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Prospective evaluation for retrievability and equilibrium duration on bull epididymal sperm cryopreservation

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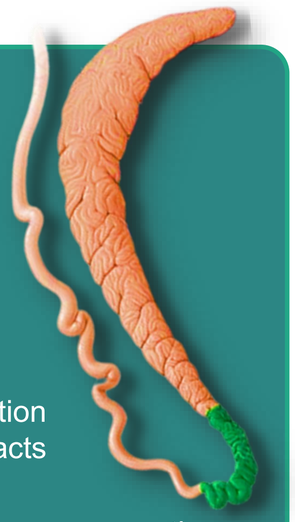
1 INTRODUCTION



- Cryopreservation of epididymal sperm is crucial for preserving the genetics of elite bulls, especially after sudden death.
- The cauda epididymis offers mature, motile sperm that can be retrieved postmortem and cryopreserved for long-term use.
- Success depends on two key factors: the sperm-retrieval technique and the equilibration time before freezing.
- Retrieval technique affects sperm yield and structure, while equilibration time influences how sperm interact with cryoprotectants, which impacts post-thaw survival.
- Evaluating these factors is essential for improving epididymal sperm cryopreservation.

2 AIM

- This study was carried out to evaluate the impact of retrieval techniques and equilibration durations on cauda epididymal sperm quality from *Bos indicus* bulls.

3 MATERIALS AND METHODS

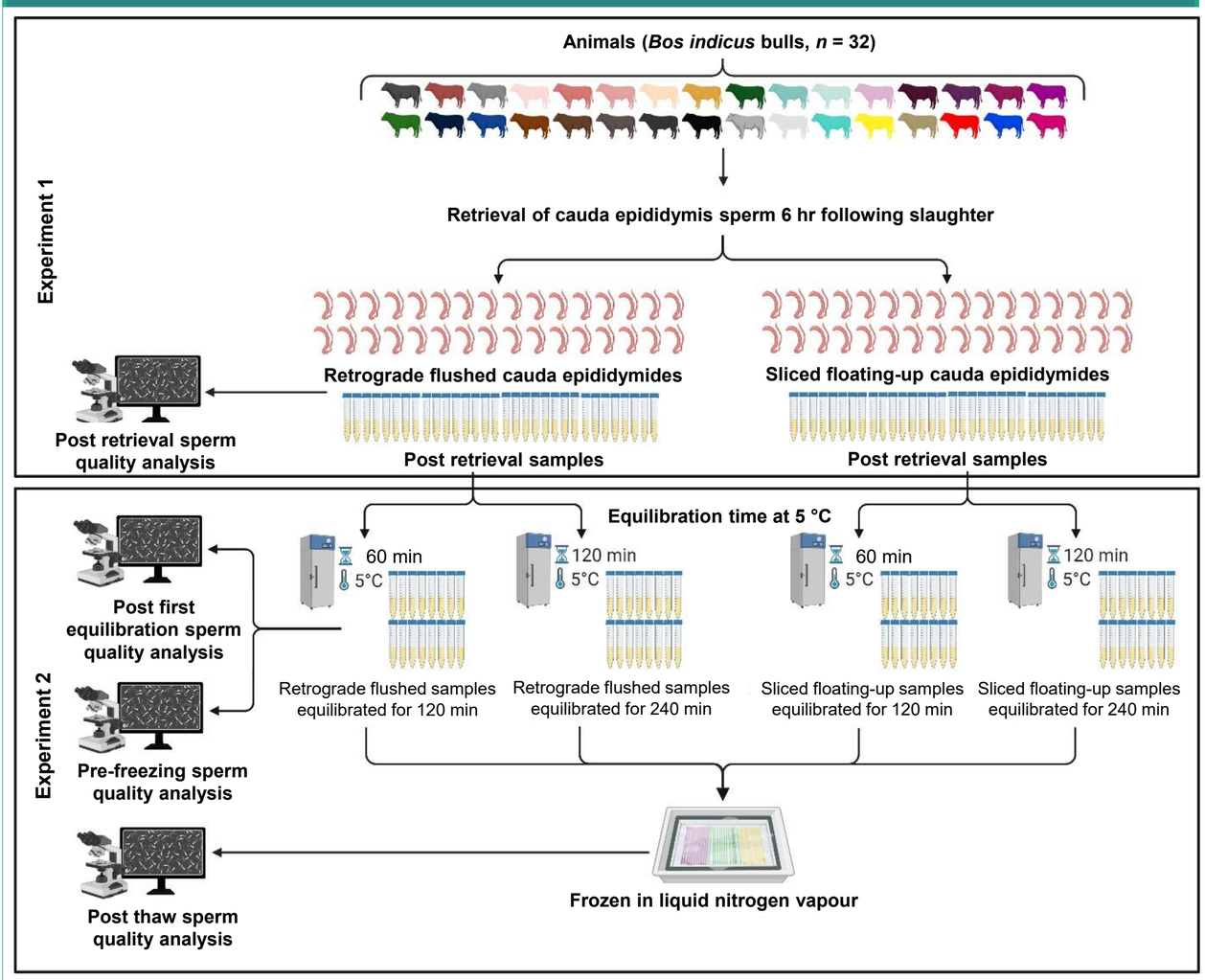


Figure 1; Schematic representation of the experimental design detailing the retrieval, dilution, equilibration, cryopreservation processing, and assessment of cauda epididymal sperm from Bos indicus during different evaluation periods; Created by BioRender.

4 STATISTICAL ANALYSIS

- Data were analyzed using ANOVA, treatment means were separated using Fisher's protected t-test least significant difference (LSD) at 0.05 level of significance (mean±SD).

5 RESULTS AND DISCUSSION

Table 1: Retrieval technique effect across evaluation periods on cauda epididymis sperm motility and kinematic parameters of *Bos indicus bulls*.

Sperm quality parameters	Evaluation periods and retrieval technique							
	Post retrieval		Post-first equilibration		Pre-freezing		Post-thaw	
	Retrograde flushing	Slicing float-up	Retrograde flushing	Slicing float-up	Retrograde flushing	Slicing float-up	Retrograde flushing	Slicing float-up
TM (%)	84.9±4.1^{a}	72.0±4.0^{b}	66.8±4.0^{c}	59.0±4.7c^{d}	70.5±3.3^{b}	66.2±3.4^{c}	54.8±4.2^{d}	42.1±5.6^{e}
PM (%)	53.5±7.1^{a}	44.5±14.6^{b}	38.6±13.2^{c}	32.1±11.8^{d}	44.1±14.0^{b}	38.1±13.0^{c}	32.1±10.8^{d}	25.2±10.1^{e}
NPM (%)	31.4±7.6^{a}	27.5±13.4^{b}	28.2±11.3^{ab}	27.0±11.6^{b}	26.4±14.7^{b}	28.1±13.3^{ab}	22.7±10.2^{c}	16.9±7.6^{d}
IM (%)	15.1±4.1^{e}	28.0±4.0^{d}	33.2±4.0^{c}	41.0±4.7^{b}	29.5±3.3^{d}	33.8±3.4^{c}	45.2±4.2^{b}	57.9±5.6^{a}
VCL (µm/s)	86.5±13.6^{a}	78.4±40.2^{b}	63.5±34.9^{c}	45.6±18.5^{e}	61.1±32.2^{cd}	59.4±27.9^{d}	39.1±12.3^{ef}	32.8±8.8^{f}
VAP (µm/s)	48.0±7.8^{a}	41.3±21.7^{ab}	32.7±19.0^{b}	23.6±10.4^{c}	31.1±17.9^{b}	28.0±13.1^{bc}	21.7±9.7^{cd}	17.4±5.4^{d}
VSL (µm/s)	36.1±9.1^{a}	29.1±19.3^{b}	22.4±16.2^{c}	14.4±9.4^{d}	21.0±15.7^{c}	16.7±10.1^{cd}	12.9±9.5^{de}	9.9±4.1^{e}
STR (%)	66.1±5.8^{a}	61.4±11.8^{ab}	57.6±11.7^{b}	51.2±9.7b^{c}	58.0±11.9^{b}	52.5±6.9^{c}	50.4±10.1^{c}	53.4±11.9^{bc}
LIN (%)	38.8±6.2^{a}	33.0±8.3^{b}	30.0±8.2^{bc}	27.3±8.0^{cd}	31.0±11.3^{c}	25.4±5.2^{d}	29.1±10.5^{bc}	29.9±10.4^{bc}
WOB (%)	56.0±4.4^{a}	52.2±5.2^{ab}	50.0±5.8^{b}	50.6±7.0^{b}	50.8±10.3^{b}	46.8±6.3^{c}	53.7±7.6^{ab}	53.8±6.8^{ab}
ALH (µm/s)	2.6±0.4^{a}	2.5±1.0^{a}	2.1±0.9^{ab}	1.5±0.5b^{c}	2.0±0.9^{b}	2.0±0.9^{b}	1.4±0.4^{bc}	1.2±0.4^{c}
BCF (Hz)	17.4±3.4^{a}	13.5±6.6^{ab}	10.8±6.8^{b}	7.0±4.7^{c}	12.6±5.7^{ab}	8.6±5.9^{bc}	7.8±6.4^{c}	4.7±3.0^{d}

TM= Total motility; PM= Progressive motility; NPM= Non-progressive motility; IM = Immotile; VCL= Curvilinear velocity; VAP= Average path velocity; VSL= Straight-line velocity; STR= Straightness; LIN= Linearity; WOB= Wobble; ALH= Amplitude of lateral head displacement; BCF= Beat cross frequency. Means±STD with different superscript letters within a row are statistically different at P < 0.05.

Table 2: Retrieval technique effect across evaluation periods on cauda epididymis sperm motility and kinematic parameters of *Bos indicus bulls*.

Sperm quality parameters	Evaluation period and equilibration time					
	Post-first equilibration		Pre-freezing		Post-thaw	
	60 (min)	120 (min)	60 (min)	120 (min)	120 (min)	240 (min)
TM (%)	59.1±4.5^{b}	69.2±2.5^{a}	63.5±2.8^{b}	70.7±2.8^{a}	44.7±7.4^{d}	52.2±7.0^{c}
PM (%)	30.1±9.3^{c}	46.2±12.8^{a}	37.1±12.9^{b}	39.5±13.9^{b}	26.2±10.8^{d}	31.1±10.7^{c}
NPM (%)	29.0±9.9^{ab}	23.1±12.9^{bc}	26.4±12.6^{b}	31.2±13.8^{a}	18.5±9.2^{d}	21.1±9.5^{c}
IM (%)	40.9±4.5^{c}	30.8±2.5^{d}	36.5±2.8^{cd}	29.3±2.8^{d}	55.3±7.4^{a}	47.8±7.0^{b}
VCL (µm/s)	44.7±19.4^{c}	67.8±38.3^{a}	52.8±23.6^{b}	64.2±28.4^{a}	33.5±11.2^{e}	38.3±10.5^{d}
VAP (µm/s)	23.4±10.9^{b}	33.6±21.4^{a}	26.0±12.2^{b}	32.4±14.2^{a}	18.7±9.8^{c}	20.4±5.9^{bc}
VSL (µm/s)	14.5±9.4^{c}	23.1±18.7^{a}	16.0±10.6^{b}	20.9±11.5^{ab}	11.6±9.3^{d}	11.2±5.0^{d}
STR (%)	52.4±8.4^{b}	57.6±12.7^{a}	51.9±9.3^{b}	57.4±10.3^{a}	54.5±13.1^{ab}	49.2±7.8^{c}
LIN (%)	27.9±7.1^{ab}	28.7±8.7^{ab}	26.3±7.6^{b}	30.9±10.4^{a}	32.2±13.2^{a}	26.8±5.3^{b}
WOB (%)	50.6±6.1^{b}	47.4±7.0^{c}	48.4±7.0^{c}	51.7±9.5^{b}	55.2±8.7^{a}	52.3±5.0^{ab}
ALH (µm/s)	1.5±0.6^{c}	2.2±1.0^{a}	1.8±0.7^{b}	2.2±0.9^{a}	1.2±0.3^{d}	1.4±0.4^{cd}
BCF (Hz)	7.1±4.9^{bc}	12.7±6.1^{a}	7.8±5.4^{b}	11.4±6.5^{a}	6.0±6.0^{c}	6.5±4.3^{c}

TM= Total motility; PM= Progressive motility; NPM= Non-progressive motility; IM = Immotile; VCL= Curvilinear velocity; VAP= Average path velocity; VSL= Straight-line velocity; STR= Straightness; LIN= Linearity; WOB= Wobble; ALH= Amplitude of lateral head displacement; BCF= Beat cross frequency. Means±STD with different superscript letters within a row are statistically different at *P* < 0.05.

6 CONCLUSION AND RECOMMENDATION

- Bull cauda epididymis sperm quality remained satisfactory when using retrograde flushing retrieval technique and equilibration for 240 minutes.
- Further research is warranted to evaluate how sub-species differences influence epididymal sperm cryotolerance in *Bos indicus* and *Bos taurus*.

7 ACKNOWLEDGEMENTS

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