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VITRIFIED EMBRYO TRANSFER OF TWO SELECTED SPANISH RABBIT LINES TO URUGUAY

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ABSTRACT

Survival rates from vitrified embryos belonging to two Spanish selected lines (V selected by litter size and R selected by growth rate) were evaluated after transfer in females from undefined genotypes in Uruguay . A total of 562 vitrified embryos were transferred in 64 nulliparous does, 340 from 38 donors of line V and 222 from 26 donors of line R . Thirty-eight recipients became pregnant (59%) and 30 give a birth (47%). The vitrified embryos of the two lines showed differences in the thawing process and survival rate at birth. The R line showed a lower percentage of transferable embryos and survival rate than the line V (85% vs. 92%, $P<0.05$; 22% vs. 43%, respectively).

INTRODUCTION

Since the 1970´s, embryos from some livestock species (cattle, sheep or rabbit) can be frozen and stored (Wilmut and Rowson, 1973; Willadsen *et al*., 1976; Banks and Maurer, 1974, respectively). This reproductive tool allows the mobility of genetic material in animal breeding, facilitating the diffusion of genetic improvement or animals of high genetic value. The efficiency of an embryo cryopreservation programme depends on the definition of optimal conditions of each step (recovery, cryopreservation and transfer). Factors as hormones used in reproductive control of donor or recipient females, or genotype of donors and recipients affect the efficacy of the programme.

In general, the global efficiency of rabbit embryo cryopreservation to reconstitute a population has been evaluated in commercial lines (García *et* al.,1998; García *et al*., 2000). In these cases, determining factors of a cryopreservation programme are studied and controlled. The aim of this work was to evaluate the possibility to establish two Spanish selected lines in Uruguay by transferring vitrified embryos to recipient does of undefined genotype.

MATERIALS AND METHODS

Animals.

Donor does were multiparous does (4th-6th parities) of two synthetic lines: line V selected for litter size at weaning for 23 generations and line R selected for growth rate from weaning (28 days) to slaughter (63 days) for 16 generations in the Animal Science Department (U.P.V-Spain). Lines and selection methodologies applied were described by Estany *et al*. (1989 and 1992).

Recipient does were nulliparous does from undefined crosses belonging to “Las Brujas” Experimental Station (INIA-Uruguay).

Embryo recovery.

Sixty four donors were mated with bucks from the same line and slaughtered 70-72 hours *post-coitum*. The reproductive tracts were removed and embryos recovered by flushing with Dubelcco´s PBS (DPBS) at room temperature (20-25ºC). After recovery, morphologically normal embryos from each donor doe were washed twice in fresh DPBS, kept at room temperature until vitrification (10 to 15 min.).

Vitrification procedure.

The vitrification procedure has been described by Vicente *et al*. (1999). The cryoprotective solution was a 1:1:2 solution (v/v/v) of dimethyl-sulfoxide (3.5 M DMSO, Sigma D5879), ethylene glycol (4.4 M EG, Sigma 9129), and PBS (D1283) supplemented with 2 g. bovine serum albumina (BSA; Sigma, A3311) per liter of cryoprotective solution.

Vitrification was carried out in two steps. First, normal embryos were pipetted into 0.2 ml of PBS medium and placed in a glass culture dish and then 0.2 ml of the cryoprotective solution was added and agitated. Embryos were left in this medium for 2 minutes. In the second step, 0.6 ml of the cryoprotective solution was added and agitated quickly. Then, embryos suspended in the final vitrification solution were loaded into plastic straws (L91 mm, IMV) and plunged directly into liquid nitrogen. The exposure time of embryos to the final vitrification solution did not exceed 1 minute. The two vitrification steps were carried out at 20 ºC.

The straws contained three sections separated by air bubbles. The first consisted of PBS in the cotton plug, the second section contained the embryos suspended in vitrification medium (0.1 ml) and the third section consisted of PBS. The straws were sealed and identified. Each straw held between 7 to 15 normal morulae from one donor doe.

Devitrification was performed by immersing the second and third sections of the straws in a water bath at 20 ºC for 10-15 sec. The cryoprotective solution was removed from the embryos in a two step dilution procedure at room temperature (20-25 ºC). Embryos suspended in the final vitrification solution were released into a glass dish containing 1 ml of 0.33 M sucrose in PBS medium. After 2 minutes, embryos were washed twice in fresh PBS medium and scored morphologically before transfer. Only embryos with an homogenous cell mass and an intact zone pellucida were transferred.

Embryo Transfer

Sixty-four nulliparous recipient does were used. Ovulation was induced with an intramuscular dose of 0.8 µg buseriline acetate (Hoescht) at 66-68 h (synchronous) or 48-50 h (asynchronous) before transfer. Only does with red vulvar lips were synchronised.

The recipients were anesthetized by a injection of a solution of ketamine at the rate of 1.2 ml kg^{-1} body weight. Oviductal embryo transfer was performed unilaterally. Four to fourteen normal embryos were transferred to each recipient doe.

Statistical Analysis

A Chi-square test with Yate´s correction was used to analyse the effect of donor line and timing of recipient synchrony on birth rate and survival rate at birth.

RESULTS AND DISCUSSION

A total of 562 vitrified embryos were transferred to 64 nulliparous does, 340 from 38 donors of line V and 222 from 26 donors of line R. Thirty -eight recipients became pregnant (59%) and 30 give a birth (47%) (Table 1). This result was low in comparison to the 70% obtained in previous works (García *et al*., 1999, 2000). However, in those studies, the recipient does were does of the line V tested as recipients.

Table 1.-Effect of line of donor on survival rate of vitrified embryos.

Donor Line	Vitrified embryos (%)	Transferred embryos n(%)	Recipient does n	Birth n(%)	Total born n(%)[1]	Born alive n(%)[1]	Kits at weaning n
V	372	340 (92)[a]	38	21 (55)	81 (48)[a]	73 (43)[a]	71
R	263	222 (85)[b]	26	9 (35)	25 (28)[b]	21 (22)[b]	21
Total	635	562 (89)	64	30 (47)	106 (40)	94 (35)	92

[1] Percentaje of born on transferred embryos in females delivering.
[ab] Values in the same column with different superscripts differ (P<.05)

The vitrified embryos of the two lines showed differences in the number of viable embryos after thawing and survival rate at birth. The R line showed a lower percentage of transferable embryos and survival rate than the line V (85% vs 92%, P<0.05; 22% vs 43%, respectively), (Table 1). On the other hand, synchrony or asynchrony did not affect the results (Table 2). The number of its born alive was numerically, better for 66-68 hours, a time closer to the embryo age.

Table 2. Survival rate of vitrified embryos by synchronous or asynchronous transfer.

Time (h)	Vitrified embryos (%)	Transferred embryos (%)	Recipient does	Birth (%)	Total born (%)[1]	Born alive (%)[1]
48-50	304	272 (89)	31	13 (42)	39 (35)	33 (29)
66-68	331	281 (85)	33	16 (48)	67 (44)	61 (40)

[1] Percentaje of born on transferred embryos in females delivering.
[ab] Values in the same column with different superscripts differ (P<.05)

In spite of low pregnancy and birth rates, the number of donor does with offspring (21) and the number of young rabbits at weaning (71) in line V were enough to line V in Uruguay. A very low number of line R donors had offspring (9) and there were few young rabbits (21), making establishment of this line more difficult. Establishment of line V, a maternal line tested as recipients will permit better results when exporting embryos to Uruguay.

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