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Changes to porcine blastocyst vitrification methods and improved litter size after transfer

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Abstract

The objective was to improve the protocol that was used to obtain the first reported piglets from transferred vitrified and warmed zona-intact blastocysts. Blastocysts were collected from super-ovulated sows and gilts, centrifuged to polarize lipid, vitrified, warmed and cultured for 24 h or transferred immediately. Removing the zona pellucida after warming increased the number of cells in the surviving blastocysts (zona-free 60.8 ± 4.3, zona-intact 39.1 ± 2.8; $P < 0.05$). Thinning the zona pellucida produced similar results to zona removal. Changing the basal medium of the vitrification and warming solutions from modified PBS to phosphate buffered NCSU-23 increased the number of cells (44.7 ± 2.2 versus 56.0 ± 3.9, respectively; $P < 0.05$). Reducing the plunge temperature of the liquid nitrogen from −196 °C to less than −204 °C improved the embryo survival rate (61.9% versus 82.9%, respectively; $P < 0.05$). These modifications were incorporated into the vitrification protocol that was used to vitrify and warm 105 blastocysts (that were subsequently transferred into four recipients). Three recipients became pregnant, farrowing three litters (average litter size, 5.3; 18.8% embryo survival in farrowing sows). Changing the warming protocol to using sucrose rather than ethylene glycol resulted in a trend towards improved embryo survival (73.5% versus 91.2%) but this was not statistically significant. Incorporating this modification, 203 blastocysts were vitrified, warmed and transferred into seven recipients. Five became pregnant and 36 fetuses were recovered (average litter size 7.2; 24.8% embryo survival in pregnant sows) at Day 40 of pregnancy. In

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conclusion, changes made to the vitrification protocol improved pregnancy rate and in vivo embryo survival compared to an earlier study using the original protocol.

Keywords: Pig; Blastocysts; Vitrification; Litter size

1. Introduction

As techniques such as genetic engineering and cloning produce pigs of extremely high genetic value, the ability to reliably cryopreserve zona pellucida intact pig embryos would be of major benefit to the pig industry. Cryopreservation allows the indefinite storage of genetic material, protected from disease outbreak and would facilitate low-cost international movement of selected genetics with a minimal risk of disease transmission.

Porcine early preimplantation embryos were found to be very sensitive to temperatures below 15 °C [1,2]. Older preimplantation stages displayed greater cooling tolerance; piglets have been produced from transferred vitrified and warmed peri-hatching blastocysts [3–5]. However, these embryonic stages are no longer protected by an intact zona pellucida, which is a prerequisite for establishing minimal disease risk and a requirement for most protocols for the international transport of embryos [6].

Nagashima et al. [7] discovered that reducing the lipid content of early preimplantation porcine embryos by centrifugation and micromanipulation improved their ability to survive freezing. Using this technique, piglets have been born from frozen and vitrified early preimplantation embryos [8,9]. However, the micromanipulation required to remove the lipid compromised the integrity of the zona pellucida. It was subsequently found that removing the lipid by micromanipulation after centrifugation was not necessary, leading to the first report of piglets born from entirely zona-intact, vitrified early blastocysts [10]. Since then, there has been considerable progress, with reports of litters of piglets from vitrified blastocysts and morulae using a variety of protocols [11–14]. While pregnancy rates of around 80% have been achieved [11–13,15], the reported rates of total embryo survival in vivo in these studies were fairly low (range, approximately 10–15%). A higher rate of embryo survival is needed to make these techniques suitable for commercial application.

This study reports on development of the vitrification protocol that was used to obtain the first reported piglets resulting from the transfer of vitrified and warmed zona-intact blastocysts. It was developed to a point where a large on-farm transfer trial was conducted, producing what was considered commercially acceptable results [15].

2. Materials and methods

2.1. Embryo collection and in vitro culture

Early blastocysts were obtained from mature Large White × Landrace sows and gilts. The sows were on their seventh or eighth parity and were being culled for age. Sows were