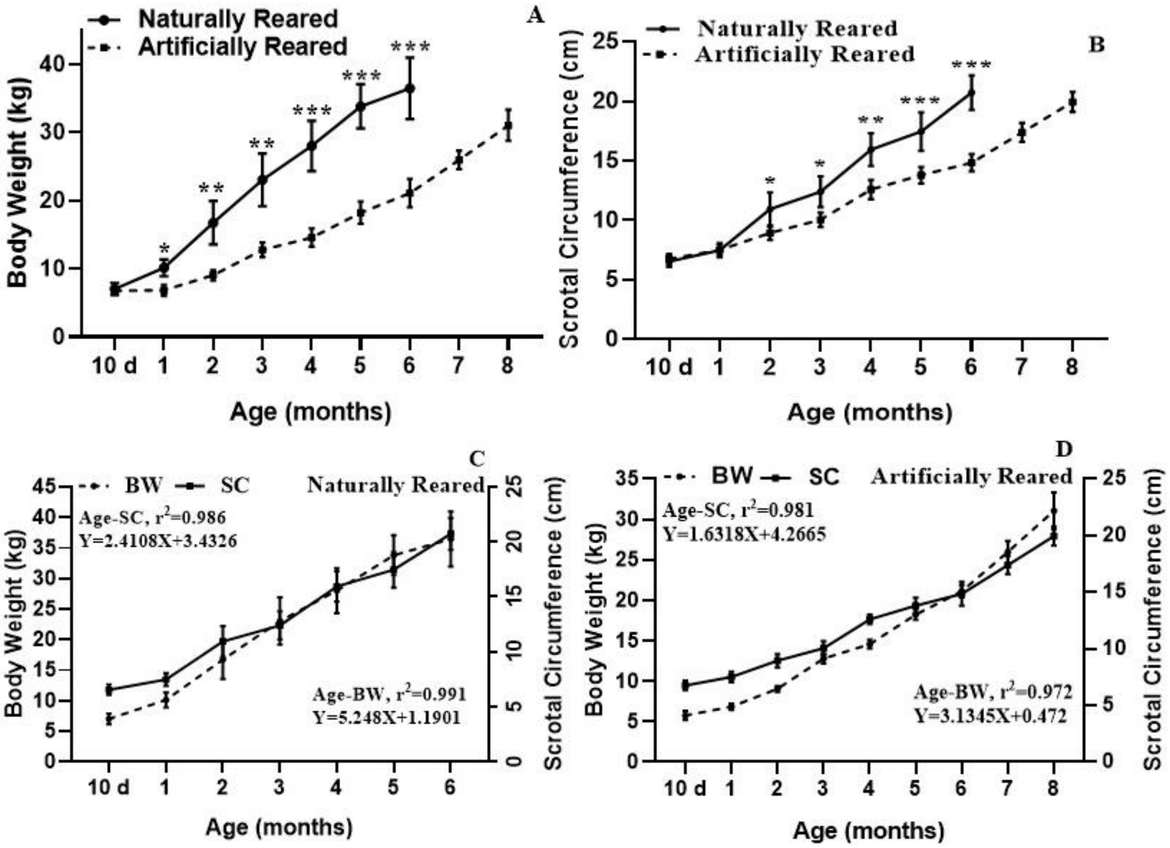



**Fig. 1:** Age related changes of body weight-BW (A) and scrotal circumference-SC (B) in kids of two groups. Correlation of SC and BW with age in Naturally Reared kids (C) and in Artificially Reared kids (D). Asterisks denote differences between the groups; ***P<0.001; **P<0.01 and *P<0.05.

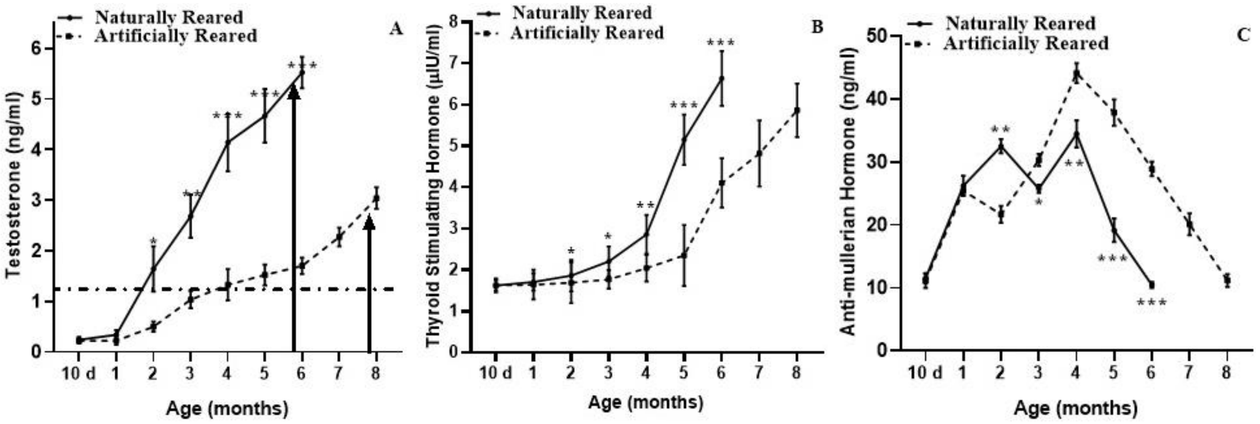



**Fig. 2:** Age related changes between kids of the two groups in: (A) serum testosterone concentrations; dotted line shows the cut off value (1.3 ng/ml) where sexual behavior initiated, while the arrow shows the age of pubertal onset; (B) serum TSH; and (C) serum AMH. Asterisks denote differences between the groups; ***P<0.0001; **P<0.001 and *P<0.01.

**Table 3:** Primers sequence used in quantitative PCR

| Genes | Forward | Reverse | Amplicon length (bps) |
|---|---|---|---|
| GAPDH | ACAAGATGGTGAAGGTCGGA | GTGAAGGTCAATGAAGGGGT | 122 |
| PRM1 | AGATACCGATGCTGCCTCAC | TTCGTCTGCGACATCTTCTT | 73 |
| ODF2 | TGCTCGGACATCAACACC | CGCCAGCCGTTTCAATAGT | 168 |
| STRA8 | CCACCCCTGAGGAGATCCTTT | GCAGCCCACTCCAAAACGC | 380 |
| SOX9 | GCAGAAGGCAAGCAAAGG | GGTGTTCAGAGAGGCACAGG | 160 |
| FSHR | CGACAAGGCAAAACGGACAC | CAGAAGACAGGCAGTGGGTT | 173 |
| HSPA8 | TCAGCGTCAGGCTACCAAAG | ACATCAAAAGTGCCACCACC | 162 |
| CYP19A1 | AAAACTCACTCCAGCTCCTTAC | TAGTCCCTACCCCACCAAAA | 176 |

present study, both NR and AR goat kids showed a linear increase in BW and SC till puberty, despite that nutritional regimens strongly affected the SC and BW. Both age and BW appeared to be strongly associated with SC in kids of both groups, as the correlation coefficient between both these parameters was always above 0.9 during the study period. Irrespective of age, SC at pubertal onset was slightly different in both groups, which is consistent with previous reports in South African bucks (Ramukhithi *et al.*, 2017).

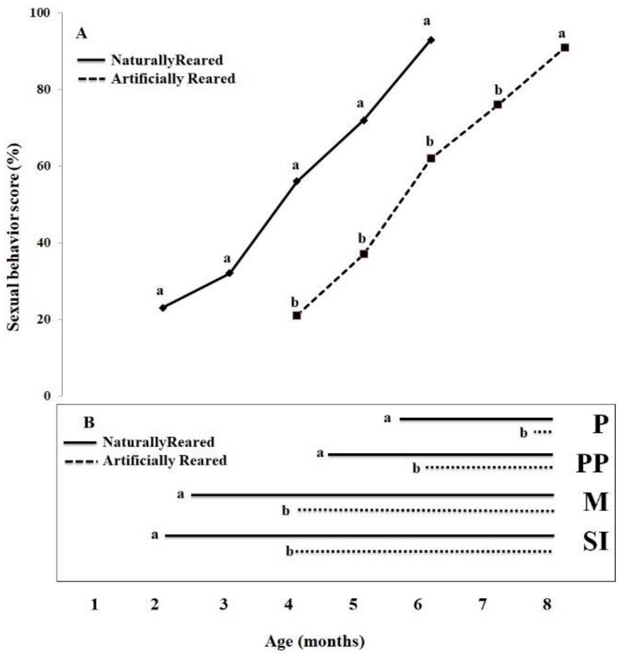
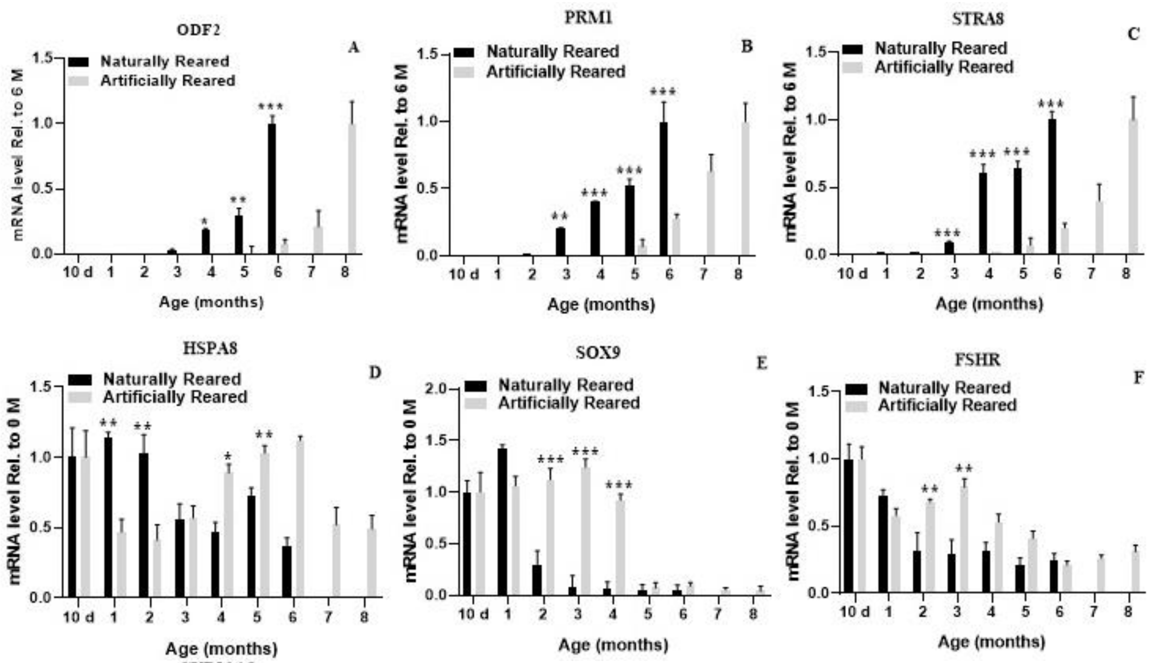



**Fig. 3:** (A) Changes in sexual behavior during pre-pubertal development in kids of two groups. (B) Schematic representation of the chronological sequence in which different behavioural components appeared before puberty. SI, sexual interest M, mounting, PP, penile protrusion, P, puberty. a, b denote differences (P<0.05) between groups

In the current study, the sexual behavior activity started at 2nd month of age in NR kids, with mounting and sexual interest being the dominant sexual behavior. A similar pattern was found in AR kids, although it was delayed till 4th month of age. A previous study on male goats has shown that mounting is the first sexual activity observed in the presence of a female goat (Ramukhithi *et al.*, 2017). It was also found that with the advancement of age, the exhibition of mounting and sexual interest persisted in both groups. However, the overall sexual activity was significantly higher in NR than in AR kids till puberty onset. The pre-pubertal sexual behavior was concomitant with the level of blood testosterone in both groups, suggesting that increased blood testosterone concentration is important for the initiation of sexual behavior before puberty in goat kids (Farshad *et al.*, 2012). These results are in agreement with previous studies in buffalo bulls (Ahmad *et al.*, 1991) and male goats (Suyadi, 2012). Testosterone production coincides with the development and activation of Leydig cells, which are critical for the attainment of puberty (Nishimura *et al.*, 2000). Blood testosterone concentration in immature South African indigenous goats is reported to be below 1 ng/ml (Ramukhithi *et al.*, 2017). Likewise, male Beetal kids in the current study did not exhibit any sexual behavior activity when testosterone concentration was below a cutoff value (1.3 ng/ml) in both groups. Pre-weaning nutrition delayed achieving this cutoff value in AR goat kids, indicating that initiation of sexual behavior is strongly linked with blood testosterone level. Previous studies have also indicated that improved early nutrition results in increased blood testosterone level in Holstein bulls (Dance *et al.*, 2015) and male goats (Arangasamy *et al.*, 2018).



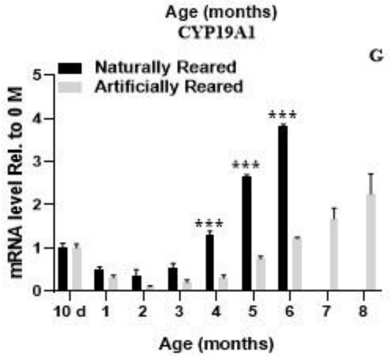




**Fig. 4:** Relative expression pattern of selected genes in kids of two groups (fold chain value; means±SEM). (A to C) Relative levels of mRNA for spermatogenic genes "ODF2, PRM1 and STRA8", where 6 and 8 months of age were adjusted to 1 in NR and AR kids, respectively. (D to G) Relative levels of mRNA for testicular growth gene "HSPA8" and Sertoli cells specific genes "SOX9 and FSHR", where 0 months of age were adjusted to 1 in NR and AR kids. Asterisks denote difference between groups; ***P<0.001, **P<0.01 and *P<0.05. Rel=relative, M=month.

Anti-mullerian hormone is released from immature Sertoli cells and its concentration increases gradually until Sertoli cells differentiation in the growing male (Rajak *et al.*, 2017). In the current study, serum AMH was low at 10d of age, gradually increased to its peak at 4 months of age, then declined sharply until puberty onset in kids of both groups. A decline in serum AMH may be explained by the arrest of Sertoli cells proliferation and start of spermatogenesis, as reported in bulls (Kitahara *et al.*, 2016) and stallions (Claes *et al.*, 2013). Moreover, higher level of AMH in AR goat kids compared with NR kids at 4 months of age may be due to higher number of immature Sertoli cells in AR goat kids (Banco *et al.*, 2012).

According to Faucette *et al.* (2014), spermatids appeared at the start of 3rd month and mature spermatozoa appeared at the age of 6 months in male goats which indicate active spermatogenesis. In this regard, the molecular pathways in the present study showed that the expression of ODF2, PRM1and STRA8 genes significantly increased at advance ages (from 4 months till end of study) in both NR and AR kids. Faucette *et al.* (2014) also observed similar expression pattern of STRA8 and PRM1 genes in Alpine goats. It has also been shown that STRA8 gene is essential for meiosis of germ cells (Bo *et al.*, 2020), expression of PRM1 gene is required for the transition into spermatids during spermatogenesis (Ganguly *et al.*, 2013), while ODF2 gene protects the tail of spermatozoa (Zhao *et al.*, 2018). The current study demonstrated that ODF2 mRNA expression was found highest at time of puberty in both groups, resulting in higher mature sperms in testis at that time in both groups. In the current study, Sertoli cells specific genes (SOX9 and FSHR) expression pattern was higher at early age in kids of both groups. The past studies documented similar expression pattern of SOX9 gene in Alpine goats (Faucette *et al.*, 2014), mice, human and rats (Barrionuevo *et al.*, 2016). Similarly, FSHR gene expression decreased with growing age in goat kids, as described by Bo *et al.* (2020), who attributed such decline in FSHR gene expression to increased population of germ cells. The CYP19A1 gene mainly controls androgen level in males. In our study, CYP19A1 expression was significantly increased from 3rd month of age till puberty onset in kids of both groups. According to Ramukhithi *et al.* (2017), higher expression level of CYP19A1 might be an indication of higher testosterone concentration in goats. In the present study, expression of HSPA8 gene was maintained throughout the study in kids of both groups. These results are consistent with those of Faucette *et al.* (2014), who also stated that continuous expression of this gene might be due to growth of testes. Although, the exact mechanism for relatively low expression of this gene in AR kids during early ages is not known but one reason could be the absence of growth promoters in milk replacer (De Palo *et al.*, 2015). However, these kids ideally represented how pre-weaning nutrition can affect the reproductive indices of future breeding goat kids.

**Conclusions:** In conclusion, initiation of sexual behavior in male Beetal goat kids occurred when blood testosterone level achieved a cutoff value around 1.3 ng/ml. The molecular events suggested the differentiation of Sertoli, Leydig and germ cells at the end of 4th month of age. Relationship of age with BW ($r^2$=0.9) and SC ($r^2$=0.9) may be used as selection indices for male Beetal kids. Findings of this study also indicate that goat kids raised on natural suckling should preferably be selected for the future breeding program.

**Acknowledgments:** The authors would like to acknowledge Higher Education Commission, Islamabad, Pakistan, for financially supporting the study by providing funds under project "HEC-NRPU (5317/Federal/NRPU/ R&D/HEC/2016)". The authors also acknowledge Dr. Muhammad Farooq for help to look after experimental kids throughout the study and Rana Akhtar for assistance in hormonal assays.

**Authors contribution:** AR, MA and HR conceived the idea, designed the study, analyzed the data and wrote the manuscript. MA executed the study, while MA and IF performed the hormone analysis. WS, MRY, NA, KJ, AI and all authors critically reviewed the manuscript.

## REFERENCES

Ahmad N, Shahab M, Anzar M, *et al.*, 1991. Changes in the behaviour and androgen levels during pubertal development of the buffalo bull. Appl Anim Behav Sci 32:101-5.

Anzar M, M Ahmad, M Nazir, *et al.*, 1993. Selection of buffalo bulls: Sexual behavior and its relationship to semen production and fertility. Theriogenology 40:1187-98.

Arangasamy A, Krishnaiah MV, Manohar N, *et al.*, 2018. Advancement of puberty and enhancement of seminal characteristics by supplementation of trace minerals to bucks. Theriogenology 110:182-91.

Argüello A, Castro N and Capote J, 2004. Growth of milk replacer kids fed under three different managements. J Appl Anim Res 25:37-40.

Ashraf M, Ullah M, Yousuf MR, *et al.*, 2020. 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. Pak Vet J 40:370-4.

Banco B, Veronesi MC, Giudice C *et al.*, 2012. Immunohistochemical evaluation of the expression of anti-mullerian hormone in mature, immature and neoplastic canine Sertoli cells. J Comp Pathol 146:18-23.

Barrionuevo FJ, Hurtado A, Kim GJ, *et al.*, 2016. Sox9 and Sox8 protect the adult testis from male-to-female genetic reprogramming and complete degeneration. Elife 5:e15635. doi: 10.7554/eLife.15635.

Bo D, Jiang X, Liu G, *et al.*, 2020. Multipathway synergy promotes testicular transition from growth to spermatogenesis in early-puberty goats. BMC Genomics 21:372. doi:10.1186/s12864-020-6767-x.

Bopape MA, Lehloenya KC, Chokoe TC, *et al.*, 2015. Comparison of electro ejaculator and artificial vagina on semen collection from south African indigenous goat following assessment by computer aided sperm analysis. Open J Anim Sci 5:210-8.

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.

Dance A, Thundathil J, Wilde R, *et al.*, 2015. Enhanced early-life nutrition promotes hormone production and reproductive development in Holstein bulls. J Dairy Sci 98:987-98.

De Palo P, Maggiolino A, Centoducati N, *et al.*, 2015. Effects of different milk replacers on carcass traits, meat quality, meat color and fatty acids profile of dairy goat kids. Small Rumin Res 131:6-11.

Delgado-Pertíñez M, Guzmán-Guerrero J, Caravaca F, *et al.*, 2009. Effect of artificial vs. natural rearing on milk yield, kid growth and cost in Payoya autochthonous dairy goats. Small Rumin Res 84: 108-15.

Farshad A, Yousefi A, Moghaddam A, *et al.*, 2012. Seasonal changes in serum testosterone, LDH concentration and semen characteristics in Markhoz goats. Asian-Australas J Anim Sci 25:189-93.

Faucette AN, Maher VA, Gutierrez MA, *et al.*, 2014. Temporal changes in histomorphology and gene expression in goat testes during postnatal development. J Anim Sci 92:4440-8.

Ganguly I, Gaur GK, Kumar S, *et al.*, 2013. Differential expression of protamine 1 and 2 genes in mature spermatozoa of normal and motility impaired semen producing crossbred Frieswal (HF× Sahiwal) bulls. Res Vet Sci 94:256-62.

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.

Livak KJ and Schmittgen TD, 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods 25:402-8.

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.

Murtaza A, Khan MI-u-R, Ahmad W, *et al.*, 2019. Follicular dynamics and changes in plasma estradiol-17β and progesterone concentrations during estrous cycle in Beetal goats. Pak Vet J 39:193-8.

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 SK, Kumaresan A, Attupuram NM, *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.

Ramukhithi FV, Nephawe KA, Chokoe TC, *et al.*, 2017. Attainment of puberty in South African unimproved indigenous bucks. Small Rumin Res 153:57-61.

Suyadi S, 2012. Sexual behaviour and semen characteristics of young male Boer goats in tropical condition: a case in Indonesia. World Acad Sci Engineer Technol 6:388-91.

Zhao W, Li Z, Ping P, *et al.*, 2018. Outer dense fibers stabilize the axoneme to maintain sperm motility. J Cell Mol Med 22:1755-68.