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Effects of cryopreservation of somatic cells of puma, *Puma concolor* (Linnaeus, 1771) aiming at the formation of cryobanks

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INTRODUCTION

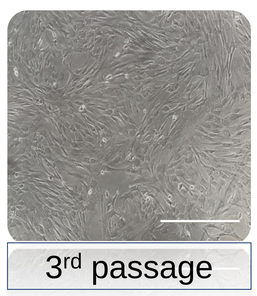
The puma is one of the largest felids in the Americas, widely distributed on the continent and with important contributions to the ecosystem, mainly as a predator. Puma populations have reduced in size mainly due to hunting in retaliation for the predation of domestic animals, burning, and being run over. A tool that is yet to be developed for this species is the establishment of somatic resource banks, such as cryobanks of somatic cells. To obtain a bank of somatic cells, the cryopreservation of these cells is essential for adequate application.

OBJECTIVE

To assess the effects of cryopreservation on the viability, metabolism, proliferative activity, levels of reactive oxygen species (ROS) and mitochondrial membrane potential (ΔΨm) in somatic cells derived from four pumas (**Fig. 1**).

MATERIAL AND METHODS

***n* = 4**



3rd passage

Cryopreservation solution
DMEM containing 1.5 M DMSO, 0.25 M sucrose, and 10% FBS

Before and after cryopreservation

EVALUATIONS

Viability	Metabolism	Proliferative activity
Trypan blue	MTT assay	Population doubling time (PDT)

Reactive oxygen species (ROS) levels	Mitochondrial membrane potential (ΔΨm)
H_2DCFDA probe	MitoTracker Red probe

Figure 1. Evaluation of cryopreservation of puma somatic cells.

RESULTS AND DISCUSION

The cryopreservation did not affect (P > 0.05) the viability, metabolism and PDT of cryopreserved cells when compared to non-cryopreserved cells (**Table 1**).

Table 1. Evaluation of cryopreservation of somatic cells obtained from puma.

	Viability (%)	Metabolism (%)	PDT (h)
Non-cryopreserved cells	79.2 ± 5.2	100 ± 13.7	31.4 ± 8.5
Cryopreserved cells	79.8 ± 4.6	100 ± 3.6	68.1 ± 18.9

The ROS levels in cryopreserved cells did not differ (P > 0.05) from those in non-cryopreserved cells. When comparing non-cryopreserved cells with cryopreserved cells, a reduction in ΔΨm was observed (**Fig. 2**).

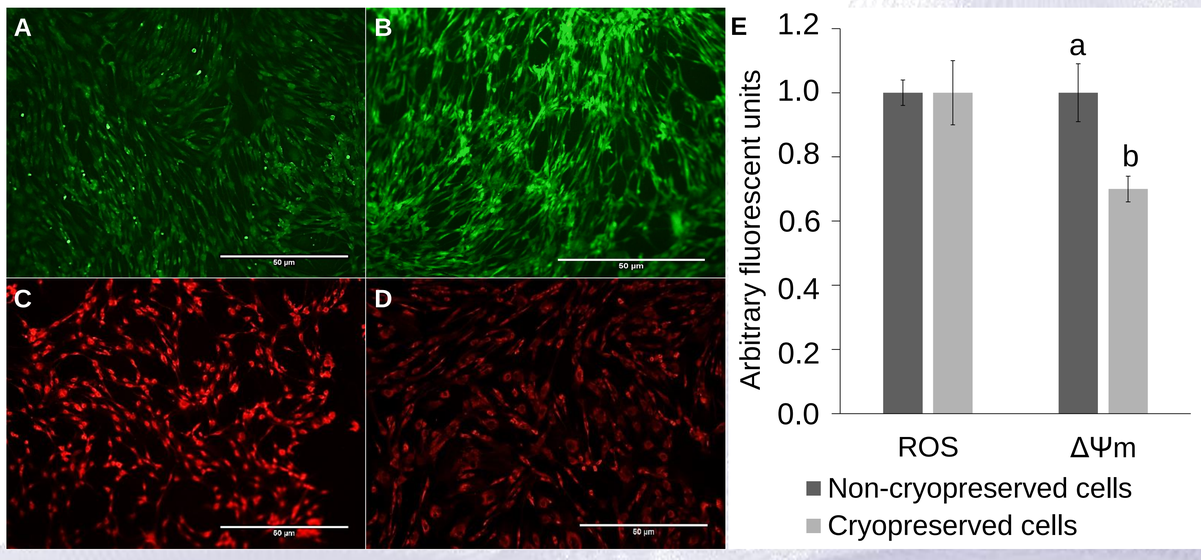


Figure 2. Influence of the cryopreservation on oxidative stress of puma cells. **(A)** Non-cryopreserved and **(B)** cryopreserved cells for evaluation of ROS levels. **(C)** Non-cryopreserved and **(D)** cryopreserved cells for evaluation of ΔΨm. **(E)** Quantification of ROS and ΔΨm levels. Bars indicate standard error. [a,b]: P < 0.05. 10× magnification.

CONCLUSION

Cryopreservation did not affect the viability, metabolic activity, or proliferative activity of the somatic cells after slow freezing. Nevertheless, cryopreservation changed ΔΨm, indicating the need for optimization of the cryopreservation protocol.

ACKNOWLEDGEMENT

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