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Development of OPS vitrified pig blastocysts: Effects of size of the collected blastocysts, cryoprotectant concentration used for vitrification and number of blastocysts transferred

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Abstract

Unhatched blastocysts from Large White hyperprolific gilts ($n = 103$) were identified, measured and vitrified using the Open Pulled Straw (OPS) technique to evaluate the effects of the collected blastocyst size and cryoprotectant concentrations used for vitrification, and the number of embryos transferred per recipient. Vitrified/warmed blastocyst viability was estimated *in vitro*, as the percentage of embryos developing after 72 h, and *in vivo*, on pregnancy Day 30. In the *in vitro* study, we compared the use of three cryoprotectant concentrations (16.5, 18, or 20% DMSO + 16.5, 18, or 20% EG + 0.4 M sucrose). Survival rates differed significantly between the control (98.3%) and the three cryoprotectant concentrations (67, 62.3, and 57%, respectively). Blastocyst size at vitrification determined the further *in vitro* development of embryos (26% survival for blastocysts 126–144 μm versus 100% for blastocysts >199 μm). For the *in vivo* study, blastocysts were vitrified using cryoprotectant concentrations of 16.5 or 18% DMSO + EG and transferred surgically in groups of 20 or 30 per recipient ($n = 40$). Recipients were slaughtered on pregnancy D30. No significant differences were detected in gestation rates (50–70%) and embryo survival rates (14.7–25%), although survival was higher ($P = 0.0003$) when 20 blastocysts were transferred compared to 30 (24.7% versus 15.5%). Our findings indicate that best results, in terms of subsequent *in vivo* embryo survival, were achieved after transferring 20 embryos at the blastocyst or expanded blastocyst stage, previously vitrified using cryoprotectant concentrations of 16.5 or 18%.

Keywords: Porcine; Blastocyst; OPS vitrification; *In vitro* development; Embryo transfer

1. Introduction

Embryo transfer and cryopreservation techniques have recently attracted the attention of the pig production industry, since these technologies allow the transport and storage of valuable genetic material with a reduced impact on the animals and minimal costs. The main advantages of these new technologies are: a minimized risk of disease transmission if the embryos are protected by an intact zona pellucida [1], reduced welfare problems and avoiding the adaptation of transferred animals to their new environment, since embryos rather than adult or prepubertal animals are transported.

The commercial and practical applications of embryo transfer in the pig have nevertheless so far been limited by the need for surgical transfer procedures

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and difficulties encountered in the long-term storage of pig embryos. This has led to the recent development of non-surgical embryo transfer procedures [2,3] and embryo cryopreservation methods have been improved [4]. Vitrification has been explored as an alternative to traditional freezing methods, which did not give satisfactory results with porcine embryos. The Open Pulled Straw (OPS) technique has the advantage that cooling and warming rates of vitrification are increased [5]. In effect, a small volume of cryoprotectant medium surrounding the oocytes and embryos and direct contact with liquid nitrogen provide very fast cooling rates and the method has been successfully used to vitrify the oocytes or embryos of several species including pigs. OPS technology has resulted in live piglets after the surgical and non-surgical transfer of vitrified/warmed embryos [6,7].

It has been established that pig embryo survival depends upon the stage of embryonic development when vitrified [8]. Several authors have reported that pig embryos at the blastocyst- or peri-hatching blastocyst stages seem to tolerate cryopreservation best [4,9].

Factors such as the cryoprotectant concentration used during vitrification and the number of vitrified/warmed embryos transferred can affect embryo survival. Better survival rates to farrowing after the transfer of vitrified morulae are obtained using low cryoprotectant concentrations [10] but to date there are no reports of the effects of cryoprotectant concentrations on outcomes using vitrified blastocyst stage embryos.

The transfer of vitrified/warmed embryos is generally performed using a pool of 16–38 embryos [11–14]. Several studies have directly or indirectly assessed the appropriate number of fresh embryos to transfer to a recipient female. These reports have indicated that the transfer of more than 20 fresh embryos per recipient may not be warranted [15–18], yet this question has not been addressed using vitrified/warmed blastocyts.

The present study was designed to establish whether blastocyst size at the time of vitrification can influence their *in vitro* development and whether the cryoprotectant concentration and number of vitrified/warmed embryos transferred affect the fetal survival rates.

2. Materials and methods

2.1. Animals

Experiments were conducted at the INRA experimental farm in Nouzilly (France). Embryo donors were Large White hyperprolific (LWh) cyclic gilts (aged from 7 to 8 months) and recipients were cyclic Meishan gilts aged from 6 to 7 months when they were introduced into the experiments.

Animals were allocated individually into crates in a mechanically ventilated confinement facility and fed with a commercial ration of 3 kg/day. Water was provided *ad libitum*.

2.2. Embryo production and collection

For the two experiments, LWh donor gilts ($n = 103$) were synchronized and superovulated. Two injections (10 and 16 h) of 175 μg of cloprostenol i.m. (Planate®, Shering-Plough Vétérinaire, France) were given on Days 13, 14, or 15 of the estrus cycle (Day 0 = first day of estrus). Superovulation was induced by injecting 800 IU eCG i.m. (Chrono-Gest® Intervet, France) 24 h after the second cloprostenol injection. Ovulation was induced by administration of 500 IU hCG i.m. (Chorulon®, Intervet, France) 48 h after the eCG treatment.

Estrus detection was performed twice a day by exposing females to a mature boar. The donors were artificially inseminated 12 and 24 h after first exhibition of estrus. Only gilts showing signs of estrus 24–36 h after hCG treatment were used as donors.

The semen used for artificial insemination (AI) was collected from adult Pietrain boars. Seminal doses (3×10^9 spermatozoa per A.I.) were prepared at INRA, UEICP (Rouillé, France). The embryo genotype was therefore Pietrain × Large White hyperprolific (P × LWh). Donors were slaughtered in the experimental slaughterhouse of the INRA, Nouzilly (France) at Day 5.5–6 of the estrus cycle. The genital tract was collected immediately after slaughter and before scalding of the gilts. The numbers of corpora lutea in the ovaries were counted. Embryos were recovered some 10 min after slaughter by flushing the uterine horns with saline solution (0.9% NaCl) containing 2% newborn calf serum (NBCS; Bio Whittaker S14416, Belgium). Embryos were evaluated under a stereomicroscope at a magnification of 60×, measured (diameter inside of zona pellucida) and classified as follows:

- Unfertilised "oocytes" or embryos with an abnormal developmental stage,
- Late morulae/early blastocysts: diameter between 126 and 144 μm,
- Early blastocysts: diameter between 145 and 162 μm,
- Blastocysts: diameter between 163 and 180 μm,
- Expanded blastocysts: diameter between 181 and 198 μm,