Pregnancy after short exposure of cryopreserved porcine embryo to cryoprotective agents

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Florence Guignot[1], Florine Dubuisson[1], Camille Voiry[1], Eric Royer[2], Anais Giovanetti[2], Christian Moussu[1], Frédéric Elleboudt[1], Jonathan Savoie[2], Henri Woelders[3], Pascal Mermillod[1], Elisabeth Blesblois[1]

[1]INRA, UMR85-PRC, F-37380 Nouzilly, France; [2]INRA, UEPAO, F-37380 Nouzilly, France; [3]Wageningen UR Livestock Research, Wageningen, the Netherlands.

The pig industry has nowadays an increasing demand for a reliable and cost-effective porcine embryo cryopreservation allowing long-term conservation, transport and widespread dispersion of high-quality genetics resources. Progress in embryo vitrification process made it possible to use the method in pigs, but lower and variable pregnancy rates are achieved with frozen embryos compared to fresh one. High concentrations of cryoprotective agents (CPAs) used for vitrification are believed to negatively affect developmental competence (Woelders et al, Cryobiology, 2018).

The aim of the present study was to test the viability of cryopreserved porcine embryo after short exposure to CPAs during vitrification process.

Embryos were surgically recovered 6 days after ovulation from Large White sows. Only embryos at the blastocyst stage were selected and vitrified in superfine open pulled straw (SOPS). Embryos were firstly placed in equilibration solution (ES) containing 7.5% ethylene glycol (EG) and 7.5% DMSO, and then in vitrification solution (VS), containing 16% EG, 16% DMSO and 0.4M sucrose. Embryos were incubated 2 min in ES and 30 sec in VS (short exposure to CPAs) or 3 min in ES and 1 min in VS (control, Cuello et al, RFD, 2010). Embryos were then loaded into straws and plunged into liquid nitrogen. After thawing, they were transferred to Talp-Hepes PVA with decreasing sucrose concentrations (0.13 and 0M) for 5 min each. In vitro and in vivo survival were tested. For in vitro survival, embryos were cultured for 3 days in 50µL of NCSU-23 + 10% FCS at 38.8°C in a humidified atmosphere of 5% CO_2 in air. For in vivo survival, embryos were surgically transferred in uterine horn of synchronised Meishan recipient (30 blastocysts per recipient).

Three hundred and four embryos were used to test in vitro embryo survival after short and control exposure to CPAs. In the first experiment, the survival rate was better with shorter exposure to CPAs (66.2% vs 45.6%, p=0.008, n=145), but in the second experiment, it was identical (47.4% vs 60.6% ; p=0.101, n=159). New embryos were produced and collected (n=157) to test in vivo survival. Transfers were performed with embryos vitrified according to short exposure to CPAs. Among 4 recipients, one is pregnant. Farrowing is expected in next weeks.

Our results show that a short exposure to CPAs is as efficient as the longer exposure usually employed for porcine embryo vitrification. As short exposure decreases the embryos contact with high level of toxic CPAs, reducing potential harmful and epigenetic modification of embryonic genome, this short exposure to CPAs should be chosen for porcine embryo vitrification.

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