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POST- THAWING CHARACTERISTICS OF GOAT SEMEN FROZEN IN PELLETS USING COOLED SURFACE OF PARAFFIN WAX COMPARED TO CONVENTIONAL METHODS

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SUMMARY

The present work aimed at evaluating post-thawing characteristics of Boer goat semen frozen in pellets using cold surface of paraffin wax (PCSPW) compared to two conventional methods of straws frozen by liquid nitrogen and pellets frozen by dry ice.

Ten pooled semen samples with >70% motility of spermatozoa were used. The same extender (Tris-egg yolk), extension rate and equilibration period were used in the three freezing methods.

The overall means of post-thaw motility, acrosome integrity, live spermatozoa and sperm recovery using PCSPW were 40.0, 36.5, 39.1 and 52.3%, respectively. The corresponding values using straws were 43.3, 47.0, 41.6 and 56.5%, respectively. The differences between these values were insignificant except in acrosome integrity. Freezing as straws or PCSPW had significantly ($P<0.05$) higher post thaw motility and sperm recovery compared to dry ice pellets.

In conclusion, freezing Boer goat semen in pellets using cooled paraffin wax could be used successfully.

Keywords: Goat, semen, freezing methods, post-thawing traits

INTRODUCTION

Using the frozen- thawed semen in artificial insemination programs allows the use of genetically superior males even in the absence of those males. The two conventional methods for freezing goat semen are straws using liquid nitrogen and pellets using dry ice, which may be limited sometimes by the inavailability of straws or dry ice. The present work aimed to freeze Boer goat

buck semen in pellets using cold surface of paraffin wax compared to the two conventional methods.

MATERIALS AND METHODS

Semen samples were collected using the artificial vagina technique from nine mature Boer goat bucks at the Institute of Animal Husbandry and Breeding Farm, Georg Augost University, Goettingen, Germany. Each ejaculate was immediately evaluated for progressive motility. Three ejaculates of 70% or more progressive motility were pooled together and considered as one sample. Ten pooled semen samples were used. Each pooled semen sample was extended using two-steps to reach 1:8 extension rate. The first step was by adding 4 volumes of diluent A to one volume of the semen sample at 37°C. The second step was addition of 4 volumes of diluent B after 30 minutes at room temperature. The composition of dilluent A & B is illustrated in Table 1. The extended semen samples were cooled gradually to 5°C within 2 hrs. then kept at the same temperature for 2 hrs as equilibration period.

Table 1. The components of egg yolk-tris-fructose extender

Components	Diluent A	Diluent B
Tris (g)	3.785	3.785
Citric acid (g)	2.115	2.115
Fructose(g)	1.00	1.00
Glycerol (ml)	----	12.00
Egg yolk (ml)	20.00	20.00
Antibiotic (ml)**	1.0 ml	1.0 ml
Glass distilled water	To 100 ml	To 100 ml

** Each 1.0 ml of the antibiotic contained 30,000 IU penicillin and 50,000 micrograms streptomycin.

Freezing techniques

Three freezing methods were evaluated, straws and pellets on dry ice as conventional methods of freezing and pellets on cold paraffin wax, as a tested method of freezing. The Salamon (1971) techniques for freezing ram semen in 0.25 ml straws or in pelleted form on dry ice were used. Awad (1989) techniques for freezing ram semen in pelleted form on cold surface of paraffin wax was used as illustrated below:

Pellets on cold paraffin wax

Some paraffin wax was melted in a bored box of aluminum foil (5x7x15cm) to make a layer of 1 cm depth. Some holes were engraved in the surface, then left to reach room temperature. The prepared paraffin wax blocks were cooled

by immersion in liquid nitrogen for 15 seconds, then placed horizontally and lowered into liquid nitrogen vapor 2-3 cm above the surface of liquid nitrogen. About 150 - 200 ul of equilibrated semen were pipetted into each hole, after 9 minutes on the vapor of liquid nitrogen, the frozen pellets were immersed in liquid nitrogen (-196°C), followed by careful packaging into small goblets of appropriate size and transferred into liquid nitrogen storage container.

Thawing of frozen semen

Three straws were thawed in a water bath at 37°C for 1 minute, then the surface of each straw was cleaned .The pellets were thawed by placing each three pellets in a clean dry test tube in a water bath at 37°C. for 1 minute.

Examination of Thawed semen

Frozen-thawed spermatozoa in straws and both kinds of pellets were microscopically tested for progressive motility and live spermatozoa according to Salamon (1976). Acrosome integrity was determined according to Shams-Borhan and Harrison (1981) after 0, 1.0, and 2.0 hours of incubation at 37°C. Sperm recovery was calculated as follows:

$$\text{Sperm recovery} = \frac{\text{Post thaw motility} \times 100}{\text{Initial motility}}$$

Statistical Analysis

This experiment was carried out to evaluate the effect of three freezing methods (straws, pellets on dry ice and pellets on cold paraffin wax) with three incubation periods of frozen thawed semen at 37°C (0, 1 & 2 hrs) on factorial design (3 x 3, with 10 replicates) was applied to analyze the data using SPSSWIN (version 3) computer program. The statistical model was

$Y_{ij} = u + F_i + l_j + F_{lij} + e_{ij}$

Fi: The effect of freezing method
lj: The effect of incubation period
Fiij: Interaction between freezing methods and incubation period
eij: Error

RESULTS AND DISCUSSION

The overall means of post-thaw motility, acrosome integrity, live spermatozoa, and sperm recovery of Boer goat buck spermatozoa using pellets on the cooled surface of paraffin wax were 40.0, 36.5, 39.1, and 52.3% (Table 2), respectively. These data were almost in the middle between the two conventional recommended methods of freezing, straws and pellets on dry ice.

The poorer performance of pelleted semen has been explained by some authors. Pontbriand *et al.* (1989) illustrated that the pellets spherical configuration causes uncontrolled temperature variations which leads to

Table 2. Means ± S.E of post-thaw motility, acrosome integrity, live spermatozoa, and sperm recovery of frozen-thawed Boer goat bucks spermatozoa as affected by method of freezing and incubation period at 37°C

Variable		After Thawing	1 Hour	2 Hours	Overall Mean
Motility (%)	Straws	52.50	42.00	35.50	43.33 ± 1.72 [b]
	Pellet/dry ice	45.50	37.00	30.00	37.50 ± 1.62 [a]
	Pellet/paraffin wax	49.00	38.99	32.00	39.99 ± 1.62 [ab]
	Mean	49.00 ± 1.34 [c]	39.33 ± 1.06 [b]	32.50 ± 1.06 [a]	
Acrosome Integrity (%)	Straws	55.70	47.60	37.80	47.03 ± 2.28 [b]
	Pellet/dry ice	43.70	31.60	24.20	33.16 ± 2.08 [a]
	Pellet/paraffin wax	48.90	36.10	24.60	36.53 ± 2.44 [a]
	Mean	49.43 ± 2.02 [c]	38.43 ± 1.99 [b]	28.86 ± 1.92 [a]	
Live Sperm (%)	Straws	47.70	41.60	35.50	41.60 ± 1.43 [a]
	Pellet/dry ice	44.20	37.40	31.00	37.53 ± 1.40 [a]
	Pellet/paraffin wax	47.40	38.10	31.70	39.06 ± 1.46 [a]
	Mean	46.43 ± 1.10 [c]	39.03 ± 0.97 [b]	32.73 ± 1.02 [a]	
Sperm Recovery (%)	Straws	68.50	54.70	46.20	56.46 ± 2.07 [b]
	Pellet/dry ice	59.64	48.37	39.11	49.05 ± 2.04 [a]
	Pellet/paraffin wax	64.05	51.00	41.87	52.30 ± 2.08 [ab]
	Mean	64.06 ± 1.61 [c]	51.35 ± 1.59 [b]	42.93 ± 1.24 [a]	

a, b, c Values with different superscripts in the same raw or in the same column of the same characteristics differ significantly ($P<0.05$) using Duncan`s test.

disturbance in internal freezing rate and crystallization patterns. On the other hand, freezing straws in liquid nitrogen vapor resulted in a constantly changing rate of cooling as the internal temperature decreases (Robbins *et al.*, 1976). This phenomenon may cause more damage to sperm cells in pellets compared to those in straws. It seems reasonable that semen within straws may be cooled uniformly than semen frozen in dry ice or paraffin wax pelleting. In other words, pellets offer less protection to spermatozoa during cooling and freezing because of the direct contact of pellets with the surface of dry ice or the cold paraffin wax. This also causes faster and less gradual changes in deep freezing temperatures in pellets than in straws. Awad (1989) stated that it could be expected that the number of injured spermatozoa by cold shock is higher in pellets than in straws. Salamon and Maxwell (1995) reported that freezing and thawing of ram semen cause ultrastructural,

biochemical and functional damage to a significant proportion of spermatozoa. These changes are usually accompanied by a reduction in motility.

Statistical analysis showed that the results of pellets on the cooled surface of paraffin wax were insignificantly lower than that obtained in straws except for acrosome integrity. Paraffin wax gave insignifiantly better results than dry ice pellets in all traits studied. This may be due to the slower cooling and freezing of pellets on dry ice than pellets on cold paraffin wax. These results suggest that the acrosomal membranes of goat buck spermatozoa were more injured in the pellets on dry ice than in paraffin wax. The injury found in this study ranged from slight swelling of acrosome to the total removal of the acrosomal membranes of spermatozoa. These observations are in agreement with Tasseron *et al.* (1977) who showed that spermatozoa were damaged during all the stages of freezing processes starting with dilution. Also the results of this study agree with the general trend reviewed in the literature that the frozen sperm cells in straws had better physical characteristics than those frozen in pellets (Fiser *et al.*, 1987; Awad, 1989). An acceptable fertility rates have been achieved from ram semen in straws previously cooled in liquid nitrogen vapor or by direct placement in dry ice (Fiser *et al.*, 1987). The present study indicated that the freezing technique of goat spermatozoa in pelleted form using the cooled surface of paraffin wax was more successful than dry ice.

The percentages of progressive motility, live spermatozoa, acrosome integrity, and sperm recovery decreased significantly ($P<0.01$) with advance of incubation time at 37°C. Frozen spermatozoa may be injured by rewarming, due to recrystalization of microscopic ice crystal to form larger ice crystals which are widely recognized to be damaging. Abdalla (1983) on goat and Khalifa and Horvath (1985) on ram semen found that the extracellular enzyme contents were significantly increased after freezing and thawing due to the damage of sperm membrane which allowed the intracellular components to go out. They also reported negative correlation coefficient between extracellular enzyme contents and physical characteristics of ram semen. On the other hand Lindemann *et al.* (1982) reported that the dead spermatozoa have a negative toxic effect on the remaining normal sperm population. The permeability of frozen sperm cell membrane increases, most of the intracellular components got out. The previous findings may explain the decrease of the life span of the frozen spermatozoa with the incubation time. The interaction between freezing methods with incubation periods in all the studied physical characteristics was insignificant. Throughout the incubation periods the freezing on paraffin wax gave insignificantly higher values of physical semen characteristics than freezing on dry ice.

In conclusion, goat buck semen could be frozen in straws or pellets using dry ice or paraffin wax on liquid nitrogen. Based on the present results, it seems more appropriate that freezing semen in pellets form by using a

paraffin wax block on the vapor of liquid nitrogen could be used successfully if straws are not available.

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صفات السائل المنوى المجمد للماعز فى حبيبات بأستخدام سطح بارد لشمع البرافين بعد الإساله مقارنة بالطرق التقليدية

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هدفت هذه الدراسة الى مقارنة صفات السائل المنوى المجمد لماعز ألبوير فى حبيبات بإستخدام سطح بارد لشمع البرافين بعد الإساله مقارنة بالطرق التقليدية وهى القصيبات المجمدة بالنيتروجين السائل والحبيبات المجمدة بالثلج الجاف.

أستخدمت عشرة عينات سائل منوى مجمعة تحتوى على ٧٠ ٪ أو أكثر حيوانات منوية متحركة، وأستخدم نفس المخفف (الترس - صفار البيض) , معدل التخفيف وفترة الأتزان فىالثلاث طرق للتجميد.

كان المتوسط العام بعد التجميد والتسييح للحيوانات المنوية ٤٠٪ حركة , ٣٦,٥ ٪ متكاملة الأكروسوم , ٣٩,١ ٪ حية و ٥٢,٣ ٪ إسترجاع بإستخدام طريقة التجميد فى حبيبات على السطح البارد لشمع البرافين. وكانت القيم المقابلة عند استخدام طريقة القصيبات هىعلى الترتيب ٣ ,٤٣، ٠, ٤٧ ، ٦, ٤١ و ٥, ٥٦ ٪. وكانت الفروق بين هذه القيم غير معنوية عدا نسبة كمال الأكروسوم. أعطت طريقة التجميد بإستخدام القصيبات أو فىحبيبات على السطح البارد لشمع البرافين نتائج أعلى معنويا (مستوى ٥ ٪) من طريقة الحبيبات على الثلج الجاف لكل من نسبة الحركة ونسبة الإسترجاع.

محصلة هذه الدراسة أنه يمكن تجميد السائل المنوى لماعز البوير بنجاح فى حبيبات على سطح بارد لشمع البرافين.