Effect of Air Space in Storage Vials on Motility of Spermatozoa in Chilled Buck Semen

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Abstract

This study was conducted in order to find out the effect of air space on the top of glass vial in which semen is stored, on the motility of spermatozoa. 45 samples collected from two bucks over a span of 6 months were used for experiment. Goat milk extender was the diluent used. Two ml each of diluted semen after noting their initial motility was stored in 2 ml and 5 ml vials. Samples were stored at 5°C and motility of spermatozoa noted at 24 and 48 hours. Semen without air space was found to preserve the motility better than semen with air space on 24 and 48 hours of incubation. This could be better attributed to reactive oxygen species production by the spermatozoa, but further investigation is needed in this aspect to confirm it.
Key Words: Sperm Motility, Oxidative Stress, Buck Semen, Reactive Oxygen Species

Introduction

One of the easiest methods of preservation of buck semen is by dilution and chilling at 5°C. Commonly Goat milk extender is used for dilution of buck semen. Such diluted semen can be preserved for 48 hours without loosing fertilizing capacity. But occasionally such stored spermatozoa used to exhibit drastic reduction in motility on 48 hours of storage. Diluted semen is used to store in glass vials. Most of these stored glass vials will be having varying amount of air space above semen. Present study is aimed to find out whether volume of air space above the stored bucks semen at 5°C, have any effect upon spermatozoa motility at 24 and 48 hour of storage.

Material and Methods

This work was conducted at Veterinary Dispensary, Athirapilly, Thrissur, Kerla. Buck semen collected from two crossbred Alpine-Malabari bucks maintained on concentrate feed was selected. One animal was two and half years old another was above six years. Another male animal housed along with these bucks were used as mount animal. For collection Danish model of Artificial Vagina (AV) modified for bucks were used.

Collected semen is evaluated without delay for physical characters. Semen with a mass activity of ++++ is used for the experiment. Diluent used was Goat milk extender. Collected semen after physical examination was diluted at the rate of 1:5 with whole Goat milk extender. After dilution semen was transferred to sterile 2 ml and 5 ml glass vials. 2 ml glass vial was filled completely with out air space (Sample A). 5 ml vials were poured with 2 ml diluted semen, so that 3 ml air space remained on the top (Sample B). After filling, initial motility of semen was noted by assessing the intensity of vibration of fat globules. Vials were closed with rubber stopper. After this glass vials with semen was kept in a water bath at 37°C and cooled slowly. Then water bath was transferred to a refrigerator and stored at 5°C. 24 and 48 hrs later motility was checked again by heating the slide to body temperature. Data collected was recorded.

Result and Discussion

45 samples collected from 2 bucks over a period of 6 months were evaluated. Average percentage of motility obtained is given in the table given below with standard error.

Table-1.

Sl. No.	Sample	Average per cent motility		
		0 h	24 h	48 h
1.	A	67.5 ± 0.19	49.2 ± 0.34	28.9 ± 0.45
2.	B	67.5 ± 0.19	38.9 ± 0.50	19.6 ± 0.33

A = Sample with out air space, B = Sample with 3 ml of air space

Table indicating average motility of spermatozoa during 0 hour, 24th and 48th hour of storage at 5°C.

All the data recorded exhibited almost similar pattern, except one experiment. The average percentage of motility was calculated from the 44 data excluding the one with drastic difference. On starting the experiment (0th hour), both samples have same motility. 24 hours later sample without air space was having an average motility of 49.2 ± 0.19 %. Sample with air space was having a motility of 38.9 ± 0.5 %. After 48 hours sample without air space was having a motility of 28.9 ± 0.45 % where as sample with air space was having a motility of 19.6±0.33 %. Difference in motility between two samples on 24 hours was 10.3%. After 48 hours the difference was 9.3%.

On analysis of result it was found that sample without air space was having a better motility than sample with air space on 24 hours and 48 hours of storage. At 24 hours of storage sample without air space was having an average of 10.3% more motility than sample with air space. On 48 hours of storage sample without air space was having an average of 9.3% more motility than sample with air space. This clearly indicates that air space kept above semen definitely reduces motility of spermatozoa.During experiment one sample exhibited a different pattern when compared to rest of the 44 samples. In this sample the motility of sample without air space was lower than that of sample with air space. In this sample during the storage the semen was in direct contact with the rubber cap. This could be the reason for this effect.

Sperm can retain their activity in a medium without oxygen for a considerable time. Sperm motility certainly does not depend on presence of oxygen. The motile activity of human sperm is not dependant on energy derived from respiration. Glycolysis is considered as the principal source of energy of spermatozoa. In moving sperm glycolysis is important both under aerobic and anaerobic conditions. In presence of oxygen anaerobic glycolysis is reduced and aerobic process increased. In absence of oxygen spermatozoa remain only in presence of sugars by aerobic glycolysis. (Anderson, 2001).Reactive oxygen species (ROS) such as the super oxide anion (O2-), the hydroxyl radical (-OH) and hypochlorite radical (-OHCl) produced by the spermatozoa and the contaminating leucocytes in the seminal fluid adversely affect sperm motility and impair their fertilizability (Verma et al., 1999).

The source of reactive oxygen species comprise an enzymatic system located in the sperm plasma membrane that utilizes NAD(P)H as substrate and a second system involving the mitochondrial electron transport chain (Vernet et al., 2001).The use of 5% O2 in incubator rather than 20% (atmospheric air) has presented excessive production of ROS by spermatozoa and related alteration of sperm function (Griveau et al., 1997).

Presence of oxygen favours the aerobic respiration and active involvement of mitochondria and electron transport chain which is a chief source of reactive oxygen species like super oxide anion (O2-). Excess production of reactive oxygen species impairs sperm functions like motility and its fertilizing capacity. So absences of air space above the stored semen vial reduces reactive oxygen species production and thus better motility, and better fertilizing capacity is retained when compared to sample with 3 ml of air space. A similar study conducted by Krzyzoslak et al. (2001), in bovine semen reported reduction of membrane integrity under aerobic condition, but he did not observed that much difference in motility of spermatozoa. In a study conducted by De Paun et al. (2003) in bovine semen no significant difference in motility and membrane integrity were observed in spermatozoa stored under aerobic condition and nitrogen gassed condition.

So air space above the semen has definite role in reduction of spermatozoal motility. So avoiding air space above the stored semen can improve the storage life and fertilizing capacity of spermatozoa. But the role of ROS is ambiguous. Since conflicting reports exist in this aspect an investigation involving measurement of production of reactive oxygen species is needed to establish a clear relationship.

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