XXIV Congresso Brasileiro de Reprodução Animal
VIII International Symposium on Animal Biology of Reproduction - Joint Meeting
CBRA

In vitro culture of fresh and vitrified preantral ovarian follicles of *Dasyprocta leporina*

N.R.N. Luz[1], E.C.G. Praxedes[1], L.G.P. Bezerra[1], A.M. Silva[1], A.F. Pereira[2], A.R. Silva[1]

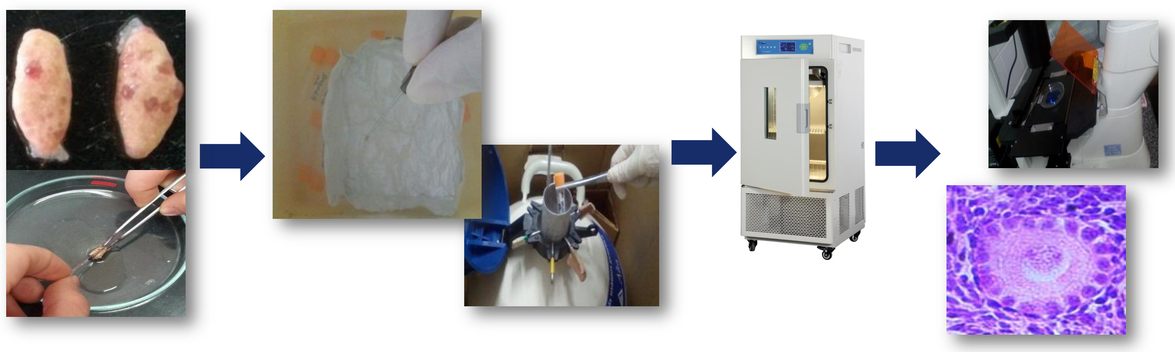
[1]Laboratory of Animal Germoplasm Conservation, UFERSA, Mossoró, RN, Brazil;
[2]Laboratory of Animal Biotechnology, UFERSA, Mossoro, RN, Brazil

1. INTRODUCTION

Dasyprocta leporina is a hystricognath rodent, with important ecological functions for acting as a seed disperser and contributing to the food chain, in addition to being used as an experimental model for endangered species. Therefore, we aimed to establish an in vitro culture system (IVC) of fresh and vitrified preantral ovarian follicles (PAFs) from agouti using TCM-199 medium supplemented with follicle stimulating hormone (pFSH).

2. MATERIAL AND METHODS

Six pairs of sexually mature persistence ovaries from agouti from CEMAS / UFERSA, Mossoró, Brazil, were collected, washed and fragmented (9.0 mm3 = 3 × 3 × 1 mm). Two fragments were prepared to a new control group, while other fragments were vitrified using a solid surface procedure. After one week, the vitrified samples were rewarmed and subjected to a 6-day IVC in an incubator at 38.5°C and 5% CO_2, using TCM-199 supplemented with 50 ng/mL pFSH.



Fresh, vitrified and cultured samples were evaluated for morphology and development (proportion among growing and primordial follicles) by a histological procedure, and viability through a Trypan blue assay. Data were expressed as means (± SEM) and evaluated by ANOVA followed by PLSD Fisher ($P < 0.05$).

3. RESULTS AND DISCUSSION

Fresh samples presented 71.8 ± 2.1% (71/98) morphologically normal follicles, similarly ($P > 0.05$) as verified for vitrified (67.5 ± 13.9%; 76/119) and vitrified-cultured (76.2 ± 7.2%; 61/82) samples. Regarding PAFs development (**table 1**), we found the proportions of 73.9 ± 18.7%, 69.7 ± 13.5% and 68.0 ± 9.4% developing PAFs in the fresh, vitrified and vitrified-cultured samples, respectively. No differences were found among treatments ($P > 0.05$).

Table 1. Means ± SEM for the normal morphology of *Dasyprocta leporina* PAFs in the fresh control group and in samples submitted to solid surface vitrification and then cultured for 6 days. %PMNF: percentage of morphologically normal follicles.

	Morphologically normal follicles(%)		
Treatments	**Primordial**	**Primary**	**% PMNF***
Fresh control	68.4 ± 6.6[b] (44/63)	73.9 ± 18.7[a] (27/35)	71.8 ± 2.1[a] (71/98)
Vitrified	74.2 ± 12.4[b] (54/79)	69.7 ± 13.5[a] (22/40)	67.5 ± 13.9[a] (76/119)
Vitrified-cultured	95.2 ± 4.7[a] (24/26)	68.1 ± 9.4[a] (37/56)	76.2 ± 7.2[a] (61/82)

a, b Different superscript letters indicate significant differences in the same column ($P < 0.05$).

For viability, fresh samples presented 88.3 ± 4.8% (106/120) viable follicles, similarly ($P > 0.05$) as found for vitrified samples 65.8 ± 11.4% (79/120); however, a decrease ($P < 0.05$) on the percentage of viable follicles was verified for vitrified-cultured samples (60.0 ± 9.2%; 73/120) in comparison the fresh control. In summary, we provide initial data regarding the establishment of a IVC system for agouti PAFs, essential for the utilization of female germplasm stored in biobanks.

4. CONCLUSION

The use of TCM-199 medium supplemented with pFSH seems to be adequate for this proposal, but more studies are applied to improve the protocol in order to provide efficient development of the PAFs of *Dasyprocta leporina* submitted to the in vitro culture system.

Acknowledgements: