*J. Reprod. Fert., Suppl.* **43** (1991), 293–295

*Printed in Great Britain*


# Gene transfer studies in cattle

K. Roschlau

*Research Centre of Animal Production, Academy of Agricultural Sciences of GDR, Dummerstorf 2551, German Democratic Republic*

*Keywords:* gene transfer; zygotes; cattle

## Introduction

Prospects of using gene transfer in farm animals have been developed from the potential possibilities of this technology for quantitative and qualitative improvement of existing traits. On the other hand this powerful technique may create animals with completely new characteristics and make them into producers of proteins of biomedical importance.

The following aspects of the technology are currently under discussion in relation to animal improvement by genetic engineering.

—Increased growth rate
—Modification of body composition to achieve a leaner carcass
—Improvement of feed efficiency
—Increased milk production
—Modification of milk composition and secretion of foreign proteins into the milk (gene farming)
—Improved fibre production
—Altered fertility
—Improved disease resistance

## Methods

In the past 5 years recombinant DNA has been successfully introduced into the germ line of rabbits, sheep and pigs (Hammer *et al.*, 1985; Brem *et al.*, 1985; Simons *et al.*, 1988; Vize *et al.*, 1988; Pursel *et al.*, 1988), but there are only a few reports of gene transfer in cattle. The integration of injected DNA was analysed in these experiments mainly in early embryos (Lohse *et al.*, 1985; Church *et al.*, 1986; Roschlau *et al.*, 1988; Biery *et al.*, 1988) but only a few transgenic calves have been born (Church, 1987; Roschlau *et al.*, 1989). Gene transfer experiments in cattle are associated with a number of specific problems that differ from those for other species. One of the main difficulties is the limited access to a large number of zygotes and to an adequate number of recipients. In addition, such experiments are very expensive and time consuming because of the long generation interval. Nevertheless, cattle may be suitable for certain practical aspects of gene transfer such as 'gene farming'. We have therefore concentrated in the past few years on the development of various methods of gene transfer in cattle. The detailed results are published elsewhere (Roschlau *et al.*, 1989).

The established technique can be characterized as follows:

(1) Zygotes are obtained from superovulated 18–23-month-old heifers of 380–420 kg bodyweight. To obtain superovulation the animals are treated with PMSG and prostaglandin (Rommel *et al.*, 1989; Roschlau *et al.*, 1989).

(2) The optimal time for zygote recovery in gene transfer experiments is 78–82 h after prostaglandin treatment.

(3) About 73% of the donor heifers react to the hormone treatment with more than 2 ovulations.

(4) An average number of 17 ovulations occurs in the ovaries of successfully superovulated heifers.

(5) After hysterectomy and flushing of the oviducts about 10 zygotes are obtained for each successfully superovulated donor.

(6) The pronuclei are visualized by centrifugation of the zygotes at 12 000 *g* for 10 min. This procedure stratifies the ooplasm and in 60% of the zygotes the pronuclei become visible by Nomarski microscopy.

(7) Foreign gene constructs are introduced into one of the two pronuclei by microinjection of 1–2 pl DNA solution.

(8) In various experiments to date with 5 different gene constructs about 900 zygotes have been injected.

(9) After gene injection and in-vitro cultivation overnight the zygotes are transferred into the oviducts of 'temporary' recipient heifers (an average of 15 zygotes per recipient) and recovered 7 to 14 days later.

(10) Rates of recovery are about 40% and are independent of the duration of the in-vivo cultivation in the oviducts. About 35% of the recovered embryos develop normally up to the morula or blastocyst stages respectively.

(11) All developed embryos recovered at an age of 14 days are analysed directly for the injected foreign DNA by dot-blot hybridization (Roschlau *et al.*, 1988). Foreign DNA can be detected in 40% of embryos. The rate of efficiency of gene transfer (the ratio of transgenic organisms to the number of injected and transferred zygotes) calculated for preimplantation on 14-day-old embryos varies between experiments in the range of 3–6%.

(12) The recovered 7-day-old morulae and blastocysts are transferred into the uteri of synchronous recipient heifers. In relation to the number of injected zygotes about 6% calves have been born.

(13) The total rate of efficiency of gene transfer at birth is 0·7% and so comparable with results known from other farm animals.

## Conclusions

It may be said that, in our hands, we have a complete series of methods for the production of transgenic bovine embryos and calves for various purposes. In future these techniques should become more efficient, leading to a higher rate of production of transgenic calves and to decreased costs. To attain these ends we are concentrating on the following topics.

(a) The isolation of oocytes from non-stimulated slaughter-house ovaries and their in-vitro maturation and fertilization to obtain more plentiful and cheaper zygotes for experiments.

(b) The endoscopic puncture of growing follicles on the ovaries of valuable breeding cows to obtain oocytes for the production of zygotes by in-vitro fertilization.

(c) The development of long-term in-vitro cultivation methods for zygotes as an alternative for the use of temporary recipients. In preliminary experiments the co-cultivation of bovine zygotes with cumulus cells or with oviduct epithelial cells as feeder layers showed the best results.

(d) The development of methods for improvement of the integration frequency of foreign DNA in zygotes. Possibly this aim can be reached by a limited increase of the mutation rate in the recipient genome. Irradiation with ultraviolet light before transformation can double the integration frequency of DNA in somatic cells (Van Duin *et al.*, 1985).

(e) The establishment of an efficient integration analysis of the embryos before transplantation. This technique includes the biopsy of 1–2 single embryonic cells and their subsequent use in the polymerase chain reaction for amplification of the transgene. In this way it will be possible to transfer only transgenic embryos, thus leading to a drastic decrease in the number of necessary recipients.

(f) The introduction of an embryo cloning technique. It would be efficient to multiply an embryo after the diagnosis of its transgenic status. There are 3 possible ways for embryo cloning: (i) splitting an embryo at the morula or blastocyst stage and transplantation of half embryos; (ii) fusion of single blastomeres of a transgenic embryo with inactivated bovine oocytes and subsequent cultivation and transplantation of the fusion products; and (iii) the disaggregation of transgenic embryos at the 8–16-cell stage followed by aggregation of these blastomeres with earlier bovine embryos and transplantation of the products.

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