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Effect of Age and Postmortem Time on Some White-Tailed Deer (*Odocoileus virginianus texanus*) Epididymal Sperm Characteristics and Response of Cryopreservation

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Abstract: Problem statement: Males rather than females of the White-Tailed deer (*Odocoileus virginianus texanus*) are more susceptible to hunting because their physical characteristics, therefore their elimination can increase a genetic degradation and a lower productivity because of the effect of gender. Thus, the aim of the current study was to evaluate the effect of age and postmortem time of White-Tailed deer on its epididymal-tail sperm motility and morphology and the response to sperm cryopreservation. **Approach:** Twenty two hunted deer were used and were classified into three age-groups: A = 1.5-3.5; B = 4.5-5.5 and C = 6.5-7.5 years old and four groups according to postmortem time: 1= 0-3; 2 = >3-5; 3 = >5-7 and 4 = > 7 h. Two samples per animal (one per each epididymal) were diluted and frozen with Triladyl® and Tris-Fructose. Thawing was done 72 h post freezing. **Results:** Age did not neither affect motility nor morphology ($p>0.05$). Postmortem time had a deleterious effect on motility ($p<0.05$), a significant drop in this trait was found after 5 h postmortem (58.1, 56.1, 37.5 and 38.3% motility in groups 1-4, respectively). However, did not affect morphology until 7 h postmortem and after 7 h an unexpected, significant ($p<0.05$) improvement was found (66.4, 74.9, 62.2 and 83.8% of normal sperm in groups 1-4. As regarding freezing ability, though without a statistical difference ($p>0.05$), the percentage of samples with acceptable motility after freezing and thawing was greater with Tris-Fructose than with Triladyl (36 Vs 18%). **Conclusion:** It was concluded that these results indicate that age as studied does not affect epididymal sperm quality in this species, while post-mortem time has a detrimental effect on motility and epididymal sperm can be successfully cryopreserved, for which Tris-Fructose would be better as an extender than Triladyl.

Key words: White-tailed deer, epidydimal sperm, freezing ability

INTRODUCTION

The White-Tailed deer (*Odocoileus virginianus texanus*), is currently one of Mexico's most important game species. Regulated hunting of this species yields important economic benefits and is too a way to preserve a natural resource. However, without a suitable planning, even regulated hunting could lead to a reduction of the species productivity or to negative genetic modifications, particularly in low-density populations were adult dominant males are eliminated (Galindo-Leal and Weber, 1988). This can occur because dominant males are more susceptible to hunting, due to their greatest mobility in search of females during the breeding season, since the latter lies within the hunting season and because owing to their physical characteristics these males are more attractive to hunters. One can assert, in general, that dominant males perform the greater numbers of mating during the breeding season and, therefore, their elimination can lead to a genetic degradation and a lower productivity. This, because if the males are hunted before they mate, a number of females could go unbred and not to reproduce that specific year, with the consequent lower number of young animals. Besides, in the absence of dominant males, a number of females could mate with other males with less outstanding characteristics, thereby leading to a genetic degradation.

There are researchers interested in the control of reproductive activity of this species, in order to contribute to its conservation and population strengthening. However, much of this study concerns

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range conditions, while there is little knowledge on wild populations, which are subject to the pressure of hunting, both regulated and not regulated. This makes necessary to undertake efforts towards genetic conservation in these populations. The use of epididymal sperm taken from outstanding recently-hunted specimens is an important possibility to this purpose. Sperm recuperated in this way could be frozen for preservation and be used to inseminate wild or semi-wild females, thus contributing to genetic conservation of the species and could contribute to minimise inbreeding through exchange of semen between different areas within Mexico.

The aims of this study were: (a) to evaluate the effect of age and time postmortem of the White-Tailed deer on its epididymal-tail sperm motility and morphology and (b) to study the response of this sperm to cryopreservation with two extenders (Triladyl® and Tris-Fructose).

MATERIALS AND METHODS

This study was done with samples obtained from 22 specimens of White-Tailed deer (*Odocoileus virginianus texanus*) hunted under regulation in northern Mexico (Nuevo Leon, Coahuila and Tamaulipas states) during November and December 2000. Immediately after hunting, the following data were recorded: time of hunting, specimen's body weight and estimated age by teeth characteristics. Afterwards the animal was taken to the laboratory, where both testicles were removed and the epididymis carefully separated from each testis for sperm recuperation, which was performed through a little incision cut in the caudal region of the epididymis.

Spermatic individual motility and morphology were indicative of sperm quality; these traits were evaluated in duplicate, taking a sample per epididymis of each animal and keeping the best result. A drop of the sample was placed on a slide and covered with a coverslip to be observed at 400× in order to evaluate the percentage of sperm motility, which was qualified from 0-100%. Sperm morphology was evaluated on a smear stained with eosine-nigrosine and observed with a phase-contrast microscope at 1000×; a hundred cells were counted per smear per sample. Three age-groups were considered for comparison of sperm quality between ages: A (n = 6), with animals of 1.5-3.5 years old; B (n = 12), 4.5-5.5 and C (n = 4), 6.5-7.5 years old. To study the effect of postmortem time on sperm quality, four groups were formed for comparison: (1) (n = 4), including animals in which the sample was evaluated from 0-3 h postmortem; (2) (n = 7), evaluation from >3-5 h; (3) (n = 8), from >5-7 h and 4 (n = 3), >7 h postmortem.

Two extenders were used for testing the response of epididymal sperm to freezing: Triladyl® (Minitüb, Germany) and Tris-fructose as used in the White-Tailed deer (Asher *et al*., 2000): Tris 3.63 g, fructose 0.50 g, citric acid 1.99 g, egg yolk 15 mL, glycerol (5%) 5 mL, G-peniciline 0.06 g streptomicine sulphate 0.1g and bidistilled water to make 100 mL. The samples for this part of the study were obtained by washing the epididymal tail with the dilution media. Once the epididymal tail was separated from the rest of the organ, the medium was infused with a 5 mL syringe from the proximal end of the deferent duct towards the epididymis, to be recuperated at the point where the epididymal tail had been cut from the body. In this way, two ml of sperm-rich liquid were recuperated into a sterile glass tube per epididymis, using one of the extender per each epididymis from each specimen, which means that each animal had one of its epididymis washed with Triladyl and the other with Tris-fructose.

Immediately after obtainment, the samples, placed into a bath at 37°C, were examined for sperm motility and concentration. Motility was evaluated as previously described and concentration determined by double counting on a Neubauer chamber. After evaluation, concentration of each sample was adjusted at approximately 70×10^6 sperm mL^{-1} by adding the needed volume of extender, which was done at the initial temperature (37°C), in order to get around 35×10^6 sperm per straw of 0.54 mL. The next step was cooling the sample, this done by placing it into a refrigerator to decrease the temperature from 37-5°C in 2 h. Then the sperm was packed in medium French straws (0.54 mL).

To be frozen, the straws were placed horizontally 5-7 cm above the surface of liquid nitrogen during 7 min, then submerged into it. The straws were stored into a liquid nitrogen thermo during 72 h, after which they were thawed into a bath at 37°C during 60 sec and evaluated for motility. Equal or greater than 50% of motility was indicative of a good ability for freezing.

Mean and Standard Error (MSE) for values of sperm quality (motility and morphology) were calculated. The effect of age and postmortem time was analyzed by ANOVA with the GLM procedure and that of the extender on freezability was analyzed by comparison with the Chi-square test.

RESULTS

Mean age and body weight of the 22 animals (±SE) were 4.8±1.4 years old and 66.8±8.7 kg, respectively.

Table 1: Epididymal tail sperm characteristics of hunted White-Tailed deer (*Odocoileus virginianus texanus*) of three age-groups and at different postmortem times (mean ± SE)

	Sperm characteristics		
Cells (%)	N	Motility (%)	Normal
Age-group (years)			
A (1.5 a 3.5)	12	46.7±6.7	69.7±4.9
B (4.5 a 5.5)	24	47.1±3.9	70.4±3.2
C (6.5 a 7.5)	8	48.8±7.5	68.6±5.8
Postmortem time (h)			
1 (0 a 3)	8	58.1±9.8	66.4±2.8
2 (>3 a 5)	14	56.1±4.1	74.9±4.7
3 (>5 a 7)	16	*37.5±4.5	62.2±3.7
4 (>7)	6	*38.3±4.4	*83.8±3.1

Asterisks (*) in a same column indicate significant differences ($p<0.05$)

Table 2: Percentages of samples of White-Tailed deer epididymal sperm, diluted with two extenders, considered suitable for cryopreservation according to rate of motility observed after thawing (suitable: ≥50% individual motility). Within parenthesis number suitable samples per total

Extender	Percentage of suitable samples
Triladyl	18.1 (4/22)
Tris-Fructose	36.3 (8/22)

Results on sperm quality according to animal's age are shown in Table 1; they indicate that age neither did not affect spermatic motility nor morphology ($p>0.05$) between 46.7 and 48.8% of motility and between 68.6 and 70.4% of normal cells. In contrast with this, postmortem time did show a detrimental effect on sperm motility ($p<0.05$); this trait dropped markedly after 5 h postmortem, showing a decrease of around 20%, going from around 57% in groups 1 and 2 (0-5 h postmortem) to around 37% in groups 3 and 4 (>5->7 h postmortem). The effect of postmortem time on sperm morphology, on the other hand, was not clear, since a difference ($p<0.05$) was found between groups 1-3 (0-7 h postmortem) and group 4 (>7 h postmortem), but this was due to an increased percentage of normal sperm found in the last group (Table 1).

As regarding spermatic freezing ability (Table 2), proportion of samples considered suitable for cryopreservation was rather low for the two treatments (near to 27% as a mean) and diluting with Tris-Fructos yielded a greater percentage of samples with good rate of survival than did diluting with Triladyl, though in spite of a difference of 18% there was not a statistical significance ($p>0.05$). On the other hand, mean percent of post-thaw motility per treatment of samples considered suitable for cryopreservation was 61.9±3.7 for Tris-Fructose and 62.5±5.4 for Triladyl ($p>0.05$).

DISCUSSION

According to several authors, age is a factor affecting epididymal sperm quality in deceased animals, together with season and postmortem time among others (Aguado *et al*., 1994; Anel *et al*., 2000). Young animals may most probably show a reduced sperm quality due both to some degree of lack of sexual maturation and to reduced sexual activity, since a strong hierarchy in this species precludes the access of young males to sexually active females (Appleby, 1983). Long-term storage of sperm into the epididymis seems related to a loss of sperm quality (Colas, 1984).

Then, the lack of an effect of age as seen in this study is somewhat difficult to explain. An earlier sexual maturation of the White-Tailed deer could provide an explanation (Galindo-Leal and Weber, 1988). A low number of very young specimens into age-group A in this study would also contribute to the explanation and this was something that happened in this study: Only one animal 1.5 years old became included in age-group A. This specimen showed the lowest sperm motility (17%) within the group, as well as a relatively high percentage of sperm abnormalities (30%). Thus, present results cannot completely rule out an effect of age on epididymal sperm quality in the White-Tailed deer.

Results of the present study on postmortem time effect on epididymal sperm motility do agree in general with previous reports on sheep (Aguado *et al*., 1994); in this case postmortem time was found to have a detrimental effect on sperm motility. However, (Aguado *et al*., 1994) observed a drop in this trait after 6 h postmortem in sheep -quite similar to the 5 h postmortem observed in this study. A probable explanation to the detrimental effect of postmortem time on epididymal sperm motility are biochemical changes occurring within the epididymis of the deceased animal; the usual descent of temperature starting at death could also participate in this effect. Differences in the time postmortem at which a significant drop in sperm motility occurs might relate to aspects such as species, environmental temperature, season and handling conditions, among others (Christian *et al*., 1993).

As for sperm morphology, the apparent improvement in the percentage of normal cells observed in this study is unexpected. Christian *et al*. (1993) have not found significant changes in sperm morphology due to postmortem time (up to the time studied), probably because structural alterations need more time after death to appear than physiological modifications. The explanation given to this result was that in the >7 h postmortem group there was a random accumulation of specimens with the greatest percentages of normal cells of the study.

A significant drop in sperm motility after freezing and thawing, as observed in this study, is a normal

outcome of these procedures. Cell metabolic changes, as well as plasmatic membrane alterations, due to the decreasing temperature during freezing and to the increasing temperature during thawing, are considered as responsible (Palacios, 1994; Hafez, 1996; Rush *et al*., 1997). In the case of epididymal sperm, the fact that the cells have not been in contact with seminal plasma might further decrease their resistance to these procedures (Parks and Graham, 1992).

Post-thaw motility of samples suitable for conservation. These data suggest that conservation of frozen epididymal sperm of the White-Tailed deer can be done with acceptable results.

CONCLUSION

It was concluded that these results indicate that age as studied does not affect epididymal sperm quality in this species, while post-mortem time has a detrimental effect on motility and that epididymal sperm can be successfully cryopreserved, for which Tris-Fructose would be better as an extender than Triladyl.

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