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EFFICIENCY OF CRYOPRESERVATION PROGRAMMES IN THE RE-ACTIVATION OF A MATERNAL RABBIT LINE

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ABSTRACT

A total of 483 rabbit embryos from a line selected for litter size at weaning were used. Embryos of generations 17 and 26 were frozen in 1993 and vitrified in 1998 respectively. Both cryopreservation programmes guaranteed the re-activation of the population of the respective generations. The survival rate at birth of generation 17 frozen embryos and generation 26 vitrified embryos were 42% (69 live pups from 17 donors) and 50% (84 live pups from 22 donors) respectively.

INTRODUCTION

Establishing rabbit embryo banks has been possible since 1974 when Banks and Maurer demonstrated that these embryos could be frozen. This reproductive technology allows the conservation of high value genotypes, easy transport of genetic material, or studies of genetic response. However, the efficiency of an embryo cryopreservation programme depends on provision of optimal conditions for each step (recovery, cryopreservation and transfer). Factors such as hormones used in reproductive control of donors or recipients females, or genotype of donors and recipients affect the efficacy of the programme (Renard *et al*., 1982, Vicente and García-Ximénez, 1993).

Another important factor could be the length of storage, but several authors show that embryos of mice can be cryopreserved for more than 20 years without affecting their viability (Wood *et al.* 1987; Glenister *et al.* 1990).

In 1992 the Animal Science Department of the Politechnic University of Valencia (Spain) constituted an embryo bank from three selected rabbit lines. Since 1992, the cryopreservation procedure has been improved in rapidity and efficacy (survival rate at birth: 26% vs 56% Vicente and García-Ximénez, 1993 vs Vicente *et al*., 1999).

The aim of the present study was to evaluate the efficiency of a cryopreservation programme in the re-activation of a representative population of a maternal line.

MATERIAL AND METHODS

Animals.

Cryopreserved embryos coming from two generations (17 and 26) of a New Zealand White line (line A) selected for litter size at weaning were used. This line and the selection methodology were described by Estany *et al* (1989).

Recipient does from line V (a synthetic maternal line selected for litter size at weaning, Estany *et al*; 1989) were used. In previous studies, does of this maternal line have been tested as recipients (Vicente and García-Ximénez, 1993).

Embryo recovery procedure.

The donor does, at the end of the selection process for the respective generation, were mated to bucks from the same line and generation, and killed 70 to 72 h after mating. The reproductive tract was immediately removed and the embryos were recovered by flushing at room temperature with Dubelcco's phosphate buffer saline (DPBS) . After recovery, morphologically normal embryos were washed twice in fresh DPBS then pooled and held at 20ºC until used.

Cryopreservation procedures.

Freezing procedure: Two hundred fifty one normal embryos from 25 donors of generation 17 (line A) were frozen. A programmable freezer was used. Normal embryos were suspended in a solution 1.5M of DMSO supplemented with 20 % heat-inactivated rabbit serum at 20ºC for 5 min. Morulae were loaded into 0.25 ml straws and refrigerated to –6ºC. After manual seeding at –6ºC, the straws were frozen at a rate of -1ºC/min to -80ºC and at a rate of -8ºC/min between -80ºC and -120ºC before being plunged into liquid nitrogen. Thawing was achieved in air at 20ºC. The cryoprotectant was then removed and the embryos were washed.

Vitrification procedure: Two hundred thirty two normal embryos from 28 donors of generation 26 of line A were vitrified in accordance with Vicente *et al*. (1999). Briefly, the vitrification procedure was carried out in two steps. In the first step morulae were placed in a vitrification solution consisting of 12.5% (v/v) ethylene glycol (EG) plus 12.5% (v/v) dimethyl sulfoxide in DPBS supplemented with 0.2% (w/v) of bovine serum albumin (BSA). The embryos were held in this medium for 2 min. In the second step embryos were loaded in a solution of 20% (v/v) EG and 20% (v/v) DMSO in DPBS medium supplemented with 0.2% BSA for 1 min. The two vitrification steps were carried out at 20ºC. Embryos were agitated quickly and loaded into 0.13-ml plastic straws, sealed with colored plastic and then plunged directly into liquid nitrogen.

The straws contained 3 fractions separated by air bubbles, the first and third fractions consisted of DPBS, the central fraction (second) consisted of embryos from a donor (4 to 16 embryos) suspended in vitrification medium.. Devitrification was performed by immersing the straws in a water bath at 20ºC. The cryoprotectants were removed from the embryos in one-step dilution; the central fraction (about 0.1 ml) was then poured and mixed into a culture dish containing 1 ml of 0.33 M sucrose in DPBS. After 5 min, the embryos were washed twice in fresh DPBS.

Embryo transfer

A total of 53 recipient does belonging to generation 22 of line V were used. Ovulation was induced in receptive recipient does with an intramuscular dose of 0.8 µg of busereline acetate (Hoescht) 60 h before transfer. Oviductal embryo transfer was performed unilaterally by laparotomy. All of the embryos from a donor doe were transferred into the oviducts of a single recipient doe. Only normal embryos were transferred. The number of embryos thus transferred was between 4 to 15.

Statistical analysis

A Chi-squared test was used to analyse the percentage of transferable embryos (non-damaged embryos after thawing/number of frozen or vitrified embryos), delivering rate (proportion of donors having offspring), survival rate (number of total pups or live pups/number of transferred embryos) global efficacy (pups born alive /vitrified embryos) between cryopreservation programmes.

RESULTS AND DISCUSSION

Percentage of transferable embryos, delivering rate and survival rate were not different between the cryopreservation programmes. After thawing, 430 of 483 vitrified embryos were recovered without morphological damage (89%), 219 from generation 17 (87%) and 211 from generation 26 (91%). Delivering rates were 68% and 79% (generation 17 and 26 respectively). The number of donor genotypes preserved by giving offspring was equivalent to the fertility rate because all embryos of a donor were transferred to one recipient doe. The survival rate at birth was 42% and 50%, for generation 17 and 16, respectively. The only significant differences observed were for global efficiency at birth between cryopreservation programmes, 27% and 36%, respectively, for the freezing and vitrification programmes. This result was similar to that obtained in previous studies for re-activation of the V line using the vitrification procedure (García *et al*., 1998). The differences observed between programmes might be due to an interaction between generation and vitrification programme, that can not be evaluated.

Table 1.-Survival and offspring rates of vitrified embryos from line A.

G	Vitrified Embryos N	Transferred embryos n(%)	Recipient does n	D (%) n	Total born (%)[1] n	Born alive (%)[1] n	Global efficiency n(%)
A17	251	219 (87)	25	17 (68)	76 (46)	69 (42)	27[b]
A26	232	211 (91)	28	22 (79)	88 (53)	84 (50)	36[a]
Total	483	430 (89)	53	39 (74)	164 (49)	153 (46)	32

G: Generation of line A, A17 and A26 cryopreserved by freezing and vitrification respectively.
D: delivering rate = offspring rate of donor females.
[1] Percentage born of the embryos transferred to the does that kindled.
[ab] Values in the same column with different superscripts differ (P<.05)

After cryopreservation, 12 of 15 males (80%) and 17 of 25 females (68%) had offspring in the re-activated population for generation 17, and 14 of 21 males (67%) and 22 of 28 females (79%) had offspring in generation 26. This suggests that the number of offspring were sufficient for re-activating the population in both generations.

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