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*Original Article*

# Assessment of Sperm Damage with use of Unconventional Practices for Freeze Thawing During Artificial Insemination in Goat

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**Abstract**

A study was conducted to evaluate the effect of different unconventional thawing procedure practised in field for artificial insemination in goat. 10 healthy Barbari bucks routinely used for the semen collection at frozen goat semen production station were selected for the experiment. 15 cryopreserved semen doses (French mini, 0.25ml capacity) of each individual buck from single ejaculate were selected. Frozen semen doses from individual buck were divided into five different group each with three straws. The straws which were frozen thawed using thawing unit were marked as positive control (C) while the straws of other respective group were frozen thawed using different thawing procedure practiced in field viz. pocket, underarm, palm and in water maintained at 37°C using thermometer. All the 3 frozen thaw straws of each group using different thawing process and later evaluated for semen quality. Significantly higher values for vitality, mitochondrial activity, motility and path velocity were observed in ideal thawing followed by thawing in water maintained at 37°C using thermometer, underarm, pocket and palm thawing. A significantly lower values for ROS+ve spermatozoa and those with the acrosomal damage were observed in ideal thawing followed by other thawing procedures. It may be concluded that thawing unit is ideal for thawing but under unavailability of thawing unit thawing procedure should be followed in water maintained at 37°C using thermometer.

**Keywords:** Goat Thawing; Semen; Seminal attributes.

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## INTRODUCTION

Thawing process is considered as main prerequisite to determine the post thaw semen quality in domestic animal. Thawing involves sequential retrieval of metabolic process by subjecting spermatozoa to a specific temperature for appropriate time (*Yánez-Ortiz et al., 2022*). Any disturbance in the time dependent temperature exposure may alter the cell physiology reflected through change in the sperm charters (*Anand et al., 2017*). Strengthening the structural integrity

of sperm cell to overcome thawing stress has beena challenge. Researches have been conducted to improve the animal health, reproductive performance through supplementation of micro and macro minerals in diet and semen extender for strengthening membrane stabilization of spermatozoa to withstand freeze thawing stress (*Mittal et al., 2014, Keshri et al., 2022, Singh et al., 2019*). Despite this, thawing process is still considered as one of the most important factors in determining the post thaw semen quality. In India, with immense pressure of AI and limited infrastructure especially availability of thawing units, different unconventional thawing procedure are being practices, that are identified as major factor responsible for loss in post thaw semen quality and poor conception. The AI workers are bound to undergo thawing using unconventional methods viz. Pocket thawing, palm thawing, underarm thawing and thawing in water maintained at 37°C through thermometer under fields condition.

Since goat AI has recently been introduced in Indian animal husbandry system, it requires better outcome in terms of conception rates for its wider acceptance among farmer community. Since the sperm membrane composition, its response to environmental stressor and degree of losses incurred vary from species to species (*Donkin* and *Berrie., 2018*), it become important to evaluate the degree of loss incurred goat semen with use of different unconventional thawing procedure practiced in field. So, taking into account the relevance of thawing in determining the post thaw sperm health and to develop an understanding about the extent of loss incurred in semen quality with unconventional thawing procedures, the study was designed to evaluate the effect of different thawing practices on the post thaw semen quality in goat.

## MATERIALS AND METHODS

The experiment was conducted at Buck frozen semen station, Department of Veterinary Physiology, College of Veterinary Sciences and Animal Husbandry, DUVASU, Mathura, Uttar Pradesh. Ten healthy Barbaribucks routinely used for the semen collection at goat semen production station were selected for the experiment. 15 cryopreserved semen doses (French mini, 0.25 ml capacity) of each individual buck from single ejaculate were selected. Frozen semen doses from individual buck were divided into five different group each with three straws. The straws which were frozen thawed using thawing unit were marked as positive control (C) while the straws of other respective group were frozen thaw using different thawing procedure practiced in field viz. T-1 in pocket, T-2 underarm, T-3 palm and T-4 in water maintained at 37°C using thermometer. All the 3 frozen thaw straws of each group using different protocol thawing were transferred to 2 ml conical plastic tube maintained at 37°C in dry bath to reduce variation. The sample were later diluted 3 times using semen diluter to reduce the concentration from $400x10^6$ to $100 x10^6$ per ml. The diluted semen was subjected to analysis for different seminal attributes. Viability, mitochondrial potential, ROS affected spermatozoa and acrosomal integrity was evaluated through flow cytometer using easy kit (IMV, France). Computer assisted semen analyzer was utilized to evaluated the path velocities and sperm kinematic. To evaluate physical seminal attribute through flow cytometer, 10 µl of semen sample was diluted in 90 µl of EBB to prepare stock solution of sample containing 10 million sperm per ml. The stock sample solution prepared was utilized to evaluate the different parameters using specially designed easy kit for each parameter and subjected to analysisunder flow cytometer. To evaluate the effect of thawing practices on sperm kinematic and path velocities, 10 µl of stock solution was placed on 8 chambered lieza slide for even distribution of sperm cell within the field. The slide was placed on the worm stage and evaluated under 10X using CASA with specific software. Data generated was statistically analysed using using SPSS 21.0 computer program package (SPSS, USA).

## RESULT

The effect of different thawing practices on the sperm character were evaluated and compared with the ideal thawing protocol. The different seminal attributes exhibited by spermatozoa has been presented in Fig. 1. A significantly higher values for viability, mitochondrial activity was observed in ideal thawing followed thawing in water maintained at 37°C by thermometer, compared to other thawing procedures. The value for viable ROS +ve spermatozoa and % viable sperm with disrupted acrosome exhibited significantly higher values in pocket, underarm and palm thawing followed by thawing in water maintained at 37°C by thermometer and ideal thawing. The values recorded for motility and path velocity of sperm after thawing have been presented in Table 1. The

total motile spermatozoa were significantly lower in all the other thawing compared to ideal thawing procedure. Although comparable values were recorded in the thawing done in water maintained at 37°C by thermometer. The values for path velocity and kinematic characters that includes curvilinear velocity (VCL, µm/sec) (average path velocity (VAP, µm/sec), straight line velocity (VSL, µm/sec), Linearity (Lin%), Straightness (Str%), Wobble (WOB%), beat cross frequency (BCF%) and maximum amplitude lateral head displacement (ALH, µm) also exhibited a similar trend with higher values in ideal thawing followed by thawing in water maintained at 37°C, underarm, pocket and

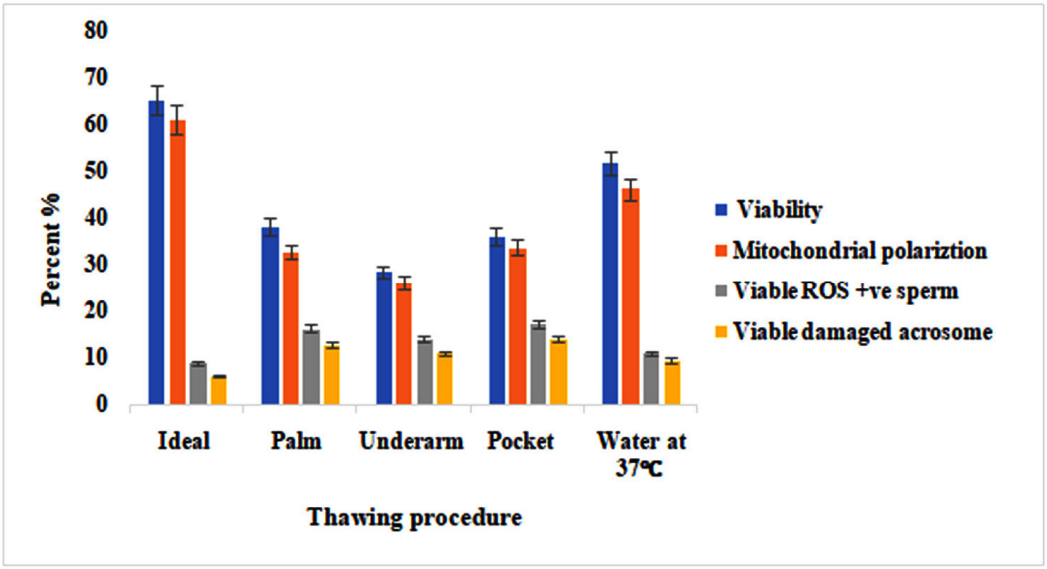


**Fig. 1:** Seminal attributes exhibited by frozen thawed spermatozoa subjected to different thawing procedure

**Table 1:** Effect of different thawing procedure on motility and path velocity of spermatozoa

| Thawing Process/ Motility Characters | Ideal Thawing | Palm thawing | Underarm Thawing | Pocket thawing | Water at 37 |
|---|---|---|---|---|---|
| Total motile | 59.22 ±3.17a | 28.84±2.36c | 32.11±2.11c | 29.41±0.94c | 48.50±1.43ab |
| Rapid progressive | 41.853±2.43a | 18.235±1.17c | 21.754±2.83c | 16.568±3.07c | 30.015±2.32b |
| VCL (µm / sec) | 75.25±5.05ab | 53.50±8.90bc | 51.25±10.71bc | 49.75±7.49bc | 65.5±4.41ab |
| VAP (µm / sec) | 50.5±8.88ab | 33.25±6.46b | 44.75±5.08b | 36.25±3.20b | 40.0±6.78ab |
| VSL (µm / sec) | 33.00±8.04ab | 31.0±8.04a | 46.00±6.63b | 32.0±3.36ab | 34.5±6.45ab |
| LINEARITY (%) | 56.40±8.16 | 52.72±3.13 | 58.82±5.28 | 51.65±2.47 | 55.12±3.44 |
| BCF (hz) | 11.00±1.56a | 7.75±0.86b | 6.67±0.80b | 7.85±0.38b | 7.82±0.75b |
| Max ALH (µm) | 10.60±1.11ab | 10.42±1.03ab | 8.95±0.92a | 11.37±0.59b | 11.32±1.46b |

*Mean with different superscripts with in rows differ significantly* ($P \leq 0.01$)

palm thawing.

## DISCUSSION

Thawing is the retrieval of metabolic processes in sperm maintained frozen at -196°C for long (*Rizkallah et al., 2022*). Thawing requires a balance between the time and temperature that initiates the sperm activity in a sequential manner to establish coordination among the different components of sperm cell. During the experiment, the proportion of viable spermatozoa were significantly ($P \leq 0.01$) lower in all the thawing processes compared to ideal thaw. The reason for the reduced viability is lack of balance between the time dependent change in temperature that result in inability of sperm to establish sequential revival and coordination of metabolism resulting in sperm death (*Menezes et al., 2019*). Percent ROS+ve sperm were significantly (P

≤ 0.01) higher in all the non-conventional thawing procedures compared to ideal thawing. The reason may be attribute to the increased ROS production by sperm under stress with metabolic destabilization especially in the middle piece with abundance of mitochondria which are major source of energy metabolism as well as ROS production (*Almansa-Ordonez et al., 2020*). During the thawing the inability to attain desired temperature in stipulated time induced stress leading to increased proportion viable ROS+ve spermatozoa. The significantly (P ≤ 0.01) higher values of acrosomal damage were recorded in groups compared to ideal thawing. Overproduction of ROS result in increased efflux of cholesterol and phospholipids and disrupts the plasma membrane of spermatozoa (*Chianese* and *Pierantoni., 2021*). Acrosome is most liable to membrane disruption by ROS once out of the male reproductive tract (*Alahmar., 2019*). Significantly (P ≤ 0.01) higher values for viable spermatozoa with disrupted acrosome in different unconventional thawing protocols may be attributed to increased ROS resulting in premature capacitation, weakening sperm membrane leading to acrosomal damage. The motility parameters were significantly (P ≤ 0.01) higher in the ideal thawing compared to other thawing processes. The motility and path velocity of sperm to be categorized in progressive slow of static depends upon the energy production and its successful transfer to sperm tail for movement (*Anand* and *Yadav., 2016*). During the experiment high ROS production in different non-conventional thawing affected the membrane as evident through values for acrosomal damage and ROS affected spermatozoa, leading to loss of selective permeability of sperm plasma membrane affecting energy production and transfer mechanism causing reduced motility (*Nowicka-Bauer* and *Nixon., 2020*).

## CONCLUSION

It can be concluded that thawing of frozen semen ideally in thawing unit give the best result in terms of post thaw semen quality. However, in context of non-conventional methods practiced in India, under unavailability of thawing units, thawing of goat frozen semen in water maintained at 37°C by thermometer can be preferred over the other non-conventional thawing practices.

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