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RESEARCH ARTICLE

Effect Specific Cooling Times on Motility and Membrane Integrity of Cauda Epididymal Bovine Bull Sperm

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Abstract

Cauda epididymides (CEs) collected post-mortem could be an important source of genetic material from male for conservation and use for assisted reproductive technologies. In this study, we characterize the epididymal sperm (ES) collected post-mortem from CEs of bull cow raised locally. Testicles (n=30) were sliced off from bull cow post-mortem, placed in a box at ambient temperature and transported to the laboratory within 2 hr. The CEs were detached from the testicle and the tail were sliced longitudinally and dropped into a 50-ml conical tube with 15 ml TRIS citric acid. The ES was collected after 10 min, washed through centrifugation and measured. The Live Epididymal Sperm Cooling Sperm 76.40±0.93, 48 hours 68.94±2.38, initial motility, 72 hours 64.23±2.47, 96 hours 60.62±2.40 and 56.62±2.31 per cent, Membrane integrity with initial motility cooling time had the lowest values compared to the gap in initial motility cooling period and 120 hours cooling time .sperm motility whereas through CASA, For CASA (VAP, VSL, VCL, ALH, WOB, LIN), there were no interactions between different cooling times. In addition, different cooling times (P>0.05) affected sperm motion kinematics, i.e. VAP, VSL, VCL, ALH, WOB and LIN, but these parameters are influenced during the cooling process. With epididymal sperm cry presets, both VSL and VAP values were the highest. Cry retained (VSL: 66.25±2.21µm / s, VAP: 81.42±1.46µm / s) compared with 48 hours (VSL: 59.09±1.52µm / s, VAP: 76.67±1.43µm / s), During the cryopreserved cooling period of 5.29±0.14µm (P<0.05), the ALH value was the lowest in epididymal sperm relative to 72 and 96 hours and 120 hours (5.03±0.13, 4.30±0.69 and 4.03±0.069µm).Nevertheless, during the cooling process of cryopreserved with the highest value at 0.80±0.026, LIN was significantly different from the cooling period (P<0.05) used for epididymal semen, and the lowest value at 120 hours was 0.77±0.026. The cooling cycle with the highest values of 0.62±0.027 at initial motility and the lowest values of 0.59±0.020 at 120 hours was significantly affected by WOB (Table 1).The VCL was significantly affected by the cooling time. The VCL value was the highest for initial motility (141.60±3.64µm / s) and the lowest for 48.72.96 and 120 hours, respectively, 128.13±3.62, 123.34±3.55, 118.85±3.51 and 109.48±3.49µm / s of bull cow.

Keywords: *Epididymal sperm, Motility CASA, Hypo-osmotic swelling, Live sperm, Epididymal Sperm Cooling and cauda epididymides.*

Introduction

Examples of an application of the epididymis post-mortem sperm collection for the preservation of endangered species as stated in ibex [1] and in the construction of semen banks [2-4]. Several domestic and wild species documented the conservation of epididymal sperm from dead or slaughtered animals: deer [5-8], cattle [9], fox [10], horse [11], camelin [12], black and white tegu [13], rhesus monkeys [14], saltwater crocodiles [15], goat [16], and sheep [17]. However, due to the lack of facilities and expertise near the farming areas of local breeds, epididymal sperm extraction is sometimes not easily practicable in marginal regions. Overall, the resistance over time of the sperm collected from the testes has been observed to be species-specific [17, 18]. It has been shown in caprine organisms Epididymal sperm can be successfully cryopreserved and used for in vitro fertilization and artificial insemination if processed immediately following animal death. Obtaining caudal epididymal sperm is an important technique for the propagation and preservation of animal specimens with high genetic values following serious injury or from dead animals [19], endangerment. Species [20] and pets [21].Moreover, if the epididymal sperm is obtained immediately after death, the gamete will remain alive for

24 to 48 hours and will be viable for fertilization [22]. In this context, assisted reproductive techniques such as artificial insemination [23], in vitro fertilization [24] and intracytoplasmic sperm injection [25], increased attention to the retrieval of active sperm from the dead animal epididdymis [26]. In different domestic and wild species, three main methods of post-mortem epididymal sperm recovery were described. The cutting method implies that numerous cuts are made with a blade on the cauda epididymidis and the sperm fluid from the tubules is collected [27-34].

These changes in temperature result in various degrees of damage to sperm cells, cell damage of varying degrees of severity is induced by distinct mechanisms at each of the cryopreservation phases, and the functional condition of the frozen-thawed cells is the result of accumulated injuries [35].

Declining temperature from normothermic to 4°C decreases cellular metabolic activity and allows some cell lifespan extension. Nevertheless, because sperm has very little biosynthetic activity and is mostly reliant on catabolic processes to work [36], the sperm cell's remaining metabolic activity leads to death due to the intrinsic aging process. Still, Reports suggest that maximum sperm cell damage occurs if sperm cells are lowered from 4°C to-10°C [37].

Until now, it has been very difficult to mimic in vitro these epididymal conditions, although some reports have shown that sperm can show some fertilizing ability after being stored in cooled epididymids of different species for a few days [38-40].In this study, the pre-freeze quality of epididymal sperm as a function of post-mortem storage was investigated to establish an optimal protocol for the recovery and cooling of bovine bull epididymal sperm (0, 24, 48, 72, 96 h). The investigation was conducted within a protocol-setting system to extract and cryopreserve viable sperm from slaughtered animal epididymis as a method for establishing semen banks for endangered races.

Material & Methods

Experimental Design

Testes were collected at the bull cow animal s anctuary (mean age: 3.04.0 years), 1.30 h on average after slaughter transported to the la boratory under experimental conditions (n = 30) at an ecological temperature of 21.5 ± 2.2 ° C (E) in the Styrofoam box, (n = 3) at a cooli ng temperature of + 4 ° C (4 °C during transp ort).

Extender Preparation

The stock extender was prepared using tris citric acid as a buffer, consisted of 1.56g citric acid, 3.0 g tris- (hydroxymethyl)-amino methane 0.2g fructose, 0.23 g/L ethylenediaminetetra acetic acid (EDTA) 7% glycerol and 20% egg yolk in distilled water [41].

Collection of Epididymal Spermatozoa

Sperm specimens were collected by retrograde injection of 2 mL of the tris citrate-egg yolk solution [42], through the ductus deferens and by collection of the epididdymal sperm from the proximal end of the detached ductus epididdymidis [43, 44]. Most of the sperm in the cauda epididymis was probably recovered by the procedure mentioned [41]. The specimens were collected directly into this medium, so it was not possible to estimate the color and purity of sperm samples.

This technique is needed because at the height of the rutting cycle sperm samples have a consistency close to that of thick honey and stick to plastic dish walls, making it very difficult to dilute subsequently.

Samples collected from different epididdymides from the same individual were combined and examined together because our previous observations showed that there appeared to be no variations in cell performance between the same individual tests [42]. Semen epididdymis was then filled in 0.25 French straws automatically.

Straws containing extended sperm epididdymal were cooled in a refrigerated cabinet (4 ° C), straws from each extender were placed at different cooled to 5 ° C within (0,48 hours, 72 hours, 96 hours and 120 hours), then each time from cooling Immediate sperm motility (CASA assessment), Evaluate live sperm and hypo-osmotic swelling test (HOST) responded sperm.

Sperm Motility

Computer Assisted Cell Motion Analyzer, Sperm Analyzer (Florida, USA, Genex) has been used to assess sperm motility characteristics. Samples of 5 µl are mounted in the Makler chamber (Makler Counting Chamber, Sefi-Medical Instruments, Haifa, Israel) to track and test approximately 400 fresh or cooling sperm (.400). The following parameters have been determined: sperm amount, curve line speed (VCL, µm/s), and amplitude of lateral head displacement (ALH, µm), the wobble of the sperm head (WOB) is measured as (VAP/VCL); the linearity of the curvilinear track (LIN) is measured as VSL/VCL.

Hypo-osmotic Swelling Test

Hypo-osmotic swelling test (HOST) as defined by Lodhi et al., 2008 evaluated sperm functional plasma membrane integrity. The preserved semen(sperm epididdymal) (50 L) was combined with 1 mL (150mOsmol kg-1) HOST (7.35g / L sodium citrate dihydrate and 13.51 g / L fructose) and put in a water bath at 37 µC for 30 minutes. Semen (15 µL) was dropped on a glass slide after incubation. Depending on the sperm tail plasma membrane status; the sperm was classified as reacted and unreacted. Viability of sperm was tested using the technique of Eosin-Nigrosin staining. Semen (SE) was combined in a ratio of 1:4 with eosine-nigrosine. Upon homogenization, the heating table (37 ° C) used to prepare and dry slide smears.

Live Sperm Percentages

Live sperm percentages were assayed by staining smears with Eosin-Nirgosin [18]. A total of 200 sperm cells were examined unsystematically. Live sperm percentage was recorded.

$$\% \text{ live} = \frac{\text{no. of live sperm counted}}{\text{Total no. of sperm counted}} \times 100$$

Statistical Analysis

Results are shown as the least square means ± standard media error (LS means ±SEM). ANOVA tested cooling effects on sperm motility, live sperm and hypo-osmotic swelling test, using post-hoc LSD testing to determine variations between combinations of treatments. Differences are found statistically significant with a P-value of 5 percent. Sperm motility qualities, live sperm motility, live sperm and Hypo-osmotic swelling test were compared using ANOVA. Calculations were performed with the statistical software package (SPSS).

Results

Live Sperm Evaluation

There have been important associations between the cooling time of the Live Sperm and the hypo-osmotic swelling. Live Sperm had the lowest values between all time values at 120 hours of cooling time (P<0.05), while Live Sperm epididymal sperm had lower values (P<0.05) than 48 hours and 72 hours at 96 hours of balancing time. Live Epididymal Sperm Cooling Sperm 76.40±0.93, 48 hours 68.94±2.38, initial motility ,72 hours 64.23±2.47, 96 hours 60.62±2.40 and 56.62±2.31 per cent were significantly different (Table 1).

Membrane Integrity (Hypo-osmotic swelling test)

Live Sperm cooling time and membrane integrity (P<0.05) were substantially interacted. Membrane integrity with initial motility cooling time had the lowest values compared to the gap in initial motility cooling period and 120 hours cooling time for Live Sperm and membrane integrity. The integrity of the sperm membrane of the epididymal sperm at initial cooling period of motility was significant (Table 1 and Figure1).

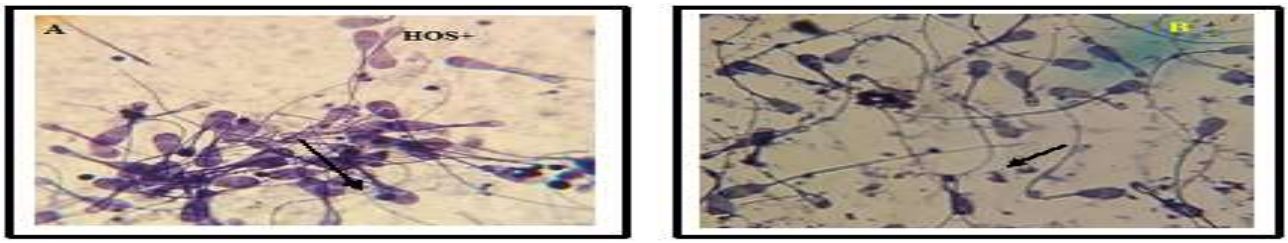

Fig. 1: Images of (A) HOST reacted (B) (+) and HOST non-reacted (-) Bovine bull spermatozoa

Table 1: Mean ±SEM live sperm and membrane integrity of epididymal sperm bovine bull using Cooling time

Live Sperm %	Cooling time	Results
	(0)initially motility	76.40±0.93A
	48 hours	68.94±2.38B
	72 hours	64.23±2.47BC
	96 hours	60.62±2.40C
	120 hours	56.62±2.31C
LSD		7.61333
HOST (%)	(0)initially motility	31.74±1.43D
	48 hours	43.04±1.43C
	72 hours	48.88±1.42B
	96 hours	54.79±1.41A
	120 hours	60.62±1.43A
LSD		5.83000

Sperm Motility (CASA Evaluation)

For other parameters tested by CASA (VAP, VSL, VCL, ALH, WOB, LIN), there were no interactions between different cooling times. In addition, different cooling times (P>0.05) affected sperm motion kinematics, i.e. VAP, VSL, VCL, ALH, WOB and LIN, but these parameters are influenced during the cooling process. With epididymal sperm cry presets, both VSL and VAP values were the highest. Cry retained (VSL: 66.25±2.21µm / s, VAP: 81.42±1.46µm / s) compared with 48 hours (VSL: 59.09±1.52µm / s, VAP: 76.67±1.43µm / s), as shown in Table 1.During the cryopreserved cooling period of 5.29±0.14µm (P<0.05), the ALH value was the lowest in epididymal sperm relative to 72 and 96 hours and 120 hours (5.03±0.13, 4.30±0.69 and 4.03±0.069µm).Nevertheless, during the cooling process of cryopreserved with the highest value at 0.80±0.026, LIN was significantly different from the cooling period (P<0.05) used for epididymal semen, and the lowest value at 120 hours was 0.77±0.026. The cooling cycle with the highest values of 0.62±0.027 at initial motility and the lowest values of 0.59±0.020 at 120 hours was significantly affected by WOB (Table 1).The VCL was significantly affected by the cooling time. The VCL value was the highest for initial motility (141.60±3.64µm / s) and the lowest for 48.72.96 and 120 hours, respectively, 128.13±3.62, 123.34±3.55, 118.85 ±3.51 and 109.48±3.49µm / s (Table 1).

Table 2: Mean± SEM CASA analysis of sperm motility parameters of epididymal sperm bovine bull using Cooling time

Variable	Cooling time				
	(0)initallymotility	**48 hours**	**72 hours**	**96 hours**	**120 hours**
VSL (µm/s)	66.25±2.21Ca	59.09±1.52Cb	55.33±1.41Cc	54.65±1.27Cc	51.20±0.76Cc
VCL (µm/s)	141.60±3.64 Aa	128.13±3.62Ab	123.34±3.55Ab	118.85±3.51Ac	109.48±3.49Ac
VAP (µm/s)	81.42±1.46Ba	76.67±1.43Bb	72.25±1.41Bb	68.59±1.38Bc	65.87±1.49Bc
ALH (µm)	5.58±0.14Da	5.29±0.14Da	5.03±0.13Da	4.30±0.69Db	4.03±0.069Db
WOB (VAP/VCL)	0.62±0.027Ea	0.61±0.023 Ea	0.59±0.026Ea	0.59±0.024Ea	0.59±0.020Ea
LIN (VSL/VAP)	0.80±0.026Ea	0.79±0.025 Ea	0.78±0.036Ea	0.77±0.027Ea	0.77±0.026Ea
LSD	15.17667	4.50400	16.92000	13.93933	14.67333

Discussion

Especial interest in the selection of sires with good sperm quality and fertility is the assessment of the functional capacity of sperm in vitro. However, further studies are needed to assess other sperm characteristics such as longevity, training, IVF sperm selection response, and zonapellucida binding capacity, including Cunha et al. [26]. Methods for sperm analysis have considerably increased in recent years. It is now possible to replace subjective motility assessment by Computer Assisted Sperm Analysis (CASA) [45, 46]. The results of this study, during cooling time within 120 hours to 48 hours of VSL (µm/s), the percent Host decreased significantly (p<0.05) were in disagreement with the findings of other scientists Swain *et al* [34] were in disagreement with the findings of other scientists Swain *et al.*[34] .Considering our results, even for a short period, bovine bull epididymides should be transported at +5°C, effects of refrigeration temperature allowed us to obtain a significant better epididymal sperm quality considering the percentage of total motility, percentage of live sperm cells because of refrigeration n total sperm motility of+ 65 percent in 120 hours compared to 96 hours. The result shows that cold storage up to 120 hours to 96 hours due to reduced sperm motility as shown in Table 2 is more resistant to cooling due to

epididymal sperm than Ejaculated semen; the values of this study were not in agreement with the results of other researchers who found that only sperm was immune. The processing temperature or post-mortem period affected live sperm cells [47, 48]. Epididymis refrigeration resulted in sustained motility and viability [49-51], the outcome of this experiment was a decreased spermatozoa metabolic rate at 5 ° C [52].

Often caused by the second result study Differences in the performance of cauda epididymal sperm cells retrieved after mortem are due to conditions of handling or species differences, under similar conditions have been reported in [53].Cold processing, however, has the beneficial effect of keeping sperm cells alive for a longer time by reducing the metabolic rate and sperm cell degeneration [54]. Hours after the death of the animal [55], remain functional. The results of this study showed that epididididymal sperm cell viability declined in a time-dependent way of storage (Table-2).Also in this study, the percentage of hypoosmotic swelling decreased significantly ($p<0.05$) from 48-72 hours, but not significantly ($p<0.05$) from 96-120 hours, as shown by the results of other scientists [56].(Table1).These results are live sperm not affected by storage temperature were disagreements with other scientists not recording live sperm cells affected by storage temperature or post-mortem [52, 56].

Conclusion

Notwithstanding reduced sperm characteristics during processing, bovine bull epididididymal sperm, stored at cooling temperature showed very good motility, viability, HOS screening, even after (0) initial motility-120 h post-mortem. By using this protocol, good epididididymal semen performance can be achieved easily at lower costs, increasing the use of this germ plasma source in gene banking [57].

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