Transfer of fresh and frozen-thawed rabbit embryos to produce live young

J. B. Hatton, A. S. Kelus and S.P. Leibo

Basel Institute for Immunology, CH-4005 Basel (Switzerland), and Rio Vista International, San Antonio (Texas 78227, USA), 2 April 1984

Summary. Live rabbits for immunological experiments were produced by transfer of fresh or frozen-thawed embryos. The transfer of 505 fresh embryos and 55 frozen-thawed embryos resulted in 141 young born alive, 81 of which lived between several months and several years. The control group consisted of 55 litters from natural matings. About 70% of the live-born rabbits of natural mating and 55% of the young delivered by embryo transfer survived for more than eight weeks. Average litter sizes were 5.7, 3.7 and 2.2 for naturally mated females, fresh embryo transfer recipients, and frozen-thawed recipients, respectively.
Key words. Rabbit embryos; embryo transfer; frozen-thawed embryos; genetically defined animals; allotype.

Embryo transfer has become a common procedure for producing live young animals, and there exists an extensive literature on embryo transfer, especially for the rabbit[1]. A common use of embryo transfer is to study the biology of reproduction or of embryology. However, another common reason for embryo transfer is to produce living young animals that will themselves become the subject of study or use. One notable example of the latter is the production of live calves in the cattle industry[12].

Our interest in embryo transfer stems from our intention to establish a bank of frozen rabbit embryos obtained from genetically defined animals. We maintain a large colony of rabbits with genetically defined immunoglobulin markers, so-called allotypes, that serve as important tools for immunological investigations[7,10]. Am embryo bank to preserve genetic markers would be less expensive and safer than is a corresponding colony of living animals.

In addition, certain immunological and physiological experiments are facilitated when a fetus develops in utero in an animal that is not its natural mother. A few successful attempts using the technique of embryo transfer to study allotype suppression have been reported[3,9,13,14]. During the past five years, we have produced 81 rabbits by embryo transfer that have served as experimental subjects in an extended study of allotype suppression.

Materials and methods. Most of the procedures used in this study were modifications of standard ones[1,4,5]. Briefly, mature female rabbits from the rabbit colony of the Basel Institute for Immunology at Rodersdorf SO, Switzerland, were superovulated by the s.c. injection of 75 IU of pregnant mare serum gonadotropin followed 66–68 h later by the i.v. injection of 50 IU of human chorionic gonadotropin (hCG). They were mated with proven males from the same colony, and embryos were collected 72 h after mating. The donors were sacrificed by cervical dislocation, their reproductive tracts were removed, and each whole oviduct-uterus was flushed with 10–15 ml of Dulbecco's phosphate buffered saline (PBS). The embryos were recovered from the solution, and classified as to their stage of development and quality. About 25 embryos were recovered from each donor, of which 70–80% were normal 8-cell to morula stage embryos.

The embryos were either transferred immediately or were frozen by standard procedures[8]. Briefly, the embryos were frozen in 0.2 ml volumes of 1.25 M dimethyl sulfoxide (DMSO) prepared in PBS and contained in 10 × 100 mm glass tubes. The tubes were cooled to −5 °C, seeded, cooled to −70 °C at about 0.5 °C/min, and then placed directly into liquid nitrogen. The frozen tubes were thawed in air at about 25 °C/min, and the DMSO was removed from the embryos by the stepwise addition of PBS to the tubes. The diluted embryos were recovered, rinsed with PBS, and transferred within a short time after recovery.

The transfers were made directly into the fimbrium of the oviduct through a flank incision. Normally, four to six embryos were transferred into each fimbrium. In some cases, however, as few as three embryos were transferred into a recipient. Pseudopregnancy was induced in all of the recipients by the i.v. injection of 50 IU of hCG. All of the recipients were in perfect synchrony with the donors; i.e. for fresh transfers, hCG was injected at the same time into both the donor and recipient animals, while for transfer of frozen embryos, hCG was given to the recipients at a time corresponding to that used for the donors whose embryos were to be thawed and transferred. Because the primary purpose of these transfers was to obtain live offspring for immunological study, all of the recipients were allowed to bear their young and to rear them until weaning. No attempt was made to determine the number of pregnancies established by the embryo transfers or the number of implantations that did not continue to term. Because both the donor and recipient animals were drawn from a common breeding colony, we have used the natural breeding performance of the colony as a control for the efficiency of the embryo transfers.

Results and discussion. The transfers reported here were performed between 1978 and 1983. The results produced by natural mating were collected from contemporary breedings. A summary of the results is presented in table 1, which shows the raw data, and table 2, which shows the values calculated from the raw data.

Sixty-eight percent of 81 females that had been mated with proven males produced litters. By comparison, 36 of 78 recipients (46%) of unfrozen embryos produced litters, and five of nine recipients (56%) of frozen-thawed embryos produced litters.

Table 1. Live rabbits produced by natural mating or embryo transfer

Category		Embryo transfer	
	Natural mating	Fresh	Frozen-thawed
No. of matings or recipients	81	78	9
No. of litters	55	36	5
Total embryos transferred	–	505	55
Total embryos in pregnant recipients	–	234	35
No. of live born	311	133	11
No. of alive at 8 weeks	216	75	6

Table 2. Efficiency of rabbit production by natural mating or embryo transfer

Category		Embryo transfer	
	Natural mating	Fresh	Frozen-thawed
Litters as percent of matings or recipients	67.9%	46.2%	56%
Live born as percent of embryos transferred	–	26.3%	20%
Live born as percent of embryos in pregnant recipients	–	56.8%	31%
Alive at 8 weeks as percent of live born	69.5%	56.4%	55%
Average No. of live born per litter	5.7	3.7	2.2
Average No. of alive at 8 weeks per litter	3.9	2.1	1.2
Overall efficiency	(100%)	54%	31%

The naturally mated females littered an average of 5.7 young; the embryo transfer (ET) recipients littered an average of 3.7 and 2.2 young from fresh and frozen-thawed embryos, respectively. Approximately 70% of the live-born young of natural matings survived for 8 weeks or longer, while only about 55% of the live young produced by ET survived for that period. The average litter size from ET was substantially smaller than from natural matings, and it may be that the ET mothers reared their young less well. If so, increasing the number of embryos transferred to each recipient female should increase the number of live offspring that survive.

The overall percentage of live-born from fresh embryos transferred (26%) was only slightly higher than the 20% produced from frozen-thawed embryos. However, almost twice the percentage (57%) of live young resulted from transfer of fresh embryos into recipients that littered young, compared to the percentage (31%) of young that resulted from transfer of frozen-thawed embryos into recipients that littered. This would suggest that there was a larger loss due to early embryonic mortality with frozen than with fresh embryos.

The overall efficiency of live young that survived for 8 weeks or longer from ET was 54% and 31% from fresh and frozen-thawed, respectively, compared to the number of live young produced by natural mating. These efficiencies are somewhat lower than those reported by others[2, 11, 15].

The purpose of our embryo transfers was to obtain living young from embryos recovered from a female of one genotype with respect to the immunoglobulin loci and transferred into a female of a second allotype. In that respect, the experiments were successful. We obtained a total of 75 rabbits from transfer of fresh embryos, and six from transfer of frozen-thawed embryos. All of those rabbits survived for months or even years. They have proved to be ideal experimental subjects for immunological studies to be reported elsewhere. Some of those animals produced by embryo transfer have even served as embryo donors themselves.

Acknowledgments. We should like to thank Drs C. M. Steinberg and L. Du Pasquier who read the manuscript critically. We are also indebted to Mr W. Hänggi of the animal colony at Roderdorf/SO for his invaluable help. The Basel Institute for Immunology was founded and is supported by F. Hoffamnn-La Roche&Co., Ltd, Basel (Switzerland).

1 Adams, C. E., Mammalian Egg Transfer, p. 29. Ed. C. E. Adams. C.R.C. Press, Inc., Boca Raton, Florida 1982.
2 Binkerd, P. E., and Anderson, G. B., Gamete Res. *2* (1979) 65.
3 David, G. S., and Todd, C. W., Proc. natl Acad. Sci. USA *62* (1969) 860.
4 Dickmann, Z., Methods in Mammalian Embryology, p. 133. Ed. J. C. Daniel, Jr. Freeman and Co., San Francisco 1971.
5 Hafez, E. S. E., Reproduction and Breeding Techniques for Laboratory Animals, p. 273. Ed. H. S. E. Hafez. Lea and Febiger, Philadelphia 1970.
6 Heape, W., Proc. R. Soc. *48* (1891) 457.
7 Kelus, A. S., and Gell, P. G. H., Prog. Allergy *11* (1967) 141.
8 Leibo, S. P., and Mazur, P., Methods ion Mammalian Embryology, p. 179. Ed. J. C. Daniel, Jr. Freeman and Co., San Fancisco 1978.
9 Mage, R. G., Transplant. Rev. *27* (1975) 84.
10 Mage, R. G., Handbook of Experimental Immunology, 4th edn. Ed. D. M. Weir. Blackwell, Edinburgh 1984 (in press).
11 Schneider, U., Jakovac, M., and Hahn, J., Fortsch. Fertilitätsforsch. 5 (1977) 168.
12 Seidel, G., Science *211* (1981) 351.
13 Vice, J. L., Hunt, W. L., and Dray, S., J. Immun. *103* (1969) 629.
14 Vice, J. L., Gilman-Sachs, A., Hunt, W. L., and Dray, S., J. Immun. *104* (1970) 550.
15 Tsunoda, Y., Soma, T., and Sugie, T., J. Reprod. Fert. *65* (1982) 483.

0014-4754/85/060755-02$1.50 + 0.20/0

Polyploidization and production of abnormal spermatids in *Psophus stridulus* (Orthoptera)

J. L. Bella, C. López-Fernández, J. de la Torre and J. Gosálvez

Departamento de Genética, C-XV, Facultad de Ciencias, Universidad Autónoma de Madrid, E-28049 Madrid (Spain), 29 June 1984

Summary. Abnormal spermatids exhibiting variation in the number of adjunct centrioles (ACs) (from two to eight) have been analyzed in a spontaneous mutant grasshopper characterized by a high tendency to form polyploid meiocytes. Results show that the observed polyploidization of these cells increases the number of abnormal gametes and, although diploid spermatids (with two ACs) are the most frequent, higher levels of ploidy are also produced. The variation in the number of ACs, the level of ploidy in the sperm and the presence of polyploid meiocytes, are topics briefly discussed.
Key words. Insect cytogenetics; spermatogenesis.

It is well known that a male, even when presenting a balanced chromosome complement, may produce sperm which in some way is not functional. During the past years, we have had the opportunity of analyzing some spontaneous mutations and chromosome polymorphisms which present a potentiality for increasing the production of abnormal spermatids in some grasshoppers[2,3]. Evidently, these analyses arise a certain interest, given that an increase in the production of non functional spermatids may have a drastic effect on the preservation of these mutations within the populations as well as on the introduction of others which appear spontaneously.

The present note deals with the production of abnormal spermatid nuclei in a male of *Psophus stridulus* presenting polyploid meiocytes. The analysis is based on only one individual, but it is necessary to bear in mind that although spontaneous polyploidization is not infrequently observed in the meiotic analysis of Orthopteroids, its low level of production does not permit us to establish a close correlation between this event and the production of abnormal spermatid nuclei. However, in the present mutant it was usual to find polyploid cells in all the follicles of the testes, favoring the possibility of establishing the correlation mentioned above.

The male used in the present study was part of a sample of 69 individuals collected in a natural population in Valle de Ordesa (Pyrenees). The testes were fixed in 3:1 ethanol:acetic acid; meiocytes were stained with lactopropionic-orcein and, in order to observe clearly both the adjunct centrioles (AC) in the spermatids and the cytoplasm of the cells, phase contrast optics were used.

The karyotype of the *Psophus stridulus* male includes 11 pairs of acrocentric autosomes plus a single, acrocentric, X-chromosome (fig., a) which implies a basic chromosome number 2n = 23. The spontaneous production of polyploid cells occurred within the cyst; consequently, different levels of ploidy were found within