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Long-term and transgenerational effects of cryopreservation on rabbit embryos

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ABSTRACT

The short-term effects of cryopreservation and embryo transfer are well documented (reduced embryo viability, changes in pattern expression), but little is known about their long-term effects. We examined the possibility that embryo vitrification and transfer in rabbit could have an impact on the long-term reproductive physiology of the offspring and whether these phenotypes could be transferred to the progeny. Vitrified rabbit embryos were warmed and transferred to recipient females (F0). The offspring of the F0 generation were the F1 generation (cryopreserved animals). Females from F1 generation offspring were bred to F1 males to generate an F2 generation. In addition, two counterpart groups of noncryopreserved animals were bred and housed simultaneously to F1 and F2 generations (CF1 and CF2, respectively). The reproductive traits studied in all studied groups were litter size (LS), number born alive at birth (BA), and postnatal survival at Day 28 (number of weaned/number born alive expressed as percentage). The reproductive traits were analyzed using Bayesian methodology. Features of the estimated marginal posterior distributions of the differences between F1 and their counterparts (F1 − CF1) and between F2 and their counterparts (F2 − CF2) in reproductive characters found that vitrification and transfer procedures cause a consistent increase in LS and BA between F1 and CF1 females (more than 1.4 kits in LS and more than 1.3 BA) and also between F2 and CF2 females (0.96 kits in LS and 0.94 BA). We concluded that embryo cryopreservation and transfer procedures have long-term effects on derived female reproduction (F1 females) and transgenerational effects on female F1 offspring (F2 females).

1. Introduction

Embryo cryopreservation and transfer procedures are widely used as assisted reproductive technologies (ARTs) in both laboratory and domestic animals. These techniques induce environmental changes that influence the relationship between genotype and phenotype by modifying the gene expression of the embryo [1–3] and may not be neutral concerning behavioral features of the individuals because of changes in maternal effects [4,5]. Some of these environmental changes have an impact on the phenotypic appearance and, perhaps, on the phenotype of their progeny (transgenerational phenotypic changes) [6]. The interaction between organisms and their environment could induce epigenetic modification that may result in the appearance of a new phenotype and could represent heritable changes in gene expression that do not involve changes in the genetic code [7].

In mammals, mothers and offspring have an extended association during gestation and lactation. For this reason, maternal effects can contribute to individual differences within a population with alternative phenotypes [8,9].

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Uterine maternal effects include heritable and non-heritable maternal attributes, independent of the direct transmission of nuclear genes that influence offspring development [10]. Postnatal maternal performance is also a significant epigenetic factor in development [11] and includes components such as litter size (LS), milk quality and quantity, and various aspects of maternal behavior. Maternal effects can condition the expression of the progeny genome [12] and, in this sense, clearly fit as epigenetic factors.

In rabbit embryos, it is known that cryopreservation causes environmental changes inducing altered gene expression patterns [13,14] resulting in reduced early fetal development and increase fetal losses [14,15], but little is known regarding long-term outcomes.

The aim of our present study was to investigate whether cryopreservation and transfer procedures of rabbit embryos could have an impact on the long-term reproductive physiology of the offspring and if these phenotypes could be transferred to the progeny.

2. Materials and methods

2.1. Animals

All experimental procedures involving animals were approved by the Research Ethics Committee of the Universidad Politécnica de Valencia.

All animals came from line V, a maternal rabbit line selected on a number of young weaned per litter [16]. Animals were housed at the experimental farm of Universidad Politécnica de Valencia. At 63 days of age, animals were kept individually under the same environmental conditions. Animals were kept under a controlled 16-hour light:8-hour dark photoperiod and fed a commercial diet.

2.2. Experimental design

Vitrified rabbit embryos were warmed and transferred to recipient females, and the resulting pregnant females were designated the F0 generation. The offspring of the F0 generation were the F1 generation (cryopreserved animals; females and males). Females (n = 65) from F1 generation offspring were bred to other F1 males to generate an F2 generation. Females (n = 50) from F2 generation were bred similarly.

In addition, two counterpart groups of animals from the same genotype and generation obtained by natural mating (noncryopreserved and nontransferred animals) were bred and housed simultaneously in the same experimental farm as F1 and F2 generations (CF1 and CF2, respectively). Each of the groups consisted of 50 females.

2.3. Embryo collection

Nonsuperovulated does were used as embryo donors. Does were slaughtered at 70 to 72 hours postcoitum. Embryos were collected at room temperature by flushing the oviducts and the first one third of the uterine horns with 5 mL of embryo recovery media consisting of Dulbecco's phosphate-buffered saline (DPBS; Sigma, Alcobendas, Madrid, Spain) supplemented with $CaCl_2$ (0.132 g/L), 0.2% (wt/vol) bovine serum albumin (BSA; Sigma), and antibiotics (penicillin G sodium 300,000 IU, penicillin G procaine 700,000 IU, and dihydrostreptomycin sulfate 1250 mg; Penivet 1; Divasa Farmavic, Barcelona, Spain). After recovery, morphologically normal embryos (morulae and early blastocysts) were vitrified. Embryos were classified as normal when they presented homogenous cellular mass and intact *zona pellucida* [17].

2.4. Cryopreservation and warming procedures

Collected embryos were vitrified and warmed using the methodology described by Vicente et al [18]. Embryos were vitrified in two-step addition procedure. The vitrification media contained embryo recovery media without antibiotics supplemented with 20% (vol/vol) dimethyl sulfoxide (Sigma) and 20% (vol/vol) ethylene glycol (Sigma) as cryoprotectants.

After storage in liquid nitrogen (<6 months), embryos were warmed by submerging the straws into a water bath at 20 °C for 10 seconds. To remove the vitrification media, the two-step procedure was used. Briefly, warmed embryos were introduced into a culture dish containing 0.7 mL of 0.33 M sucrose and 0.2% BSA in DPBS, and after 5 minutes, embryos were washed in 0.2% BSA in DPBS before transfer.

2.5. Embryo transfers

After warming, embryos were evaluated morphologically and only those without damage in mucin coat or zona pellucida were transferred. Multiparous nonlactating females were used as recipients. Between 60 and 64 hours before transfer, recipient does were synchronized by intramuscular administration of 1 μg buserelin acetate (Hoechst, Marion Roussel, Madrid, Spain). Only females that presented vulva color associated with receptive status were induced to ovulate. Asynchronous transfers were carried out by endoscopy as described by Besenfelder and Brem [19], and the mean number of transferred embryos per doe was 8.6.

2.6. Traits measured in experimental groups

Transfer results were assessed on the basis of pregnancy rate (proportion of pregnant females at 12 days after transfer), fertility at birth (birth rate, proportion of females that gave birth after transfer), embryo survival in pregnant females (number of total born/total transferred embryos expressed as percentage), and number of born alive at birth (BA).

In female F1, F2, and their counterparts (CF1 and CF2), the reproductive traits studied were LS (number of total born at birth), BA, and postnatal survival at Day 28 (PS, number of weaned/number born alive expressed as percentage). The reproductive traits were controlled from the first until the fourth parity order. Hence, records of 640 parities from 839 matings were controlled (180, 157, 137, and 166 parities from F1, CF1, F2, and CF2 females, respectively).