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Thermal insults inflicted to bovine frozen semen straws owing to mishandling in liquid nitrogen refrigerator and its impact on seminal characteristics

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Semen straws are highly vulnerable to thermal insults since the surface area to volume ratio of the straw is high. Exposure of semen straws to temperatures above – 130°C (critical temperature) and subsequent re-cooling resulted in irreparable sperm damage. The gravity of the damage depends on how high the temperature rises above – 130°C and also the duration of exposure at this temperature.

Liquid nitrogen refrigerators of 7.9 litres capacity are being used for semen storage in all the 2,990 artificial insemination centres in Kerala. These refrigerators are being refilled with liquid nitrogen once in 21 days. The neck of the refrigerator has a length of 16 cm and is the working area where semen straws are routinely handled and exposed to potentially damaging temperatures. When fully filled with liquid nitrogen, the temperature at the middle of the neck of the refrigerator is – 130°C. Saacke (1978) reported that it takes less than ten seconds for seminal temperature to rise above – 130°C. Hence canisters containing semen straws are to be handled only at the lower one third of the neck of the refrigerator for short periods of time in order to avoid thermal insults. From the above observations, it is evident that faulty semen handling procedures could result in damage to the spermatozoa leading to lower conception rates.

Studies pertaining to the effect of mishandling semen straws in field refrigerators of 7.9 litres capacity are not available. Hence the present study was undertaken to assess the magnitude of damage inflicted to frozen semen straws by thermal insults owing to mishandling in field refrigerator and its impact on seminal characteristics.

A thermodynamically efficient refrigerator having a holding capacity of 7.9 litres was used for the study. The refrigerator was maintained with full liquid nitrogen throughout the study period. Twenty doses of semen each, produced from eleven bulls (6 purebreds and 5 crossbreds) of varying levels of freezability of the Mattupatti sperm station were used for the study. The semen straws were taken from the bulk storage refrigerator. Straws (20) of one bull were stored in the refrigerator at a time; taking care to ensure that 5 semen straws were stored in each canister position. The semen straws of each canister position was subjected to separate treatments; i.e. one canister to treatment 1, another to treatment 2, next to treatment 3 and the final to treatment 4. The succeeding paragraphs describes these treatments in detail.

Treatment 1: Barth (1993) reported that sperms in the neck of the semen tank were not to be handled for more than 5 sec to avoid re-crystallization. Hence, in the first treatment, the canister containing semen straws was handled at the lower one third of the neck of the refrigerator for 5 sec with the intention of avoiding re-crystallization. By keeping the canister closer to the refrigerant, the semen doses were maintained well below the critical temperature.

Treatment 2: The semen straws were handled at the upper level of the neck of the refrigerator for 30 sec. The canister was then put back into position and refrigerator closed. After 5 min, the straws were again handled at the upper level of neck for 30 sec and kept back in position. After 5 min the procedure was again repeated.

Treatment 3: The semen straws were handled at the upper level of the neck of the refrigerator for 45 sec. The semen straws were exposed to the upper level of the neck of the refrigerator two more times, giving 5 min interval between subsequent exposures as done in treatment 2.

Treatment 4: The semen straws were handled at the upper level of the neck of the refrigerator for 60 sec. The procedure was again repeated two more times, giving 5 min interval between subsequent exposures as being done in treatment 2 and 3.

In the treatments 2, 3 and 4, the semen straws were deliberately subjected to temperature above the critical temperature for varying duration of time (30–60 sec) to simulate mishandling of semen straws. The straws were also subjected to additive warming by exposing each canister to higher temperature 3 times.The semen straws of all the eleven bulls were subjected to the above treatments in the refrigerator.

Immediately after the completion of each treatment, 1 semen straw was thawed and examined for the post-thaw

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Table 1. Mean of the seminal characteristics in the different treatments

Seminal characteristics CASA reading	Treatment 1 (15 Sec)	Treatment 2 (30 Sec)	Treatment 3 (45 Sec)	Treatment 4 (60 Sec)
Motility	89.63± 6.09	89.09± 2.33	88.91± 2.51	87.36± 5.64
Progressive motility	68.36± 7.57	66.64± 6.98	56.64± 11.37	50.55± 11.51
Path velocity	81.15± 9.97	79.25± 8.62	81.2± 7.77	77.65± 6.91
Progressive velocity	74.77± 9.29	73.85± 7.94	74.76± 7.85	71.3± 5.42
Track speed	106.9± 16.77	101.46± 14.51	109.62± 11.93	104.01± 18.93
Lateral amplitude	4.41± 0.87	4.29± 0.82	5.01± 0.91	4.7± 1.13
Beat frequency	25.73± 4.48	24.52± 4.55	23.58± 2.79	23.55± 4.62
Straightness	91.91± 2.12	92.82± 1.78	91.73± 2.28	91.91± 3.33
Linearity	71.09± 6.09	73.64± 4.43	69.36± 6.85	70.55± 9.44
Flow cytometer (%)				
Cell viability	79.34± 7.33	75.7± 5.97	71.75± 9.08	75.07± 7.42
Mitochondrial membrane potential	78.22± 17.85	69.47± 12.16	50.75± 21.97	50.67± 26.72
Live sperm with unreacted acrosome	85.91± 7.12	63.31± 10.35	52.43± 8.05	51.16± 5.81
Sperms with altered chromatin structure	9.07± 0.40	9.44± 0.45	9.47± 0.28	9.58± 0.20

motility and sperm kinetic parameters like average path velocity, progressive velocity, track speed, beat cross frequency, lateral amplitude, straightness and linearity using the computer assisted semen analyzer (CASA).

One straw each were used for assessing seminal characteristics like sperm viability, live sperms with intact acrosome, mitochondrial membrane potential and integrity of sperm chromatin structure using the Flow Cytometer. The mean values of the seminal attributes for each treatment were worked out and statistical analysis of the data was carried out as per standard procedures. The study was conducted during November to December 2016. The average ambient temperature during the study period was 24ºC.

The mean value of the seminal characteristics of the straws subjected to mishandling is depicted in Table 1. The mean post-thaw motility showed a decreasing trend with the increase in exposure time. Analysis of the post-thaw motility revealed a significant difference ($P<0.05$) between the treatments. These results were in accordance with the findings of Holt (2000) in related studies. Whereas the variation in sperm kinetic parameters between the treatments was not significant. The live sperms in treatment 1 gradually reduced up to treatment 3. But the live sperms in the semen samples exposed to Treatment 4 were relatively higher, indicating variation in quality between individual straws. The variation between the treatments was insignificant.

The mitochondrial membrane potential showed a decreasing trend on exposure of semen to higher temperature for longer duration of time. Statistical analysis revealed that the reduction was significant ($P<0.05$), except the variation between the treatments 3 and 4. The live sperms with unreacted acrosome showed a reducing trend and varied significantly ($P<0.05$) between the treatments. These results were in accordance with the findings of earlier studies (Saacke *et al.* 1978, Saacke 1983).

The results of the sperm chromatin assay showed an increasing trend when straws were exposed to higher temperature for longer duration. The increase in the sperms with altered sperm chromatin integrity was statistically significant between treatments.

The present study revealed that the seminal characteristics like post-thaw motility, mitochondrial membrane potential and live sperms with unreacted acrosome decreased owing to thermal insults on account of mishandling of semen straws in the refrigerators. Whereas a significant increase in sperms with altered sperm chromatin integrity was observed. The progressive motility also decreased when the semen doses were subjected to higher temperature. The magnitude of the damaging effects increased with longer exposure time. Kumaresan *et al.* (2001) reported a high correlation between percentage of spermatozoa with normal acrosome and fertility. Ballachey *et al.* (1988) had reported that the sperm chromatin assay is highly correlated to fertility in cattle. The study revealed that the aforementioned seminal attributes had been significantly affected owing to the mishandling of semen straws in the refrigerator.

In the simulation study, the treatments were undertaken with the refrigerators fully filled with liquid nitrogen and in each canister position, only five semen straws were stored at a time. Thereby the semen straws were adequately protected in the refrigerant when exposed to higher temperatures. Under field conditions, working evaporation loss of the refrigerator is more, level of liquid nitrogen inside the refrigerator decreases over time and more number of semen doses are being packed in each goblet. Under the above circumstances, the initial temperature of the straws would be higher at exposure time. Thereby the gravity of the damage inflicted on the semen straws would be of epic proportion. Hence it is of paramount importance that at most precautions be taken while handling semen straws in field refrigerators.

SUMMARY

The present study was aimed to assess the magnitude of damage inflicted to frozen semen straws owing to mishandling in liquid nitrogen refrigerator by conducting simulation studies. Semen straws of eleven bulls (20 straws each) of Mattupatti semen station was used for the study. Twenty straws of one bull were stored in the refrigerator at a time; five semen straws in each canister position. The semen straws of each canister position was subjected to separate treatments; i.e. one canister to Treatment 1 (5 sec exposure at the lower one third of the neck of refrigerator); another to Treatment 2 (30 sec exposure at the upper level of refrigerator neck); next to Treatment 3 (45 sec exposure at the upper level of refrigerator neck), and the final straw to Treatment 4 (60 sec exposure at the upper level of refrigerator neck). After treatment, one straw was used for assessing the post-thaw motility and sperm kinetic parameters using CASA. The second, third, fourth and fifth straws were used for the assay of live sperm, mitochondrial membrane potential, live sperm with unreacted acrosome and sperm chromatin structure respectively using flow cytometer. Statistical analysis revealed that post-thaw motility, mitochondrial membrane potential and live sperms with unreacted acrosome decreased owing to mishandling and the difference between treatments being significant. There was significant increase in the sperms with altered sperm chromatin integrity. Hence at most precautions should be taken while handling semen straws in field refrigerators.

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