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Effect of Various Collection Frequencies on the Quality of Kamori Goat Semen

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ABSTRACT

In present study the fresh semen of Kamori buck was evaluated to observe the effect of collection frequencies on its quality, thus a total of six healthy bucks of Kamori goat breed of age 6-8months purchased from local market were divided into three groups (A, B and C) 2bucks in each. The semen was collected from bucks of group A two times, group B four times and group C six times per week to find out the influence. The color of semen was noted as creamy white in semen of Kamori buck of both groups A and B while milky color group C. The volume of Kamori bucks semen was observed significantly high (P<0.05) in group A (1.3±0.030ml) than B (0.653±0.039ml) followed by group C (0.426±0.037ml). The pH of Kamori buck semen was found remarkably varied (P<0.05) among the groups of buck, average pH of Kamori buck semen was examined markedly lower (6.91±0.017) for group A compared to that of group B (7.13±0.018) and group C (7.22±0.028). The higher wave motion was observed in semen of bucks of group A in contrast to that of group B and group C. The motility rate recorded as 92.187±0.2, 68.640±1.07 and 57.281±1.37% in Kamori buck of group A, group B and group C, where the lowest rate (P<0.05) of sperms was in group C than group B and highest in group A. The groups of buck were noted significantly varied from each other for morphology and viability percent and this variation was observed in similar fashion as 92.812±0.2 and 92.800±0.22%, respectively for group A, 68.92±1.07 and 68.20±1.10%, respectively for group B and 58.260±1.41% and 56.552±1.37%, respectively for group C. The semen membrane integrity was noted significantly high (P<0.05) for group A (93.250±0.23%) moderate for group B (70.140±1.0%) the lowest for group C (59.145±1.38%). Significantly higher concentration of sperm (3.7950billion numbers/ml) was measured in semen of buck of group A compared to that of group B (2.3800 billion numbers/ml) and C (1.8550 billion numbers/ml).

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KAM: Conceived and designed the study, collected semen, analyzed data and wrote the manuscript; AAM: Assisted in study design and supervised laboratory procedures; AAM: Collected data and performed statistical analysis; NA: Participated in animal and sample handling; MA: Reviewed the manuscript and improved it technically; ASM: Supported laboratory analysis and data validation; MMR: Assisted in literature review and referencing; SHA: Supported data interpretation and manuscript revision; MAYR: Contributed to proofreading and final editing of the manuscript.

Key words
Kamori breed, Semen, Collection, Frequencies, Effect

INTRODUCTION

In Pakistan livestock is one of the largest sectors of agriculture, currently shares 60.1% in agriculture value and contributes 11.5% in national gross domestic products. The country has 80.3 million populations of goats (GOP, 2022) and it is third largest goat producing country after China and India. Primarily goat are kept for meat and milk purpose, its meat is tender, leaner and give preference over beef and chicken meat that easily available at considerable prized in Pakistan. Goats are the second most important milk producing animals after dairy cattle in both temperate and tropical region. The animals having small size, fewer months for maturing age (6-10) and less capital investment than cattle are raised as good source of incoming and employment (Kumbhar *et al*., 2016). There are 37 goat breed found in Pakistan among them 14 breeds are Pateri, Tapri or Lappi Barbari, Chapper or Kohistani or Jabli, Kamohari, Sindhi Desi, lehri, Barbri, Bugi Toori, Bujri, Jattan, Kacchan, Kurri, and Thari are found in Sindh

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province of Pakistan (Khan *et al*., 2018). In goat farming the buck selection is the starting point, male selection is even more relevant since a buck will produce thousands of children a year by artificial insemination (AI), and the selection of breeding males to enhance the genetic makeup is an important tool in reproduction (Bezjian *et al*., 2013). Breeding bucks of high genetic worth, propagates improvement at accelerated rate in goat herd (Farshad *et al*., 2009). Unfortunately, there is a intense shortage of bucks all over the nation, particularly in rural region where more than 80% of goats are raised by farmers. Due to shortage of breeding bucks available in the locality about 30 % of the female goat remain unmated (Manjusha *et al*., 2019). There are a few goat farmers who breeding bucks along with their female goat population in a flock. The genetic worth of those breeding bucks is however often very low or uncertain. In addition, use of same buck in breeding for a longer periods leads to inbreeding problems and reduces reproductive efficiency. The risk of infectious venereal diseases also increases (Husain, 2007). One more important factor that affects the quality of semen is frequently collection of semen. There is greater selection pressure on males in breeding than along the females because one male can be mate with numerous female. The goal is to mate with as many goats as possible in order to make the most of a good buck. Care must be required to ensure that increased use does not negatively impact buck semen qualities (Kaya *et al*., 2002). The most practical way is to obtain best semen quality and quantity by mange the frequency semen collection. Therefore, this study was design to evaluate the effect of various frequencies on the different quality characteristics of fresh semen of Kamori buck.

MATERIALS AND METHODS

Procurement and maintenance of bucks

A total of six healthy bucks of Kamori breed at the age of 6-8 months, were purchased from local market of Hyderabad, Sindh for the current study. They were kept in separated cage with sufficient ventilation at Sindh Agriculture University Tandojam's Faculty of Animal Husbandry and Veterinary Sciences Department of Animal Reproduction. Seasonal green grass, wheat grain 500g, wheat bran 250 g were offered to each buck, they were also allowed for 1hour grazing at evening and the water was available at *ad libitum* throughout the study period. The bucks were maintained and trained for semen collection at Livestock Farm Department of Animal Reproduction, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University Tandojam for 2 months.

Training of bucks and collection of semen

The bucks were trained between 6:00 am to 7:00 am in the morning, during a two-month period. During this training, bucks were encouraged to mount on some other bucks that were being utilized as dummy. In first month of training, the buck was just allowing to mount on other buck, the penis prepuce of bucks was checked by holding in hand for any abnormality. The 2nd month of training the penis of buck was inserted in artificial vagina with the help of hand during mounting.

The artificial vagina was washed and sterilized one night before semen collection and kept into the sterilizer. The temperature of artificial vagina was maintained at 42 -45 ºC by filling warm water, pressure of air maintained are 35 mmg and inner sleeved of artificial vagina was greased with petroleum jell.

The bucks were divided into three groups A, B and C two bucks in each group, where the semen was collected by artificial vagina method from bucks of group A, B and C with frequency of two times, 4 times and 6 times per week, respectively. Following methodology/ protocol was utilized for evaluation, extension and freezing of the semen.

Semen was collected early in the morning with the help of artificial vagina by mounting on another buck. Semen was collected 2 times from group A (only on Monday and Thursday), 4 times from group B (Monday to Thursday) and 6 times from group C (Monday to Saturday).

The evaluation of fresh semen

Immediately after collection, semen was taken into the lab and maintained in a water bath at 37 °C till further examination and following characteristics were observed with respective methods/techniques.

Mass activity: Evaluation of mass activity was performed by putting 15µl fresh semen on prewarmed (37ºC) slid and assess without cover slip under low magnification at 10X using phase contrast microscope. The grading of mass activity was done on the basis of wave pattern as follows:

Waves are absent, spermatozoa nonmotile 0; Waves are absent, spermatozoa motile, +; Scantily differentiable waves in motion, ++; Waves patent, moderate motion, +++; Dark, clear waves in fast motion, ++++.

Volume of semen: The glass graduated collection tubes was used to measure and record the semen volume in millilitres (Goswami *et al*., 2020).

Color of semen: The color of semen was determined by visual examination direct from collected tube and was categorized as white, milky, creamy, yellowish, watery and translucent as described by Hafez and Hafez (2000).

pH of semen: Semen pH were measured by using Digital pH metres (Eutech).

Sperm motility: A drop of the diluted semen was put on a previously heated at glass slide (37ºC) and was covered with a cover slip after being diluted with normal saline at a 1:100 ratio. The sperm motility was observed under phase-contrast microscope (40 ×) (Iqbal *et al*., 2015). For motility, 100 spermatozoa were counted randomly moving in forward direction and result were expressed in motility percentage.

Morphology and viability of sperm: On a prewarmed glass slide, a 10µL of dilute sample taken was mixed with two to three drops of eosin nigrosin stain. After 3 min at 37°C, another slide was used to create a thin smear. A lot of the stain was rinsed with water, and then the water was removed by immersing it in ethnol. Under a phase contrast microscope, a dried film was analysed for living dead organisms and morphology (40x). Sperm without stain penetration were regarded as live (Ahmad *et al*., 2011). The composition of used eosin nigrosine stain was eosin 0.67g, nigrosine 5.0g and distilled water 100ml.

Plasma membrane integrity: Hypo-osmotic swelling test (HOST) solution (with composition of fructose 1.35 g, Tris sodium citrate 0.73 g and distilled water 100 ml) To evaluate the integrity of the plasma membrane, the hypo-osmotic swelling test (HOST) was carried out. One ml of the hypo-osmotic solution was incubated with a 100µL sample of semen for an hour at 37 °C. Single drops of the incubated semen were placed on prewarmed glass slid, then examined using a phase-contrast microscope at 40X. 100 spermatozoa were counted, swelling of spermatozoa in response to HOST solution was considered as normal (Ijaz *et al*., 2009).

Concentration of sperm: Concentration of spermatozoa was determined by using hemocytometer as described by Farshad *et al*. (2009) fixing solution. The fixing solution was prepared with 30ml of sodium chloride (3%), 4ml of Formaldehyde (37%) and 1000 ml of distilled water. 9.99 milliliters of fixing solution and 0.01 ml of diluting semen were taken in glass tube, allowed for five min of fixation time. A drop of solution was placed onto each of the hemocytometer's two chambers, covered with a cover slip, and viewed under phase-contrast microscope at 40X. Sperm were counted in 5 squares: 1 central square, 4 corner squares, and 2 other squares. The number of cells was calculated using the formula below.

Sperm cell/ml = n×5×df×10×1000

Where N is number of sperm counted, 5 is number of chamber counted on hemocytometer, df is degree of free dome that equal to 100, 10 is depth of the counting chamber, and 1000 is unit conversion µL to ml.

RESULTS AND DISCUSSION

Macroscopic examination of semen

Table I shows that the color of semen was observed as creamy white in Kamori buck of both Groups A and B but it found milky in color when semen was collected with 6 frequencies of ejaculation (Group C). Sancho *et al*. (2004) are also agreed with these results, who found that the frequency of ejaculations affected the semen color of the West African dwarf goats. They further stated that the color of semen was either creamy or milky. Similar observations were reported by Shamsuddin *et al*. (2000). Okere (1986) stated that the color of semen significantly

Table I. Effect of various frequencies on the macroscopic quality and microscopic parameters of Kamori buck semen.

Quality parameters	Different groups of buck			LSD (0.05) SE±
	Group A	Group B	Group C	
Macroscopic features				
Color	Creamy white	Creamy white	Milky	-
Volume (ml)	1.3±0.03[a]	0.65±0.04[b]	0.43±0.04[c]	0.0449, 0.0226
pH	6.90±0.02	7.12±0.02	7.22±0.03	0.0703, 0.0163
Microscopic parameters				
Wave motion	++++	+++	+++	-
Motility (%)	92.18±0.21[a]	68.64±1.07[b]	57.28±1.37[c]	2.7403, 0.6369
Morphology (%)	92.81±0.20[a]	68.92±1.08[b]	58.26±1.42[c]	2.8897, 0.6716
Viability (%)	92.8±0.22[a]	68.20±1.12[b]	56.55±1.37[c]	4.1516, 0.9649
Membrane integrity (%)	91.25±0.23[a]	66.14±1.01[b]	54.14±1.39[c]	3.3737, 0.7841
Spermatozoa concentration (Billion No./ml)	3.97±0.004[a]	2.38±0.09[b]	1.86±0.07[c]	0.2130, 0.0495

[a, b,c] value with different superscripts within a row shown significant difference at P<0.05. Group A, semen collected 2 times a week; Group B = semen collected 4 times a week; Group C = semen collected 6 times a week.

differed when collected from Buck at week 1, 2 and 3. It has been reported that the semen color in ruminant species is generally related to the concentration of sperm (Sancho *et al*., 2004).

The volume of the semen was markedly high (P<0.05) in group A (1.3±0.030ml), as compared to bucks from group B (0.653±0.039ml) and group C (0.426±0.037ml). Volume of semen is important variable in reproductive performance and evaluation of semen (Ax *et al*., 2000). Findings of current experiment show that the semen volume of Kamori bucks was significantly (P<0.05) affected by more number of ejaculations per week. The semen volume was significantly dropped when the ejaculation was performed six times per week followed by four times and two times ejaculation frequencies. These results are in line with the observations of Ritar *et al*. (1992). They found decline in semen volume to a greater extent by successive ejaculates. Further, Kaya *et al*. (2002) stated that significant lower volume at increased per week semen collection frequency was noted. While Bingol *et al*. (2006) reported that semen volume dropped over the days and ejaculate number on the similar day. Thwaites (1995) reported that the decrease in volume of semen from 25 to 53perecnt observed by minimized interval and maximized frequency of collections.

Table I shows the effect of ejaculations frequencies per week on the pH value of semen. The mean value of pH for semen of Kamori buck was examined as 6.91±0.017 at frequency of 2 times per week (group A) which was found significantly lower in group B (7.13±0.018) and group C (7.22±0.028). This range of pH had also been reported by Al-Samarrae (2009) i.e. 6.9 to 7.2 but Madhuri *et al*. (2012) stated that the good quality semen is always slightly acidic in nature. Noteworthy, other study recorded the range of pH value from 6.4 to 6.9 of semen of West African dwarf buck reported by Okere *et al*. (1986) who investigated the effect of frequent ejaculation on the semen characteristics. Aisen and Venturino (2008) found a similar result, observing pH 6.8±0.2 for the first ejaculation and reported that increase in the frequency of semen collection cause a rise in the pH of semen.

Microscopic examinations of semen

The results of current study presented in Table I show the wave motion of the semen collected from Kamori bucks affected by more collection frequencies. The higher wave motion was observed in semen group A than that of group B, group C. Simultaneously in different studies reported by various researchers (Shamsuddin *et al*., 2000; Sancho *et al*., 2004; Bingol *et al*., 2006) the decreased waves motion was observed when the frequency of goat semen collection increased per week in goats. The mass activity of semen is markedly affected by increased ejaculations per week (Thwaites, 1995; Kaya *et al*., 2002). The motility percentage (Table I) was observed 92.187±0.2, 68.640±1.07 and 57.281±1.37% in Kamori buck semen group A, B and C. The lowest motility rate (P<0.05) of sperms was observed in group C than that of group B followed by group A. These findings of current study are in line with the results of Watson (1990) who observed that sperm motility was affected by collection interval because of the prolonged collection interval caused spermatozoa in the epididymis to degenerate resulting in lower sperm quality. According to Kaya *et al*. (2002) when an increase in ejaculation number occurred, the decrease in spermatozoa motility in German Mutton Merino x Native Akkaraman rams was found. However, Gubartalla (1998) stated that more frequency of semen collections markedly diminished the individual motility.

The Kamori buck semen morphology 92.812±0.2% was found with two times ejaculation, 68.921±1.07% with four times and 58.260±1.41% with six times ejaculation frequency per week. Other studies for instance conducted by Kaya *et al*. (2002) and Gubartalla (1998) also reported that the more ejaculation frequency significantly decreased the percent of normal sperm morphology. It has been reported that the grater ejaculation frequency caused markedly increase in abnormal spermatozoa (Sancho *et al*., 2004), had negative effect on abnormal spermatozoa (Khalifa *et al*., 2010) and/or rapid release of immature spermatozoa and fertility (Oyeyemi and Akusu, 1998). Nevertheless, Yotov *et al*. (2011) discovered that the highest survival rate of Pleven black head ram sperm was found in the second ejaculate. Further Table I indicates that the viability of Kamori buck semen was found significantly high (92.800±0.22%) when semen collected two times a week followed by semen collected four times (68.203±1.10%) and six times (56.552±1.37%) per week. This reduction in viability observed because of the more time semen collection consequences significant reduction in the rate of live spermatozoa and also an increase in the abnormalities (Gubartalla, 1998).

In present study the results regarding the semen membrane integrity of Kamori buck was noted significantly different (P<0.05) for ejaculation frequencies. The maximum semen membrane integrity was recorded as 93.250±0.23% when semen collected 2 times per week (group A), while it found intermediate (70.140±1.0%) in semen collected 4times per week (group B) and significantly lowest was observed (59.145±1.38%) when collected six times per week (group C). It is of interest to note that the study reported by Ari *et al*. (2011) reveals that membrane integrity reduced at fourday intervals as opposed to one day interval of semen collection in

tushin rams. This significant reduction in the membrane integrity had also been observed by Kaya *et al*. (2002) with increased semen collection. While Oliveira *et al*. (2012) stated that the ejaculation repetition did not harm the plasma membrane but rather factors related to animal individuality.

The sperm concentration in semen of Kamori bucks was recorded remarkably different ($P<0.05$) among various ejaculation frequencies (Table I). Significantly higher concentration of sperm (3.7950 billion numbers/ml) was measured in semen collected two times per week followed by four times ejaculation frequency (2.3800 billion numbers/ml) and in semen collected six times ejaculation frequency (1.8550 billion numbers/ml). These findings of current research regarding the sperm concentration of Kamori buck semen collected with various frequencies are also in consistent with many other studies reported by various authors, who stated that the sperm concentration found to be significantly reduced by the increase semen collections and/or ejaculations (Shamsuddin *et al*., 2000; Kaya *et al*., 2002; Gundogan, 2007; Yotov *et al*., 2011; Khalifa *et al*., 2010). However, according to Leon *et al*. (1991) and Sharma *et al*. (1991) the sperm concentration may vary depending on collection frequency. It might be due to the decrease in the viability, sperm motility and increase in the abnormal morphology rate of semen caused the more collection frequencies.

CONCLUSIONS

It could be concluded that the color of Kamori buck semen found creamy white both Groups A and B it was changed to milky in Group C. The increase in semen collection frequencies (group B and group C) had significant effect on the volume, pH, wave motion, motility, viability, morphology, membrane integrity and sperm concentration of Kamori bucks semen and these characteristics except pH found markedly reduced in contrast to semen collection with frequency of 2time per week (group A). In case of pH significant increase was observed by increase semen collection frequency in Kamori buck. Further it is suggested that the semen should be collected from buck twice a week to ensure the acceptable fertility rate.

DECLARATIONS

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IRB approval

The study was approved by the Institutional Review Board (IRB) of Sindh Agriculture University, Tandojam.

Ethical statement

All procedures involving animals were conducted in accordance with the ethical standards of Sindh Agriculture University, Tandojam. Proper animal welfare and handling protocols were strictly followed during the study.

Generative AI and AI-assisted technology statement

No generative AI or AI-assisted tools were used in the generation of data, analysis, or preparation of results in this study. AI assistance was used only for English language editing after the completion of the manuscript.

Statement of conflict of interest

The authors have declared no conflict of interest.

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