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Open pulled straw vitrification of goat embryos at various stages of development

A.N. Al Yacoub, M. Gauly, W. Holtz *

Department of Animal Science, Georg-August-University, Goettingen, Germany

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Abstract

This investigation addresses the question whether it is possible to apply the open pulled straw (OPS) vitrification method, found to be effective for cryopreserving caprine (*Capra aegagrus hircus*) blastocysts, to other embryonal stages. Morulae, blastocysts and hatched blastocysts were cryopreserved by way of OPS vitrification and blastocysts and hatched blastocysts by conventional freezing. Morulae were not included with conventional freezing because in our experience the survival rate is very low. To assess the viability of the cryopreserved embryos, they were transferred to synchronized does; in most cases, two embryos per doe. After OPS vitrification, of nine does receiving morulae, not a single one became pregnant; of 11 does receiving blastocysts, nine (82%) became pregnant (all of which kidded and gave birth to, on average, 1.8 kids); and of nine does receiving hatched blastocysts, three (33%) became pregnant (two of which [22%] kidded, giving birth to a single kid each). After conventional freezing, of 10 does receiving blastocysts, five became pregnant (four of which [40%] carried to term and gave birth to a pair of twins each); and of nine does receiving hatched blastocysts, three (33%) became pregnant (and gave birth to a single kid each). Embryo survival (kids born/embryos transferred) after vitrification for morulae, blastocysts, and hatched blastocysts was 0, 70% (16 of 23), and 13% (2 of 16), respectively, and after conventional freezing for blastocysts and hatched blastocysts was 42% (8 of 19) and 19% (3 of 16), respectively. The difference in pregnancy and kidding rate between vitrified and conventionally frozen blastocysts was significant, and so was the difference in pregnancy rate between hatched and nonhatched blastocysts, regardless whether OPS-vitrified or conventionally frozen. The results of the current study indicate that OPS vitrification is a very effective means of cryopreserving caprine blastocysts. Unfortunately, the superiority of OPS vitrification over conventional freezing does not apply to caprine morulae and hatched blastocysts.

Keywords: Cryopreservation; Embryo transfer; Goat; Open pulled straw; Vitrification

1. Introduction

The cryopreservation of gametes and embryos can be instrumental in the preservation of genetic variability, conservation of populations threatened by extinction, propagation and transport of superior breeding stock, and, in some situations, solution of fertility problems. In cattle breeding, the cryopreservation of supernumerary preimplantation embryos from superovulated donors has become an important tool in reproduction management although the transfer of cryopreserved embryos generally yields somewhat lower pregnancy rates than of fresh embryos. The vitrification technique prevents cell damage caused by intracellular ice crystal formation. It implies the use of a viscous medium containing high concentrations of penetrating cryoprotectants and an extremely rapid cooling rate, bringing about a glass-like solidification of liquids without ice crystals [1,2].

* Corresponding author. Tel.: +49 551 395605; fax: +49 551 395587.
E-mail address: wholtz@gwdg.de (W. Holtz).

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Despite the fact that vitrification is faster, cheaper, and more effective [1,3,4], conventional freezing still is the prevailing means of embryo cryopreservation in both large and small ruminants.

In many parts of the world, goats are an important domestic species, supplying milk, meat, and fiber; in addition, the goat may serve as a convenient model for other ruminants. Bilton and Moore [5] were the first to successfully cryopreserve caprine embryos. The first successful vitrification of goat embryos was reported by Yuswiati and Holtz [6]. In a previous study [1], it was shown that caprine blastocysts subjected to the open pulled straw (OPS) vitrification technique established by Vajta et al. [7] produces significantly better pregnancy and embryo survival rates than those cryopreserved by conventional freezing.

With conventional freezing, goat morulae are known to have poor postthaw survival rates [8,9]. There is no information available on the vitrification of goat morulae or on other stages than blastocysts.

2. Materials and methods

2.1. Animals

The experiment was conducted on Boer goats (*Capra aegagrus hircus*) from our own breeding flock at Goettingen, Germany (51°46′N, 9°41′E), during the breeding season (October to January). The goats were group-housed in open barns with straw bedding and an outdoor concrete run. They were fed a daily ration of 600 g concentrate, consisting of equal parts of a pelleted diet for breeding ewes (16% crude protein, 10.2 megajoule metabolizable energy/kg supplemented with 43 mg/kg Se, 12 mg/kg I, and 5000 mg/kg Zn), oats, and dried sugar beet pulp and had free access to wheat or barley straw, salt lick, and water. Once daily, the complete flock was routinely tested for estrus with an aproned male.

2.2. Donor preparation and superovulation

The estrous cycles of donor does with serum progesterone concentrations in excess of 5 ng/mL (assessed by ELISA [10,11]) were synchronized by treating them with dinoprost (5.0 mg im; Dinolytic; Pharmacia and Upjohn, Erlangen, Germany) followed, 7 d later, by im administration of 0.004 mg of the GnRH analog buserelin (1 mL Receptal; Intervet, Unterschleissheim, Germany). Superovulation was induced by six sc administrations of 4, 4, 2, 2, 2, and 2 Armour units of porcine Follicle Stimulating Hormone (pFSH) supplemented with 40% porcine Luteinizing Hormone (pLH) [12] at 12-h intervals, beginning 5 d after the dinoprost treatment. Along with the last two pFSH administrations, the does received two im doses of 5.0 mg dinoprost. To induce ovulation, 18 h after the last treatment with dinoprost the does were administered 0.004 mg of the GnRH analog buserelin im (1 mL Receptal) or 500 IU im human chorionic gonadotropin (hCG; 1 mL Chorulon; Intervet, Beaucaucé, France) or 1 mL sterile physiologic saline solution. The does were tested for estrus with an aproned male at 6-h intervals and hand mated twice daily as long as they would permit a male to mount. To counteract occasionally occurring premature corpus luteum (CL) regression, 12 h after the last mating the donors were provided with a progestogen-containing ear implant (3.3 mg norgestomet; Crestar; Intervet, Beaucaucé, France), which was removed 20 h before embryo collection together with the im administration of 5.0 mg dinoprost.

2.3. Embryo collection

To obtain morulae, blastocysts, and hatched blastocysts, does were flushed on Days 6, 7 to 8, and 8.5 to 9 after the last mating, respectively, applying the transcervical flushing technique described elsewhere [13,14]. The flushing medium consisted of Dulbecco's phosphate buffered saline (PBS) supplemented with 2% bovine serum albumin (A9647; Sigma, Steinheim, Germany) at a temperature of 39 °C. The embryos were recovered from the flushings under a stereomicroscope (M8; Wild, Heerbrugg, Switzerland) and classified according to their morphologic appearance [15].

2.4. Cryopreservation of embryos

Morphologically intact embryos at the morula, blastocyst and hatched blastocyst stage classified as ''very good'' or ''good'' were randomly assigned to either conventional slow freezing or vitrification by the OPS method as described elsewhere [1]. Only blastocysts and hatched blastocysts were subjected to conventional freezing, whereas vitrification was conducted on morulae, blastocysts, and hatched blastocysts.

Embryos to be conventionally frozen were washed three times, for 3 min at a time, in M2 medium [16]. They were then sequentially transferred for 10, 10, and 20 min to M2 medium supplemented with 0.5, 1.0, and 1.5 M ethylene glycol (E9129; Sigma), respectively. Two embryos at the same stage of development from the same donor doe were loaded into 0.25-mL straws (Minitüb, Tiefenbach, Germany) labeled with donor number, stage and quality of the embryos, and date. Up