Programa de Doctorado en Ciencia y Tecnología de la Producción Animal
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Cryopreservation of rabbit embryos using Minimum volume vitrification devices

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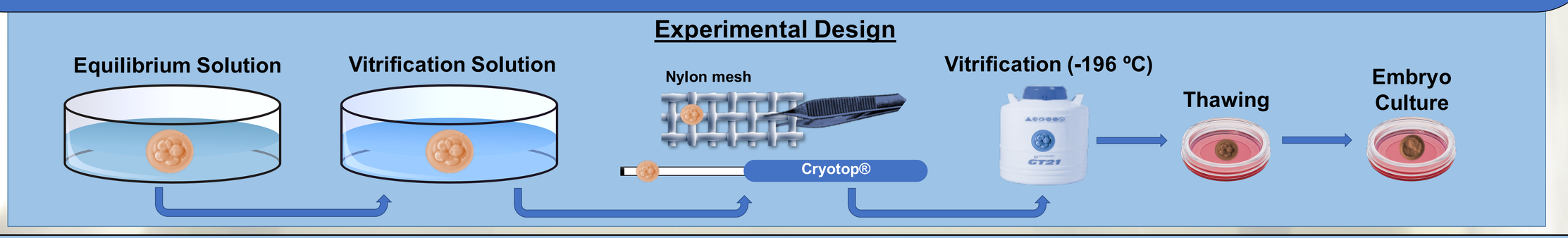
INTRODUCTION

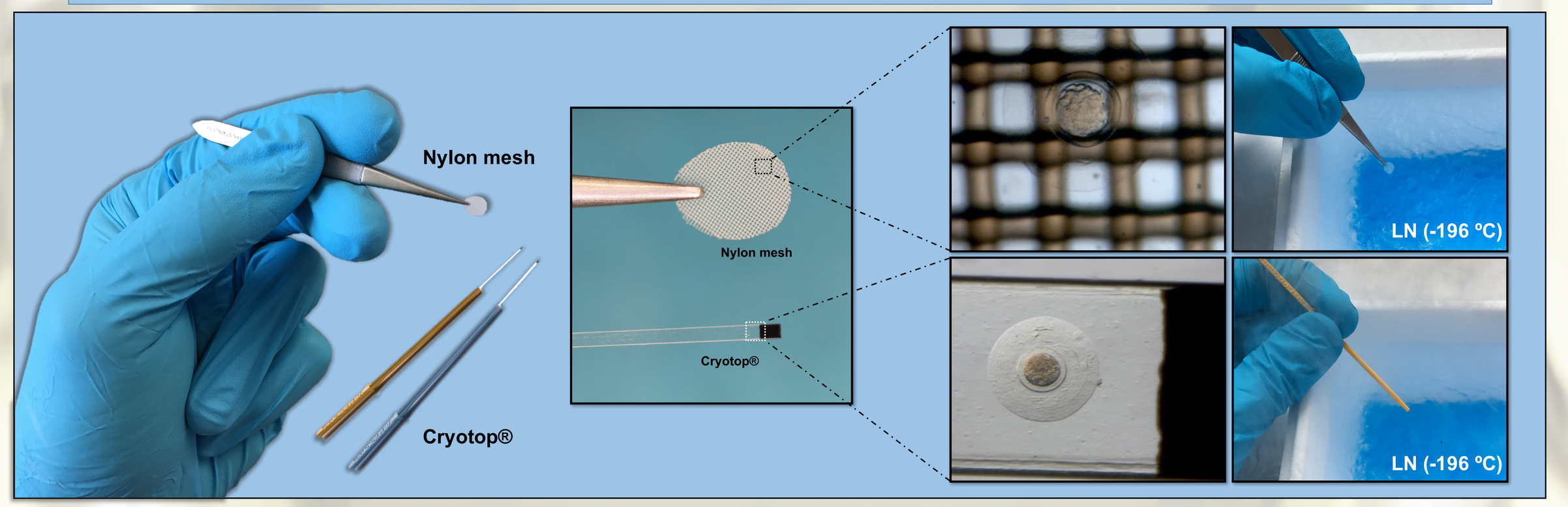
Nowadays, vitrification is being widely utilized in livestock and human embryos with very satisfactory outcomes by means of methodologies that use a minimal volume, which allow to increase the cooling/thawing rate improving the outcomes. Many techniques have been developed to reduce sample volume with an explosion of methods appearing in the literature over the last decade. With this aim, more than 20 vitrification devices are commercially available, but these devices are rather expensive since it was intended for human use. In addition, devices designed to reduce the volume are difficult to produce in-house. This study was therefore designed to compare the efficacy of the Cryotop® and nylon mesh devices for embryo vitrification by analysing the subsequent in vitro development rate in rabbit.

MATERIALS & METHODS

To assess this issue, nulliparous donors were superovulation with one subcutaneous injection of corifollitropin alfa (3 µg, Elonva®). Sixty hour after, does were inseminated (AI) and the embryos were collected 72 h after AI. Then, rabbit embryos were vitrified using cryotop® (n=110) and nylon mesh (n=103). Fresh embryos (n=110) were used as a control.

Embryos were vitrified in a two-step addition procedure; equilibrium (10% EG + 10% Me2SO + 10% Dextran) for 2 minutes and vitrification (20% EG + 20% Me2SO + 10% Dextran) for 1 minutes. When the embryos were loaded in the corresponding device, embryo vitrification was achieved by direct plunging in liquid nitrogen (LN). After thawing, the vitrification medium was removed rinsing the embryos into a solution containing DPBS with 0.33 M sucrose for 5 min. Then, embryos were cultured for 48 hours, evaluating the in vitro developmental rate until hatching/hatched blastocyst. A p value of less than 0.05 was considered to indicate a statistically significant difference.





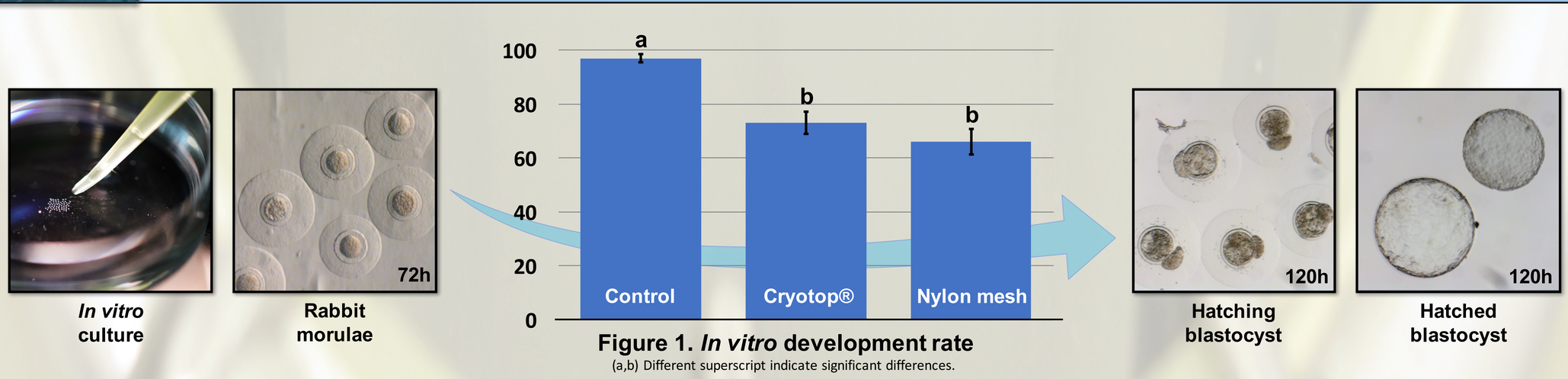


Figure 1. *In vitro* development rate
(a,b) Different superscript indicate significant differences.

The rates of embryo development (Figure 1) were similar between vitrified groups (Cryotop® and nylon mesh devices), which are lower than in the fresh group (Cryotop®: 73 ± 4.2%; nylon mesh: 66 ± 4.7%; control: 97 ± 1.6%; P<0.05). We conclude that nylon mesh could be applicable in rabbit embryos vitrification and our results suggest that it could also be widely applicable in others species, at negligible cost.

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