FOUNDATION OF A MATERNAL RABBIT LINE USING HYSTERECTOMIE AND EMBRYO CRYOPRESERVATION

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Abstract - The foundation process of a maternal rabbit line using high selection intensities is described. In a first step (June, 1993), 47 males (VHH) were obtained by hysterectomy of hyperprolific does mated with the best V line males. In the second step, VHH males were used to mate 136 hyperprolific does. In July, 1994, 1102 normal embryos had been recovered and vitrified from 103 hyperprolific does. After thawing and transfer, 519 young born alive from 94 hyperprolific does and, at 63 day old, 470 rabbits from 87 hyperprolific does were obtained. The results assure the foundation of the maternal line with the necessary genetic diversity.

INTRODUCTION

Litter size traits have been common objectives in selection programmes trying to develop specialised dam lines in rabbits. The conventional approach to improve genetically litter size has been the use of family information within small population, applying low selection intensities. The results of this approach have been lower than expected (ROCHAMBEAU, 1988; ESTANY *et al.*, 1989) and other genetic alternatives to improve litter size are currently being investigated. To apply very high selection intensities on reproductive performance of females has been revealed as a promising way to select litter size in pigs (SORENSEN and VERNESEN, 1991; HERMENT *et al.*, 1994).

The objective of this work is to describe a procedure carried out at the Department of Animal Science (Universidad Politécnica de Valencia) to found a maternal rabbit line using high selection intensities on the females involved in the process. A very important aspect, when selection is performed on animals spread in different farms, is the health troubles that can occur when the selected animals are put together. Consequently, in the description of the procedure, a relevant part will be the one concerned with the reproductive technologies used to avoid these troubles and to overcome the needs of time and housing facilities required while the high performing females were detected. In the following, the maternal rabbit line will be called HH line.

MATERIAL AND METHODS

Genetic aspects

The foundation of the HH line lay on the detection of does named hyperprolific, screening a large population of commercial rabbits, spread in different Spanish farms of size between 150 and 2200 does.

The hyperprolific does met one or both of the following criteria:

-to have 17 or more young born alive in any parity.

-to have an accumulated number of young born alive in all of its recorded parities equal or higher to the thresholds showed in table 1 (accumulative criteria).

The accumulative criteria intended to achieve a proportion selected of 0.01 in a population with a mean of 9.5 young born alive (8.5 in the first parity), a standard deviation of 2.81 and a repeatability of 0.2.

Table 1 : Accumulative criteria for the hyperprolific females.

Np	1	2	3	4	5	6	7	8	9	10
NAc	16	29	42	55	68	81	94	107	120	133

Np: number of parities. NAc: accumulated number of young born alive.

The genetic superiority of a female, having three parities and satisfying the accumulating criteria is 1.12 young born alive/litter if the heritability assumed is 0.1, and the repeatability and standard deviation of the ones showed before.

The expected superiority of the progeny of these females is 0.56. A first step of the process was to obtain male progeny (VHH) of different hyperprolific females mated to the best bucks, of the V line, a line reputed as having a good prolificacy (BASELGA *et al.*, 1992). The genetic value of the VHH males is expected to be higher than 0.56.

In the second step, a new and large set of hyperprolific females was mated to VHH males to obtain progeny that it is considered as the generation 0 of the HH line (HHO). The expected genetic value of HHO individuals, coming from hyperprolific dams and grand-dams of three parities would be higher than 0.84.

Reproductive Technologies

First step : Obtaining VHH males.
- Hysterectomy
To obtain VHH males, hyperprolific females were moved from the commercial farms to a very small farm (twenty individual cages) five Km far from the farms of the Department of Animal Science, in order to be mated to the best bucks of the V line. The pregnant dams were hysterectomized at the end of gestation and their progeny fostered by females of the V line placed in the farm of the Department. This was the way to prevent the introduction of foreign diseases, and to avoid the spread of pathogens of different hyperprolific dams between their offspring.

Second step: Obtaining Generation 0
The second step was to make a relatively high number of VHH males (47) to a large number of new hyperprolific females (136) to produce a large number of offspring reaching reproductive maturity coetaneously.
This process required time to detect the hyperprolific females, and to mate them to the VHH males in the small farm cited before.
It was necessary to apply a cryopreservation program in order to prevent diseases and wait to have enough housing facilities in the farm of the Department for this progeny. Then, the stored embryos were transferred to recipient does of the V line and the produced animals were used as founders of the HH line and others were moved to commercial farms in order to be compared with the populations from which the hyperprolific females were selected. Next, we describe the cryopreservation program.

- Embryo Recovery
Immediately after mating, females were injected intravenously with 25 IU hCG (Coriogan, Ovejero Laboratories, León, Spain). Donors were killed 70 to 72 h after mating. The reproductive tract was then removed, and embryos were recovered by flushing the oviducts and 1/3 of uterine horns with Dubelcco's PBS supplemented with 20% heat-inactivated rabbit serum (PBS1 medium). After recovery, morphologically normal embryos were washed twice in fresh PBS1, pooled and kept at 20°C until used.

- Vitrification procedure.
Vitrification was carried out in 2 steps. First, embryos were pipetted into 0.2 ml of PBS1 medium and placed in a plastic culture dish and then 0.2 ml of a solution, 25% (v/v) EG plus 25% DMSO (v/v) in PBS1, were added and diluted quickly. Embryos were left in this medium for 2 minutes. In the second step, 0.6 ml of the same initial cryoprotective solution (25/25, EG/DMSO) were added and diluted quickly. Embryos suspended in the final vitrification solution were loaded into 0.25-ml plastic straws (IMV, L'Aigle, France) and then plunged directly into liquid nitrogen. Embryos were exposed to the final vitrification solution for a total of 1 min. The two vitrification steps were carried out at 20°C and the final vitrification solution contained 20% (v/v) EG and 20% (v/v) DMSO in PBS1 medium.
The straws contained 3 fractions separated by air bubbles, the first fraction consisted of PBS1 in a cotton plug, the second fraction the embryos suspended in vitrification medium (0.1 ml) and the third fraction consisted of the PBS1 medium. Straws were sealed with coloured plastic for identification. When the number of embryos by doe were higher than 9, embryos were held in two straws.

- Embryo transfer.
Ovulation was induced in recipient does of V line with an intravenous dose of 25 IU hCG at 64-66 h before transfer. All recipient does were multiparous females (4th or 5th parity).
The recipients were anaesthetised with an injected solution of 5:1 (v/v) ketamine HCl:promethazine at the rate of 1.2 ml kg^{-1} body weight. Oviductal transfer was by ventral midline laparotomy.
Donor does with low number of embryos (<8) were transferred into opposite oviducts of recipient. Eighth recipient does were slaughtered the 29th day of gestation to identify the offspring.

RESULTS AND DISCUSSION

Table 2 shows the efficiency of the first step and the number of animals involved. The overall efficiency to obtain offspring was 71% for the hyperprolific dams, and 56% for the sires.

Table 2 : Number of animals and efficiency of the first step.

Type of animal	N° detected	N° mated (%)	N° with offspring (%)
V line males	16	16 (100)	9 (56)
Hyperprolific females	32	28 (87.5)	20 (71)

The total progeny obtained from the twenty successful matings was 218 born alive from which forty-seven males were chosen at 63 days old to be used in the second step.

One hundred and thirty-six hyperprolific females were selected and one hundred and thirty-two (97%) were effectively mated to VHH males and frozen embryos were got from one hundred and three females (overall efficiency of 76%). The average of normal embryos recovered by female was 10.8. The number of VHH males with frozen embryos was 27 (57%).

It is known that the use of cryopreservation and transfer of embryos in rabbits is an acceptable procedure to get offspring (RENARD *et al.*, 1982; VICENTE and GARCIA-XIMENEZ, 1994), and some results concerning recovery efficiency of this work have been given by GARCIA-XIMENEZ *et al.* (1995).

Table 3 shows the efficiency of second step from the hyperprolific female side, and we observed a global efficiency of 64%.

Table 3 : Number of hyperprolific females across the process.

Selected does	Does with vitrified normal embryos	Does with normal embryos post-thawing	Does with offspring at birth	Does with 63 days old offspring
136	103 (76%)	100 (73%)	94 (69%)	87 (64%)

The efficiency of the second step from the embryos and progeny side is shown in table 4, being the overall efficiency 43%.

Table 4 : Number of embryos and individuals across the process.

	Normal embryos	**Vitrified embryos**	**Total born**	**Alive born**	**Weaned**	**63 day old**
No.	1102	1068 (97%)	550 (50%)	519 (47%)	494 (45%)	474 (43%)
mean	10.8	10.5	5.9	5.6	5.3	5.0
sd	3.6	3.3	3.2	3.2	3.1	3.0

The percentage of vitrified, total and alive born, weaned and 63-day-old rabbit was calculated on recovered normal embryos.
No.= number. sd = standard deviation.

The results have been satisfactory in relation to the main objective of founding the line, because enough number of animals have contributed to the foundation to assure the necessary genetic diversity that will allow selection in the next future. Nevertheless, some hyperprolific females used in the process were very old and in bad health condition, lowering the global efficiency of the procedure.

The time required for the selection of the first hyperprolific females in the first step (June, 1993) until the last vitrification (July, 1994) was 13 months. The embryo transfer began in January, 1995, finishing in April, 1995. Current work in this line is the comparison of HHO females with the females belonging to the commercial population from which the hyperprolific females were selected.

*This population, from a genetic point of view is heterogeneous and not well known. This is an important fact that differentiates our approach from the pig experiments involving hyperprolific sows (SORENSEN and VERNESEN, 1991; HERMENT *et al.*, 1994). Consequences of this fact is, that we have not corrected doe performances by farm, and that some heterosis will be expected for the HH line at the beginning. Nevertheless, the does used in the farms supplying the hyperprolific females, accommodate the current standards to produce rabbits efficiently.*

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Spanish Abstract - Se describe el proceso seguido para la constitución de una nueva línea de aptitud maternal en conejo, utilizando elevadas intensidades de selección. En una primera etapa (junio de 1993) se obtuvieron 47 machos cruzados (VHH) por histerectomía de conejas hiperprolíficas montadas por los mejores machos de la línea V. En una segunda etapa, se detectaron 136 nuevas conejas hiperprolíficas de la población controlada, que fueron cubiertas con los machos VHH y de las que se recuperaron y vitrificaron embriones de 72 horas. En julio de 1994 se habían almacenado 1102 embriones normales procedentes de 103 conejas hiperprolíficas. Tras su desvitrificación y transferencia, se obtuvieron 519 nacidos vivos correspondientes a 94 de las conejas hiperprolíficas, de los que, al final del engorde, sobrevivieron 470 gazapos (correspondientes a 87 conejas hiperprolíficas). Estos resultados aseguran la suficiente variabilidad genética para dicha línea.