International Journal of Veterinary Sciences and Animal Husbandry

**ISSN:** 2456-2912
VET 2023; 8(4): 37-41

www.veterinarypaper.com
Received: 08-04-2023
Accepted: 17-05-2023

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# Preservation of Konkan Kanyal buck semen at refrigeration temperature

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**DOI:** https://doi.org/10.22271/veterinary.2023.v8.i4a.567

**Abstract**
Semen evaluation is essential to evaluate the buck potential of fertility before it is used to produce semen and perform artificial insemination. Therefore present study was carried out to investigate the preservation of the Konkan Kanyal buck semen at refrigeration temperature in egg yolk extender. Total seven semen samples, were collected from Konkan Kanyal bucks using an artificial vagina method. The collected semen sample was evaluated for macroscopic, microscopic and sperm function tests. For dilution 20% egg yolk extenders were used and the diluted semen samples were stored at 4°C, for 4, 12, 24, 48, and 72 hours. The post-preservation evaluation semen and CASA parameters revealed a decline in semen parameters with longer storage times. From the present study, it was concluded that, Tris egg yolk extender is a suitable dilutor to preserved Konkan Kanyal buck semen at acceptable range for Artificial Insemination (AI) upto 72 hours of refrigeration temperature.

**Keywords:** Buck semen, Tris, Egg yolk, Refrigeration temperature, CASA

## 1. Introduction
The goat plays an important role in the country's rural economy as a source of meat, milk, skin, hair, wool, and manure. It commonly known as "poor man's cow," became a significant source of income for poor people due to its low input, high prolificacy, short generation interval, and ease of marketing. The Konkan Kanyal goat is a meat-type breed with a twinning percentage of about 66% that is adapted to the Konkan region of Maharashtra's high rainfall, hot, and humid climate. In May 2012, the NBAGR recognized it as a distinct breed. Not all goat breeds can survive in areas with heavy rainfall, but this unique population of goats, produced by Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth (DBSKKV), Dapoli, and Dist. Ratnagiri of Maharashtra, through intensive selection, survives well and performs effectively in such environments on its native tract. The Maharashtra State's Goat Breeding Policy recently included the Konkan Kanyal goat breed. The habitat of Kanyal goats is located in the Sindhudurg district in the Konkan region, between 15°37′ and 16°40′ North latitude and 73°19′ and 74°18′ East longitude.
The main problem in any livestock production including goats, is scarcity of good breeding stock which is important to propagate the particular species. The key method for genetic enhancement is the selection of breeding males, which is considerably more essential because a buck can produce thousands of kids each year through artificial insemination (AI) [1]. In order to determine a buck's capacity for reproduction, breeding bucks must be evaluated based on the quality of their semen. To fertilize large numbers of does with semen of outstanding bucks is achieved by preservation of semen at a liquid unfrozen state using reduced temperatures or by another method in a frozen state which involved preservation at sub-zero temperatures.
The use of appropriate extenders to preserve semen is essential for the success of AI in goat reproduction. It increases the volume of semen, allowing for more insemination per ejaculation [2]. It extends the life span of spermatozoa without compromising productivity and also protects the spermatozoa against cryogenic damage by providing sufficient pH, buffering capacity, and osmolality.

Tris-citric acid acts as a superior diluent for goat spermatozoa and provides the best buffering mechanism Currently, egg-based extender is extensively used for semen extension and storage because of it helps to keep sperm motility and lifespan, as well as the integrity of the mitochondrial and acrosomal membranes, during physical and chemical stress [3] because of its low-density lipoprotein (LDL), which protects the sperm phospholipids during cryopreservation [4]. Keeping in view the present study has been planned with the objectives of evaluation of post-preserved quality semen samples of Konkan Kanyal buck at various time intervals.

## 2. Materials and Methods

The present study was carried out in the Department of Animal Reproduction Gynaecology and Obstetrics, Mumbai Veterinary College, Mumbai, Maharashtra (India) from August 2022 to February 2023. In the present study, seven Konkan Kanyal breed bucks were chosen from the Instructional Livestock Farm Complex, Mumbai Veterinary College, Goregaon (E) Mumbai, and the livestock owner of Raigad district for this study. All selected bucks were mature and healthy. They were dewormed, and vaccinated as per standard schedule. Semen samples were collected from all selected bucks using the Artificial Vagina method in an aseptic and hygienic manner and kept in a water bath at 37 $^0$C until analysis (Fig 1). Immediately after collection, the macroscopic and microscopic examination of the neat semen sample were done as described by [5]. The semen sample was diluted with Tris containing 20% egg yolk (TEY) at 1:10 dilution [6]. After dilution, the diluted semen is kept in vials at 4 $^0$C in refrigeration temperature to equilibrate for 4 h. The equilibrated semen sample was thawed and evaluated for semen parameters and results were noted to know the short-term storage quality of semen sample. The diluted samples in other vials were stored at 4 $^o$C in refrigeration and post-thaw evaluation of various semen parameters were done at 12, 24, 48 and 72 hours of preservation.

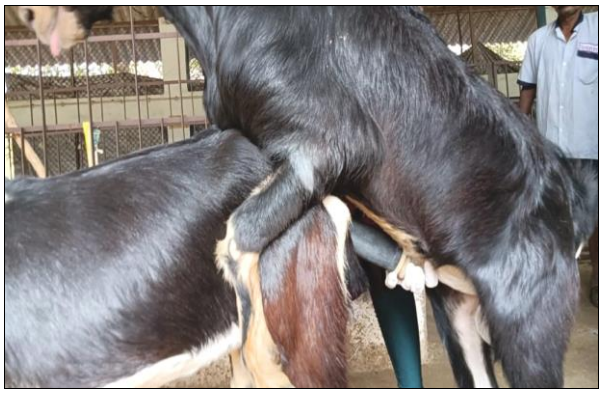

**Fig 1:** Semen collection by artificial vagina method from Konkan Kanyal buck

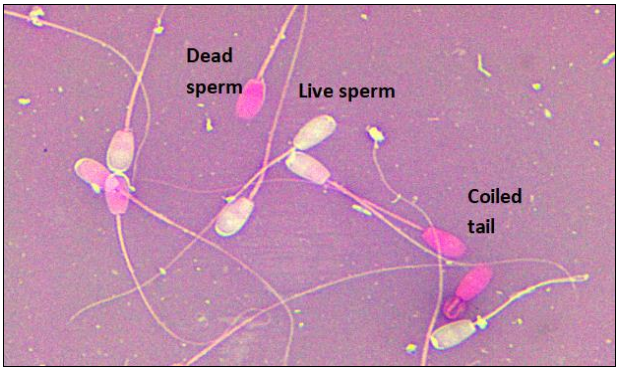


**Fig 2:** Live and dead sperm, tightly coiled sperm tail

The sperm motility was recorded from 0 to 100 based on the percentage of progressively straightforward motile sperm [5]. The nigrosin–eosin stain method was used to assess the viability of spermatozoa in samples. Sperm with partial or complete purple staining are considered as dead and those with strict stain exclusion are considered as alive (Fig 2) [7]. The smears prepared for live sperms were used again for the determination of abnormal sperms. Sperms defects such as abnormalities of the head, middle piece and tail (Fig 2) [8].

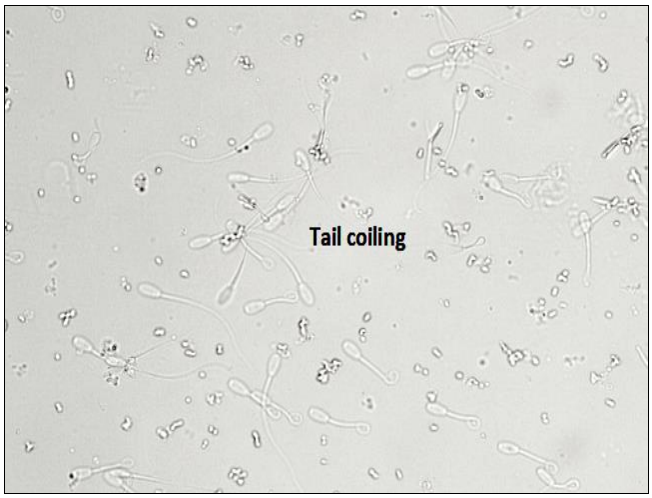


**Fig 3:** Sperm tail coiling in hypo-osmatic swelling test

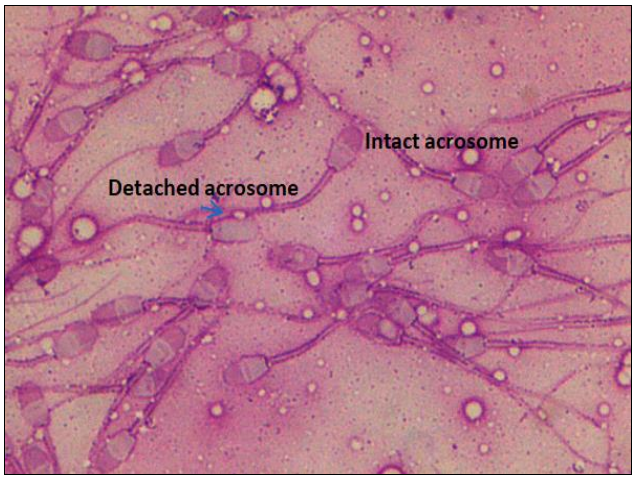


**Fig 4:** Intact and non-intact acrosomal of sperm

The functional integrity of the sperm plasma membrane was studied as per the method described by Revell and Mrode (1994) [9] using a Hypo-Osmotic solution. For each sample, the percentage of spermatozoa with tail coiling (HOST+ve) was recorded as intact plasma membrane functionality (Fig 3). The acrosomal membrane integrity of spermatozoa was determined by using Giemsa stain suggested by Hancock, (1951) [10]. Spermatozoa with a purple-stained head were counted as intact acrosomes, whereas those with a white-stained head were counted as non-intact spermatozoa (Fig 4) [11]. The sperm motility and motion parameter (Fig 6) including Total motility (TM), Progressive motility (PM), Average Path velocity (VAP), Straight Line velocity (VSL), Curvilinear velocity (VCL), Amplitude of lateral head displacement (ALH), Beat/Cross frequency (BCF), Straightness (STR), Linearity (LIN) was carried out by microscopically and by Computer Assisted Semen Analysis (CASA; Hamilton-Thorne, IVOS, Beverly MA) at a different time interval at 12, 24, 48 and 72 hours of preservation (Fig

5). The results were presented as Means ± SD. Statistical differences in semen parameters were evaluated via t-test and completely random design in Sigma Plot 15.0 online software $p<0.05$ was regarded as statistically significant in each case.

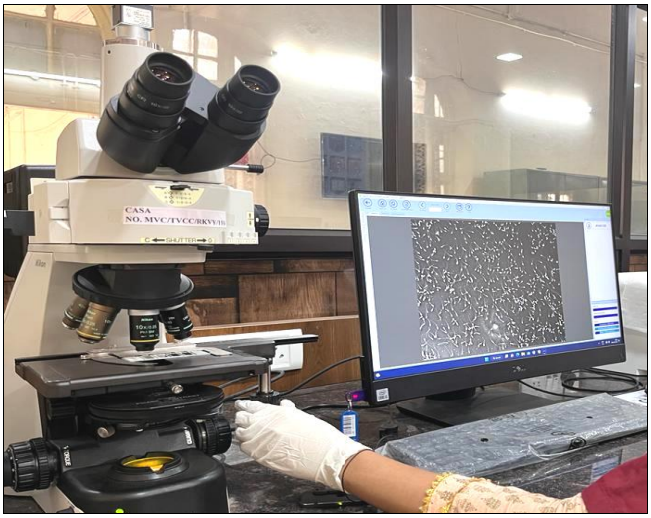

**Fig 5:** Evaluation of extended semen by CASA

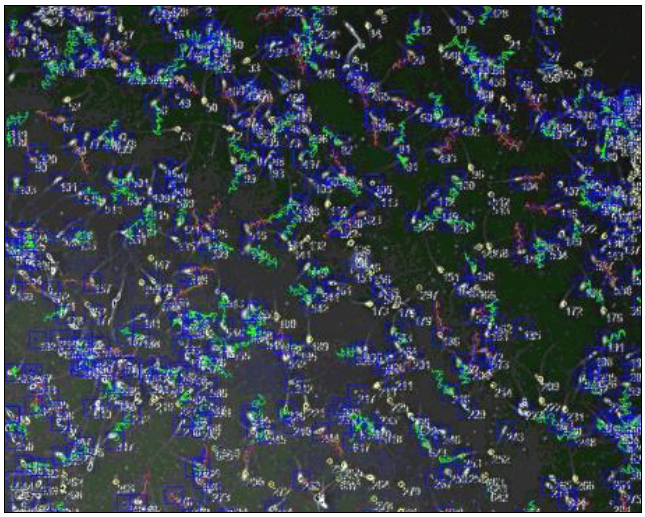

**Fig 6:** CASA motility and motion analysis

## 3. Results

**Effect of Tris egg yolk extender on sperm parameters for short-term storage at refrigeration temperature.**

Table 1 contains the findings of short-term storage of semen quality. It was observed that all semen parameters showed significant differences ($p<0.05$) except a non-significant difference was noted in the motility percentages after short-term storage. All the semen parameters were within the acceptable limits required for AI in goats.

**Table 1:** Comparison of several parameters before and after dilution

| Parameters | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Motility (%) | | Live (%) | | Morphological abnormal (%) | | Plasma membrane integrity (%) | | Acrosomal integrity (%) | |
| Before | After | Before | After | Before | After | Before | After | Before | After |
| 87.86±1.01 | 84.29±1.70 | 82.95±0.77 | 78.9±1.30* | 3.14±0.22 | 4.71±0.61* | 84.74±0.41 | 79.8±0.70* | 97.78±0.29 | 96.5±0.17* |

Values in rows with superscript* are statistically different ($p<0.05$).

Table 2, showed that the post-preservation sperm motility percentage was non-significant ($p>0.05$) up to 48 hours while motility significantly ($p<0.05$) declined from 48 to 72 hours. The live sperm percentage non-significantly declined ($p>0.05$) for the first 24 hours, but after 24 hours live sperm percentages highly decline. The abnormal sperm percentage revealed non-significant differences ($p>0.05$) for 12 to 24 hours and 24 to 48 hours of preservation. The plasma membrane integrity percentage showed a significant difference ($p<0.05$) at 12 to 24 and 48 to 72 hours while a non-significant difference was noted between 24 to 48 hours. The acrosomal sperm percentage showed a non-significant difference up to 24 hours while a significant increase ($p<0.05$) was noted from 24 to 72 hours.

**Table 2:** sperm parameters in TEY extenders preserved at various time intervals

| Parameters | Hours of preservation | | | |
|---|---|---|---|---|
| | 12 h | 24 h | 48 h | 72 h |
| Motility (%) | 82.14±2.64$^{d}$ | 75.71±2.77$^{de}$ | 67.14±2.14$^{e}$ | 47.86±4.21$^{f}$ |
| Live (%) | 74.52±1.42$^{d}$ | 70.56±1.45$^{d}$ | 63.17±1.00$^{e}$ | 52.78±2.19$^{f}$ |
| Morphological abnormal (%) | 5.86±0.88$^{f}$ | 7.00±0.85$^{ef}$ | 8.86±1.14$^{de}$ | 11.14±0.96 |
| Plasma membrane integrity (%) | 74.24±1.25$^{d}$ | 66.42±1.08$^{e}$ | 59.90±1.74$^{f}$ | 50.06±2.58$^{g}$ |
| Acrosomal integrity (%) | 94.78±0.63$^{d}$ | 88.92±1.12$^{e}$ | 82.02±2.11$^{f}$ | 75.01±2.45 |

Values in the same row with super script $^{d, e, f, g}$ show statistically significant differences ($p<0.05$)

Table 3 showed the CASA parameters, the TM percentages were significantly reduced from 12 to 72 h. PM and LIN percentages were significantly reduced from 12 to 48 h after that the reduction was non-significant. VAP, VCL and ALH were significantly reduced up to 2 4h and non-significant reduction was noted up to 72 h. VSL and BCF were showed non-significant reduction after 24 h up to 72 h.

**Table 3:** Effect of seminal plasma on CASA sperm motility parameters preserved at various time intervals

| Parameters | Hours of preservation | | | |
|---|---|---|---|---|
| | 12 h | 24 h | 48 h | 72 h |
| TM (%) | 90.26±1.90$^{a}$ | 80.24±1.99$^{b}$ | 68.84±2.06$^{c}$ | 44.47±2.60$^{d}$ |
| PM (%) | 42.56±7.58$^{a}$ | 25.66±3.36$^{b}$ | 21.09±4.05$^{bc}$ | 10.52±3.42$^{c}$ |
| VAP (µm/s) | 41.68±5.94$^{a}$ | 30.04±1.76$^{ab}$ | 28.63±3.99$^{b}$ | 21.33±4.14$^{b}$ |
| VSL (µm/s) | 28.06±4.04$^{a}$ | 20.00±2.22$^{b}$ | 18.33±2.31$^{b}$ | 13.70±1.94$^{b}$ |
| VCL (µm/s) | 77.39±11.29$^{a}$ | 57.57±3.83$^{ab}$ | 54.42±4.84$^{b}$ | 44.60±8.90$^{b}$ |
| ALH (µm/s) | 2.31±0.20$^{a}$ | 1.93±0.12$^{ab}$ | 1.60±0.12$^{b}$ | 1.42±0.23$^{b}$ |
| BCF (Hz) | 8.80±0.97$^{a}$ | 7.07±0.84$^{a}$ | 6.37±1.01$^{ab}$ | 4.42±0.42$^{b}$ |
| STR (%) | 54.66±1.74$^{a}$ | 52.11±1.57$^{a}$ | 49.81±2.11$^{a}$ | 50.08±5.17$^{a}$ |
| LIN (%) | 31.25±1.49$^{a}$ | 28.91±1.50$^{ab}$ | 26.57±1.12$^{bc}$ | 24.90±1.05$^{c}$ |

Values in the same rows with superscripts $^{a, b, c, and\ d}$ show statistically significant differences ($p<0.05$)

## 4. Discussion

Assessment of semen for a variety of parameters aids in identifying and eradicating cases of subfertility or apparent male infertility [12]. Semen dilution is performed to extend sperm lifespan and permit more accurate sperm quality assessments, semen dilution is a routinely used procedure in

many species. Due to the commercial use of cool-shipped and frozen-thawed sperm, dilution is particularly essential for buck semen storage. Most goat farms are situated in rural locations, far from the laboratory, or in regions with adverse weather conditions. In goat practice, most of the time semen is collected on the farm, and evaluation is done at a laboratory that is far away from the goat farm. For this reason, semen must be kept in storage for a short or moderate duration. Furthermore, to cryopreserve semen from extremely valuable bucks, short-term semen storage is necessary for farms. Therefore determining the impact of different extenders on semen parameters before and after semen dilution for a short period was the purpose of the current study.

Table 1 indicated that the sperm motility, live sperm percentages, morphological sperm abnormalities, sperm plasma membrane integrity, and acrosomal integrity sperm percentages declined following dilution when a neat semen sample is chilled at 4°C and preserved up to 4 hours. But preserved the semen parameters within the acceptable percentages for that specific semen parameter. Similar findings were reported by [13]. The "dilution effect" may be responsible for all semen parameters dropping following after dilution. A considerable reduction in sperm motility as a consequence of semen dilution. Many species (rabbit, ram, bull, boar, dog, stallion, and human) have been reported to exhibit the "dilution effect". When neat semen is diluted, along with the dilution effect, the neat semen during the cooling phase there is damage to the phospholipid bilayer in the plasma membrane of spermatozoa there is loss of selective permeability, extensive rearrangements, and leakage of the sperm plasma

The motility, live sperm percentage, abnormal sperm percentage, plasma membrane integrity and acrosomal integrity were maintained for up to 72 hours in the TEY extender within the normal range. The present observation was supported by [15], they reported that the motility was higher and steady over time for semen samples stored at 5 °C, but later there is a distinct drop over the period of time. The processing of the semen also leads to fluctuations in temperature, pressure, osmolality, and pH which can damage seminal plasma membranes and limit the fertilizing life of the processed spermatozoa [16]. There was a difference between the present observations and the observations recorded by other scientists may be due to different egg yolk concentrations [17], season [18], breed [19], individuals [20] and interaction between extender and temperature [15].

The results of the CASA study showed that the motility and various motion parameters were reduced with increasing duration of storage, the similar findings were also reported by [21]. The variation in spermatozoa's velocities may be due to spermatozoa's lower resistance to hypotonic stress, as well as changes to intracellular pH and potassium levels that alter mitochondrial activity and cell physiology [22].

At the time lowering the temperature from 20 to 5 °C, resulting in cold shock and there is the ageing of spermatozoa during storage. These are the main cause of changes that result in sperm membrane injury in processed spermatozoa. The phase transition of membrane lipids, which causes phase separation and a loss of selective permeability, is likely responsible for cold shock. The ratio of unsaturated: saturated fatty acids in the phospholipids and a low cholesterol content, which results in a less stable sperm membrane, are associated with the sensitivity to cold shock in cold shock-sensitive spermatozoa, which causes them to lose plasma membrane phospholipids.

The beneficial effect of TEY extender might be due to egg yolk, which contains phospholipids and lecithin, could potentially protect the sperm membrane from cold shock when added to extenders [15] and also gives the more stability to plasma membrane which improves the velocity characteristics, and preserving cell viability for longer periods [23].

## 5. Conclusion

From the present study, it may concluded that Konkan Kanyal bucks semen may be preserved for a short time (4hrs.) as well as upto at 72 h at refrigeration temperature without affecting the normal acceptable range limit for AI in goats.

## 6. Acknowledgements

For providing animals to conduct the study, the authors are grateful to the Instructional Livestock Farm Complex, Mumbai Veterinary College, Goregaon Mumbai, and the livestock owner of Raigad district.

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