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In vivo development of vitrified rabbit embryos: Effects of vitrification device, recipient genotype, and asynchrony

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ABSTRACT

This study was designed to evaluate the effects of vitrification device, recipient genotype, and recipient asynchrony on implantation rate, offspring rate at birth, and fetal losses of rabbit embryos. Morphologically normal embryos (N = 787) recovered at 72 hours of gestation were kept at room temperature until transfer or vitrification. Vitrified embryos in Cryotop and ministraw devices were transferred into females induced to ovulate 60 hours (asynchrony) or 72 hours (synchrony) before transfer. In addition, recipient genotypes were analyzed (maternal and paternal genotype). The number of implanted embryos was estimated by laparoscopy as number of implantation sites at day 14 of gestation. At birth, total kits born were recorded. Fetal losses were calculated as the difference between total born at birth and implanted embryos. Our data show that a combination of Cryotop device and recipient asynchrony at −12 hours provides the most successful rate of offspring at birth, although a similar implantation rate was obtained with both devices. Thus, low fetal loss rates were observed for embryos vitrified in Cryotop independently of recipient synchrony, and embryos vitrified in straws revealed a two-fold higher rate of fetal losses. Moreover, when an asynchrony between vitrified embryo and recipients was applied, higher rates of embryos developed to term were obtained regardless of the device used. Finally, we found a highly significant association of the recipient genotype with implantation rate, offspring rate at birth, and fetal losses. In conclusion, the current study findings show that Cryotop enhances offspring rate because it is associated with a lower rate of fetal loss. This study thus provides additional evidence that recipient genotype and recipient asynchrony affect offspring rate at birth and indicates that the genotype of the recipient and the recipient asynchrony have a significant effect on implantation rate and fetal losses after vitrification.

1. Introduction

The rabbit breeding industry is increasingly using selected lines [1]. Generation and characterization of these lines require great effort and they must be kept in stock even if not needed for commercial use [2]. Embryo cryopreservation can be used as a tool in setting up genetic resource banks for biodiversity preservation in animal breeding and laboratory products (transgenics, clones), protecting against loss caused by disease or hazards. From a genetic standpoint, the cryopreservation of inbred strains is useful to establish control populations to study the genetic drift and gain when selection programs are applied [1,3,4]. Successful vitrification of mammalian embryos, including rabbits, has been the subject of intense research over many years [5]. The efficiency of vitrifying embryos is shown by survival rates at birth, ranging between 25% and 65% [1,6–10]. Moreover, our previous results showed that storing vitrified embryos in liquid nitrogen is an effective long-term storage option that maintains similar pregnancy rate, fertility, and survival at birth for at least 15 years [11].

Factors that influence the high variability of embryo survival among experiments include the concentration and

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composition of the vitrification solution, the procedure used to equilibrate embryos in this solution, the cooling and warming conditions, and the procedure used to dilute embryos from the vitrification solution [12]. However, the embryo implantation rates are also attributed, among other factors, to the transfer technique, which depends on several anatomical, physiological, and mechanical aspects [13]. Specifically, the efficacy of cryopreservation programs depends on several factors, such as embryo stage or genotypes of donors and recipients [14]. Numerous studies have investigated the effect of genotype as a variable in embryo cryosurvival [15–21]. However, little consideration has been devoted to recipient genotype and its influence on transferred cryopreserved embryos. To the best of our knowledge, only Vicente and García-Ximénez [22] have reported the effect of recipient doe genotype on survival rate at birth of frozen rabbit embryos. In contrast, because of its essential importance in establishing and maintaining pregnancy, the mechanisms of synchrony have been intensively studied since the first description by Chang in 1950 [23]. However, when a cryopreservation procedure was applied, freezing and thawing induced a delay in the resumption of normal metabolic and synthetic activities in the thawed embryos, as demonstrated in frozen-thawed mouse embryos after transfer [24]. In early studies, it was suggested that the transfer of frozen-thawed rabbit embryos into asynchronous recipients could resolve this problem [25,26]. From that moment on, all studies were carried out using asynchronous recipients [8,10,11,27–29]. However, to the best of our knowledge, no studies have examined the effect of recipient asynchrony on the transfer of vitrified embryos.

The latest approach to improving the vitrification system consists of minimizing the volume to allow extremely high cooling and warming rates [5,30–33]. Though the 0.25-mL volume straws limit the cooling rate to less than 2500 °C/min [12], vitrification using Cryotop increases the cooling rate approximately nine-fold (22,800 °C/min). Likewise, the warming rate is 42,100 and 1300 °C/min for Cryotops and straws, respectively [34]. However, in rabbit only Hochi et al. [35] obtained offspring derived from vitrified-warmed zygotes using the Cryotop method.

This study was therefore designed to investigate the effect of vitrification device, recipient genotype, and recipient asynchrony on implantation rate, offspring rate at birth, and fetal losses of rabbit embryos.

2. Materials and methods

All chemicals, unless otherwise stated, were reagent-grade and purchased from Sigma-Aldrich Química S.A. (Alcobendas, Madrid, Spain). The Ethics and Animal Welfare Committee of the Universidad Politécnica de Valencia approved this study. All animals were handled according to the principles of animal care published by Spanish Royal Decree 1201/2005 (BOE, 2005; BOE is the official Spanish State Gazette).

2.1. Animals

Rabbit from two selected lines, maternal and paternal, were used. The maternal line was based on New Zealand White rabbits selected since 1980 by a family index for litter size at weaning [36]. The paternal line is a synthetic line selected since 1990 by individual selection on daily gain from weaning to slaughter age (28 and 63 days [37]). Animals were housed individually at the Polytechnic University of Valencia experimental farm under a controlled 16-hour light:8-hour dark photoperiod and fed a commercial diet.

2.2. Embryo recovery

A total of 74 nulliparous female animals were used as donor female contemporaries to recipients (37 from the maternal line and 37 from the paternal line). Female animals were treated with 25 IU of eCG intramuscular (Intervet International B.V., Bowmeer, Holland) to induce receptivity. After 48 hours, female animals were artificially inseminated with a heterospermic pool of semen from male animals of the same line to randomize male effect. At artificial insemination time, female animals were administered 1 μg of buserelin acetate (Hoechst Marion Roussel S.A., Madrid, Spain) to induce ovulation and slaughtered 72 hours later. Embryos were collected at room temperature by flushing the oviducts and uterine horns with 10 mL of embryo recovery media consisting of Dulbecco phosphate buffered saline (DPBS) supplemented with 0.2% (wt/vol) bovine serum albumin (BSA), and antibiotics (penicillin G sodium 300,000 IU, penicillin G procaine 700,000 IU, and dihydrostreptomycin sulphate 1250 mg; Penivet 1; Divasa Farmavic, Barcelona, Spain). After recovery, morphologically normal embryos (morulae and blastocysts) from both lines were classified as normal (presenting homogenous cellular mass, mucin coat, and spherical zona pellucida according to International Embryo Transfer Society classification) and pooled to randomize embryo effect.

2.3. Vitrification and warming procedures

Embryos were vitrified using the vitrification procedure described by Vicente et al. [8] using two devices; French ministraw (IMV, L'Aigle, France) and Cryotop (Kitazato Co., Fuji, Japan). The Cryotop consists of a flat rectangular leaf of polypropylene measuring 20 × 0.7 × 0.1 mm attached to a thin, 5-cm long handle. Embryos were vitrified in a two-step addition procedure. At vitrification time, embryos were transferred into equilibration solution consisting of 10% (vol/vol) ethylene glycol and 10% (vol/vol) dimethyl sulfoxide dissolved in base medium (BM; DPBS supplemented with 0.2% [wt/vol] BSA), at room temperature for 2 minutes. The embryos were then transferred to vitrification solution consisting of 20% (vol/vol) ethylene glycol and 20% (vol/vol) dimethyl sulfoxide in BM. Next, the embryos were loaded into 0.125-mL sterile plastic ministraws between two drops of DPBS separated by air bubbles, sealed with a sterile plug or loaded into the Cryotop device and directly plunged into liquid nitrogen within 1 minute.

After storage in liquid nitrogen, embryos were warmed according to the device used. When the French ministraw device was employed, embryos were placed at 10 cm from vapor nitrogen until the vitrified fraction begin to crystallize (20–30 seconds) and warmed by submerging the straws in a water bath at 20 °C for 10 seconds. To remove the vitrification media, the two-step procedure was used. Briefly,