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Short-term in vitro culture of canine ovarian tissue after cryopreservation by different techniques

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INTRODUCTION

The application of different ovarian tissue vitrification techniques has been highlighted as an alternative to promote the formation of female germplasm banks. Given the scarcity of studies in canine species, this study aimed to use *in vitro* culture as an evaluation tool for different ovarian tissue vitrification techniques as ovarian tissue cryosystem (OTC) and needle immersion vitrification (NIV) in female dogs.

METHODS

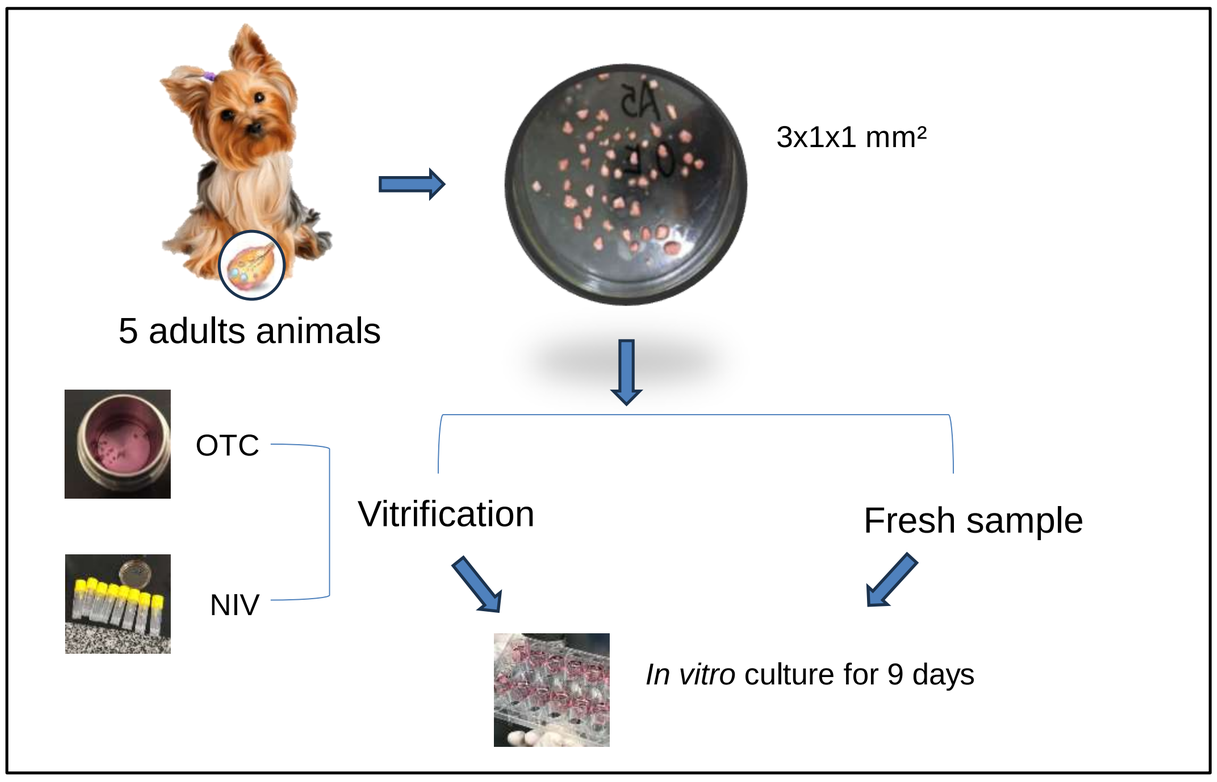

Figure 1. Experimental Design showing canine ovarian fragments in fresh control group and subjected to two vitrification methods as ovarian tissue cryosystem (OTC) and needle imersion vitrification (NIV) followed of in vitro culture for 9 days.

Vitrification of canine ovarian fragments was conducted as reported on Figure 1. After *in vitro* culture, the fresh and vitrified fragments were evaluated for preantral follicles viability using a Trypan blue die and morphology through classic histology. Ultrastructural integrity of the ovarian tissue was checked by scanning electronic microscopy. Data were expressed as mean and standard error and compared by the Tukey test ($P < 0.05$).

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RESULTS & DISCUSSION

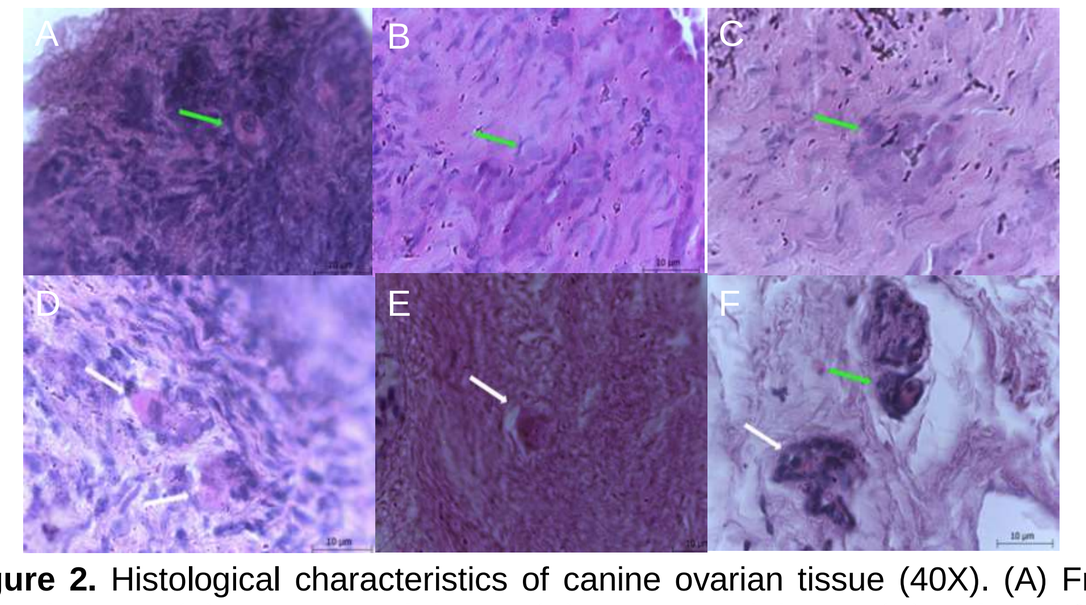

Figure 2. Histological characteristics of canine ovarian tissue (40X). (A) Fresh control tissue, (B) OTC control tissue, (C) NIV control tissue, (D) Cultured fresh tissue, (E) Cultured OTC tissue (F) Cultured NIV tissue. Green arrows indicate intact primary follicles and White arrows indicate degenerated primary follicles

For viability, the control group presented 77.2 ± 2.81%, similar to that control cultured for 09 days (72.6 ± 1.8%), and also similar to both OTC (85.7 ± 3.6%) and NIV (75.7 ± 2.8%) treatments immediately after thawing. After 09 days culture, however, viability in the OTC group dropped to 64.6 ± 16.2% ($P < 0.05$), while NIV maintained 72.8 ± 7.2% viability.

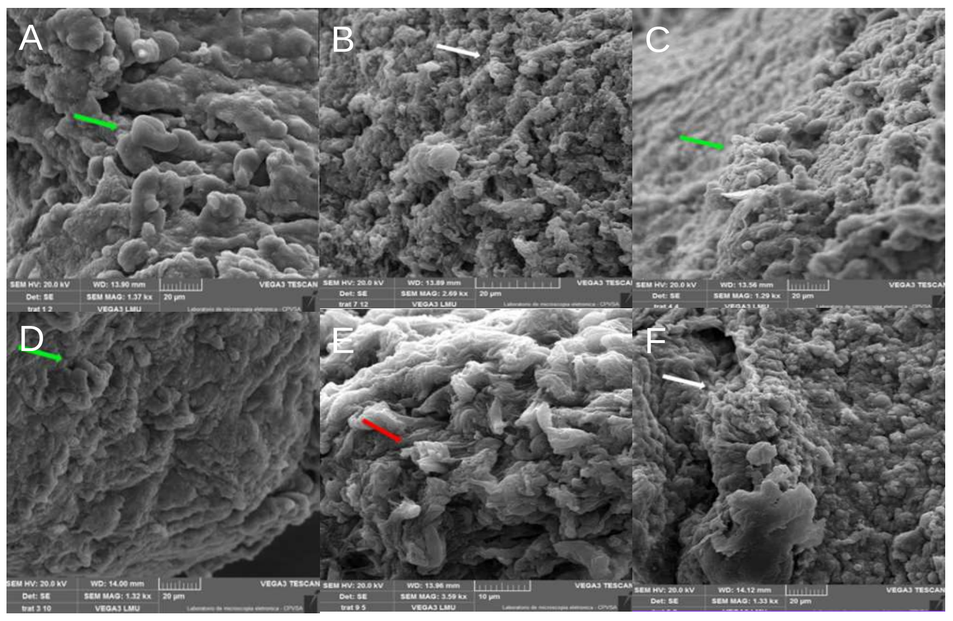

Figure 3. SEM ultrastructure PFs. (A) Fresh control, (B) OTC control tissue, (C) NIV control tissue, (D) Fresh cultived, (E) OTC cultived, (F) NIV cultived. Green arrows indicate tissue with ovarian stromal organization and White arrows indicate reduction in the size of ovarian stromal cells, Red arrow indicate ovarian stromal disorganization

CONCLUSION

In conclusion, NIV technique provided a most efficient preservation of canine ovarian tissue than OTC.