13 Production of Transgenic Cattle Expressing a Recombinant Protein in Milk

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Transgenic technology has been applied with some success in all of the major agricultural species. However, its application in cattle has been relatively slow due to the greater technical, logistical and financial challenges encountered in working with this species. Sustained efforts to produce transgenic cattle have concentrated on producing valuable biomedical proteins in milk rather than modifying agricultural production traits. The overall aim of the work described here was to enhance the nutritional value of cow's milk for human consumption by expressing human α-lactalbumin (the major whey protein in human milk) in the milk of transgenic cattle. To this end, we sought an efficient system in which to generate healthy, transgenic calves from microinjected zygotes. Embryo production and transfer were performed continuously over a period of 5 months. Zygotes for microinjection were generated by *in vitro* maturation and fertilization of abattoir-derived oocytes. To evaluate the effect of fetal calf serum (FCS) on embryo development, pregnancy rate, calving rate and birthweight, some embryos were cultured with or without FCS from day 5 to day 7 or 8 post-insemination (p.i.). Of 11,507 injected zygotes, 1011 (9%) developed to the compact morula or blastocyst stage. After non-surgical embryo transfer of 478 embryos to recipient heifers (one embryo/recipient), 155 recipients (32%) were pregnant at day 30 of gestation and 90 (19%) produced calves (including one set of identical twins). Most (97%; 88/90) calves were delivered by scheduled Caesarean section at about day 274 of gestation. Mean (range) calf birthweight was 41.83 (29.09–60.45) kg. The male:female sex ratio was 1.44:1 (59% male:41% female) and did not differ from the expected ratio of 1:1 ($P>0.05$). Perinatal calf survival was high (90/91; 99%). Under the conditions of these experiments, the presence of FCS in embryo culture medium did not affect ($P>0.05$) embryo development, pregnancy rate, calving rate, birthweight or perinatal survival. Nine (10%) of the calves were transgenic. All transgenic calves were born healthy. A female calf, induced to lactate, expressed human α-lactalbumin in her milk at a level of 2.4 mg ml^{-1}.

 Transgenic Animals in Agriculture
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Introduction

Transgenic technology has been applied in domestic livestock to enhance food and fibre production, promote disease resistance and to produce recombinant proteins from the mammary gland. Reports abound on the integration, expression and transmission of various transgenes in pigs, sheep and goats (reviewed in Wall, 1996). In cattle, however, transgenic technology has developed at a much slower pace. While sporadic efforts to produce transgenic cattle have appeared in the literature since the mid-1980s, transgene expression has been reported in only one case (Bowen *et al*., 1994) and no account of germline transmission has been published.

Several factors have conspired to limit the application of transgenic technology in cattle. Economically important traits (e.g. lactation, growth) are polygenic, and current transgenic technology is limited to the modification of monogenic traits. Single genes with a major influence over production traits have not yet been identified. Technically speaking, cattle are a notoriously difficult species in which to make transgenics, for the following reasons.

1. Collection of zygotes from single ovulating or even superovulated donors is a tedious, labour-intensive, expensive and frequently unrewarding exercise (reviewed by Eyestone, 1994; Wall, 1996), though recently these '*in vivo*' approaches have largely been supplanted by the more tractable method of producing embryos *in vitro* (Krimpenfort *et al*., 1991).
2. Cattle are a monotocous species, a condition that severely limits the number of embryos that can be transferred to a single recipient; moreover, pregnancy rates obtained after transfer of only one or two DNA-injected embryos per recipient is about half of that for non-injected embryos (reviewed by Eyestone, 1994).
3. The transgene integration frequency reported for cattle (0–6%; reviewed in Eyestone, 1994; Wall, 1996) is lower than that reported for other agricultural species (5–30%; reviewed in Brem, 1992; Wall, 1996). Thus, a rather large and expensive herd of recipients is required to support any serious effort aimed at making transgenic cattle.
4. The relatively long generation interval in cattle (*c*. 2 years) adds to production expenses and delays development of new transgenic strains.

Despite these impediments, transgenic cattle are being developed for applications where single-gene modifications are sufficient, namely for the production of medically important recombinant proteins in the milk. Indeed, one of the first applications suggested for transgenic technology was that of 'genetic farming' or the production of recombinant proteins of biomedical value in the tissues of transgenic animals (Palmiter *et al*., 1982), particularly the mammary gland (Lathe *et al*., 1985). The mammary gland is, in many ways, ideally suited for producing recombinant proteins: its capacity for protein production is extremely high; it can perform many of the complex, post-translational modifications required for biological activity of many

proteins of biomedical interest; it secretes proteins in an exocrine fashion, thus minimizing the risk of a bioactive recombinant protein upsetting the host's own physiology; and finally, milk is a well-characterized, familiar milieu from which to isolate the protein of interest.

Several groups have reported that calves resulting from *in vitro*-produced embryos tended to be larger than ordinary calves (Sinclair *et al*., 1994; Behboodi *et al*., 1995; Kruip and Den Daas, 1997). Similar observations have been made on lambs born from embryos cultured *in vitro* from early cleavage to the blastocyst stage (Thompson *et al*., 1995; Walker *et al*., 1996). Thompson *et al*. (1995) presented evidence suggesting that the presence of fetal calf serum (FCS) in embryo culture medium was related to the production of large offspring from cultured embryos. In the present report, we compared birthweights of calves born after culture in medium supplemented or not with FCS from days 5 to 7 or 8 post-insemination (p.i.).

The aim of this paper is to describe some of our experience in the production of transgenic cattle bearing a transgene for human α-lactalbumin, the major whey protein of human milk. Although the objective of this effort was to enhance the nutritional value of cows' milk for human consumption, a so-called 'nutraceutical' application, the methods employed and the results obtained here should apply to the production of transgenic cattle for more classical agricultural applications as well. A final comment will be made regarding the future of transgenic technology in cattle in view of recent breakthroughs in sheep regarding somatic cell nuclear transfer.

Methods

Zygote production

Zygotes were obtained by *in vitro* fertilization of *in vitro*-matured oocytes recovered from the ovaries of slaughtered cows and heifers. The animals from which the ovaries were obtained were primarily of the Holstein breed. Due to the lack of a suitable local abattoir, we imported oocytes from laboratories in Madison, Wisconsin (Bomed, Inc.) and Elizabethtown, Pennsylvania (Emtran, Inc.). Briefly, ovaries were recovered within 30 min of slaughter and transported to the respective laboratories in physiological saline at 25–35°C. Cumulus–oocyte complexes (COC) were aspirated from 2–8 mm diameter ovarian follicles between 4 and 10 h after slaughter and placed in maturation medium (TCM-199, buffered with 25 mm Hepes, supplemented with 10% FCS, 5 μg ml^{-1} bovine LH, 5 μg ml^{-1} FSH and 50 μg ml^{-1} gentamicin and equilibrated with 5% CO_2 in air; Sirard *et al*., 1988). Medium containing COC was placed in tightly capped, polystyrene tubes and shipped in a portable, battery-powered incubator (Minitub USA, Middleton, Wisconsin) by overnight express (Long *et al*., 1994) to our laboratory in Blacksburg, Virginia. Upon arrival, COC were rinsed in Hepes-

buffered TALP medium (TL-Hepes; Parrish *et al*., 1986) and placed in fertilization medium (TALP, supplemented with 2–20 μg ml^{-1} heparin; Parrish *et al*., 1986). Oocytes were fertilized with frozen–thawed semen from a Holstein bull of proven fertility. After thawing, the proportion of motile sperm was enhanced and standardized by the 'swim-up' technique (Parrish *et al*., 1986) and added to fertilization medium to a final concentration of 1 million sperm ml^{-1}. Sperm and COC were co-incubated at 39°C in a humidified atmosphere of 5% CO_2 in air, starting at 22 h post-onset of maturation. Fertilization frequency was assessed histologically on a subset of each batch of oocytes as described by Parrish *et al.* (1986).

Pronuclear DNA injection

Zygotes were removed from fertilization medium 16–18 h p.i., placed in 2 ml TL-Hepes in a 15 ml capped, conical tube and agitated for 2–5 min on a laboratory 'vortexing' mixer apparatus. To visualize pronuclei, zygotes were centrifuged at 13,000 *g* for 4–8 min to displace opaque cytoplasmic lipid inclusions (Wall and Hawk, 1988). Microinjection was performed on an Olympus IMT-2 inverted microscope using Nomarski differential interference contrast optics. Zygotes were stabilized by gentle suction on a holding pipette and the pronuclei brought into focus. Several picolitres of a solution containing the transgene construct were then injected into one of the pronuclei through a finely pulled glass needle. Microinjection was considered successful if the pronucleus swelled upon delivery of the DNA solution and remained swollen after removal of the injection needle. Detailed protocols for microtool manufacture and pronuclear injection may be found in Hogan *et al.* (1986) and Pinkert (1994).

Embryo culture

After microinjection, embryos were cultured in modified synthetic oviduct fluid (SOFM, also supplemented with amino acids; Gardner *et al*., 1994) for 6 or 7 days. In some cases (see below), FCS (10% v/v) was added to the media on day 5 p.i. About 30 non-injected control embryos were cultured in parallel with each set of microinjected embryos to control for the effect of injection on development.

Effect of culture medium and FCS on embryo development calf birthweight

The effect of FCS supplementation on embryo development, pregnancy rate, calving rates and calf birthweights was examined in a subset of microinjected embryos included in this study. FCS has been reported to have biphasic

effects on bovine embryo development *in vitro*. Early cleavage stage development was suppressed by the presence of 10% FCS (Bavister *et al*., 1992; our unpublished observations) while it enhanced blastocyst formation by day 7 p.i. (van Langendonckt *et al*., 1996; our unpublished observations). In the present experiment, FCS was added to the culture medium on day 5 to avoid early, embryosuppressive effects while exploiting its embryotrophic effect on the blastocyst stage. Embryos were assigned randomly to SOFM medium supplemented or not with 10% FCS.

Embryo transfer

Oestrus was synchronized in recipient heifers by intramuscular injection of prostaglandin F2-α and diagnosed twice daily by visual observation of mounting behaviour among the treated animals. Embryos were evaluated for development to the compact morula and blastocyst stages on day 7–8 p.i. Good and excellent quality embryos were placed in TL-Hepes, loaded into 0.25 cc semen straws (IMV International, Minneapolis, Minnesota) and transported to our recipient facility (30 min transit time) at 30–35°C. Embryos were transferred non-surgically to heifers in which oestrus had occurred within 24 h of the embryos' insemination *in vitro*. Each heifer received only one embryo.

Pregnancy diagnosis and monitoring

Initial pregnancy diagnosis was performed on day 30 of gestation by transrectal ultrasound (Kastelic *et al*., 1988). Pregnant recipients were monitored in this fashion weekly until the third month of gestation, after which pregnancies were checked by rectal palpation of the uterus. Recipients were maintained on pasture supplemented with medium-quality grass hay, grain, vitamins and minerals throughout gestation.

Calving and perinatal care

To minimize calf morbidity and mortality associated with dystocia, all recipients were scheduled for elective Caesarean section 5–7 days prior to their calculated due dates. Four weeks prior to their calculated due dates, pregnant recipients were taken off pasture and placed in a smaller paddock where they were observed several times a day for signs of impending parturition. Two weeks before their calculated due date, recipients were placed in a box stall in the Large Animal Clinic at the Virginia–Maryland Regional College of Veterinary Medicine where they were observed for signs of impending parturition every 6 h. At the discretion of the attending

veterinarian, recipients entering labour during this period were either allowed to deliver vaginally (with assistance as required) or submitted for emergency Caesarean section. Approximately 1 week before their calculated due dates, recipients were induced into labour and subjected to elective Caesarean section on the following day.

Within 20 min of birth, newborn calves were towelled dry, fed 1 l of colostrum, treated around the navel with an iodine solution and given an intramuscular injection of selenium and vitamin E. Calves were fed a total of 8 l of colostrum during the first 48 h of life, then placed in hutch-style housing and fed milk replacer twice a day for 6 weeks.

Screening of calves for transgene integration

The transgenic status of calves was determined by analysing samples of blood and ear tissue. Within 48 h of birth, 15–20 ml of blood was collected by jugular venipuncture into a heparinized vacuum tube. Ear tissue was sampled with the aid of a porcine ear-notching instrument and placed into tissue lysis buffer at room temperature. DNA from both tissues was purified and subjected to PCR and Southern blotting to detect the presence of the transgene.

To test whether blood and ear were representative tissues on which to base transgene screening, we analysed six additional tissues from 30 calves diagnosed as negative for the transgene. Samples of skin, skeletal muscle, liver, lung, rudimentary mammary tissue and gonads were obtained at necropsy and processed as described above for ear tissue. PCR was used to detect the transgene in purified DNA from each tissue.

Evaluation of transgene expression

Transgene expression was evaluated in one transgenic heifer by inducing her into lactation at 6 months of age. Lactation was induced by the method of Smith and Schanbacher (1973). The concentration of human α-lactalbumin in the lacteal secretion was determined by Western blotting that employed a polyclonal antibody to human α-lactalbumin, pre-absorbed with bovine milk proteins to eliminate cross-reactivity with bovine α-lactalbumin.

Statistical analysis

Proportional data were compared by the chi-squared test; continuous data were compared by one-way analysis of variance (ANOVA). Both analyses were performed using the statistical analysis package supplied with Microsoft Excel.

Results

Embryo production and DNA microinjection

The results of a 5-month microinjection campaign are summarized in Table 13.1. Overall, 20,918 presumptive zygotes were examined for the presence of visible pronuclei; of these, 11,507 (55%) were successfully microinjected. Fertilization frequency, assessed histologically, ranged from 78% to 100%. On any given day, between 5% and 30% of the injected zygotes lysed within several hours of injection. Ultimately, 1011 (9%) embryos developed to the compact morula or blastocyst stage by day 7 or 8 p.i. By comparison, 27% of contemporaneously cultured non-injected control embryos developed to the compact morula and blastocyst stages during this period.

Establishment of pregnancies and calves

Of 478 embryo transfer recipients, 155 (32%) were diagnosed pregnant on day 30 of gestation and 90 (19%) produced calves (Table 13.1). Thus, 65 (32%) pregnancies were lost between day 30 and calving. Embryo developmental stage at the time of transfer had no effect on either pregnancy rate or calving rate ($P>0.05$). Mean (±SD) gestation length was 274±3.16 days. While most calves were delivered by scheduled, Caesarean section, three (3%) were delivered spontaneously at days 255, 259 and 262 of gestation. Although the male:female ratio was 1.44:1 (59% male:41% female), the sex ratio did not differ ($P>0.05$) from the expected ratio of 1:1. Mean (±SD) weight of the calves at birth was 41.83±6.02 kg (range: 29.09–60.45 kg). Birth weight of calves was not affected by sex or transgenic status ($P>0.05$). Mean (±SD) birthweight of the three calves that delivered spontaneously was 30.68±2.25 kg.

One heifer delivered normal, healthy twin bull calves, the apparent result of spontaneous twin formation after transfer of a single embryo to the recipient. Two calves (2%) were born with congenital skeletal abnormalities. One (1%) of the spontaneously calving heifers delivered a dead calf; a post-mortem examination concluded that the calf died from complications due to dystocia. None of these three calves was transgenic.

Transgenic calves

Nine (10%) of the 91 calves were transgenic (Table 13.1). The sex ratio (male:female) among transgenic calves was 1.25:1 (56% male:44% female) and did not differ from the expected ratio of 1:1 ($P>0.05$). All transgenic calves were normal and healthy at birth.

Analysis of various somatic tissues taken from 30 calves initially identified as non-transgenic for the presence of the transgene in ear and

Table 13.1. Summary of data from a transgenic cattle production campaign. Embryo production and microinjections were performed over a 5-month period.

No. injected/total (%)	No. developed (% of injected)	No. transferred (% of injected)	No. pregnant/ no. recipients (%) day 30	No. calves/ no. recipients (%)	No. transgenic/ no. calves (%)
11,507/20,918 (55)	1011 (9)	478 (5)	155/478 (32)	91/478 (19)	9/91 (10)

27% of non-injected control embryos developed over this period.

blood revealed no evidence of the transgene in any other tissue examined, indicating that analysis of these two tissues is sufficient for predicting the absence of the transgene in other tissues.

Effect of FCS during embryo culture on embryo development, pregnancy, calving and birthweights

The results of this experiment are summarized in Table 13.2. The presence of FCS in embryo culture medium between days 5 and 7 or 8 p.i. did not affect the proportion of embryos that developed to the compact morula or blastocyst stage by day 7 or 8 ($P>0.05$). Similarly, FCS did not affect pregnancy rate at day 30 of gestation, nor did it affect calving rate, birthweight or sex ratio ($P>0.05$).

Transgene expression in milk

A total of 91 ml of a lacteal secretion was obtained over a 7-day induced lactation period. The secretion contained human α-lactalbumin at a concentration of 2.4 mg ml^{-1}.

Discussion

The work presented here confirms previous reports (Krimpenfort *et al*., 1991; Hill *et al*., 1992; Bowen *et al*., 1994; Hyttenin *et al*., 1994) on the feasibility of generating transgenic cattle on a large scale from relatively inexpensive and plentiful *in vitro*-produced ova. The proportion of transgenic calves born in this study (9/91; 10%) was higher than previously reported for cattle (0–6%: reviewed by Eyestone, 1994). Finally, the human α-lactalbumin transgene was shown to be expressed in the mammary gland of a heifer, demonstrating production of a recombinant protein in bovine milk.

Pronuclei were visible for microinjection in only 55% of the zygotes examined, despite the fact that histological evaluation revealed that between 78% and 100% of the oocytes had been fertilized. The reasons for this discrepancy are unknown, but we have observed that oocytes matured in transit yield zygotes in which pronuclei are more difficult to visualize compared with those matured under the more controlled conditions of a laboratory incubator. Pronuclei are prominent under Nomarski optics only during a rather brief time 'window' (approximately 16–24 h p.i.), whereas pronuclei are histologically identifiable outside of this window. Asynchronous pronuclear development, perhaps caused by suboptimal oocyte maturation conditions in transit, may have led to reduced pronuclear visibility at any one time.

As reported for other species (Wall, 1996), pronuclear injection of bovine zygotes reduced subsequent development to the blastocyst stage compared with non-injected controls (Table 13.1). Moreover, transfer of apparently normal, healthy, day 7 or 8 p.i. embryos to synchronous recipients led to lower pregnancy rates at day 30 of gestation (32%) compared with those generally achieved with non-injected *in vitro*-produced embryos of similar morphological quality (57% in our laboratory). Furthermore, the incidence of fetal loss between day 30 of gestation and calving was higher (42%; 65/155) than that reported for pregnancies from non-injected, *in vitro*-produced embryos (20%; Reichenbach *et al*., 1991). Thus, pronuclear DNA injection leads to losses throughout early embryo development and gestation in cattle. The reasons for these losses are unknown, but may stem from lethal insertional mutations or other physical damage to DNA resulting from the force of microinjection.

A number of workers have reported that calves resulting from *in vitro*-produced embryos were associated with higher mean birthweights (Behboodi *et al*., 1995; Kruip and Den Daas, 1997), longer gestation intervals (Kruip and Den Daas, 1997), more frequent dystocia (Behboodi *et al*., 1995; Kruip and Den Daas, 1997) and higher perinatal mortality (Behboodi *et al*., 1995; Kruip and Den Daas, 1997) compared with ordinary calves. In view of these reports, and considering that we chose to transfer Holstein embryos into Holstein × Angus or Hereford heifers, we opted to deliver all calves by elective Caesarean section in an attempt to alleviate some of the perinatal problems experienced by other groups. Indeed, the perinatal losses recorded in our study (1%) were at least as low as those expected for ordinary calves delivered naturally (3–7%; Hawk and Bellows, 1980; Behboodi *et al*., 1995; Kruip and Den Daas, 1997). Mean birthweight reported in this study (41.8 kg) was similar to those reported elsewhere for Holstein calves born after natural mating or artificial insemination (42.3 kg: Salisbury and Vandemark, 1961; 42.8 kg: Kruip and Den Daas, 1997) or conventional embryo transfer (42.7 kg: Kruip and Den Daas, 1997). However, it must be noted that in our study calves were delivered after a mean gestation of 274 days, which is about 5 days shorter than a natural-length gestation for a Holstein calf (Salisbury and Vandemark, 1961; Jainudeen and Hafez, 1980) and that birthweights were no doubt somewhat lighter than if the calves had been delivered after a full, natural gestation.

Considering the relative ease and economy of generating transgenic cattle from *in vitro*-produced embryos, it is difficult to imagine ever contemplating the formerly used approach based on *in vivo*-generated zygotes. Nevertheless, the *in vitro* approach is far from efficient. For example, only 0.08% (9/11, 507) of the injected zygotes in our study yielded transgenic calves. Opportunities for improvement include treatments to increase zygote developmental potential, embryo development in culture and embryo developmental potential after transfer to recipients. Since the mechanism of transgene integration after pronuclear injection is unknown

(Bishop, 1997), it is unlikely that rational approaches will be developed for improving the frequency of integration (Wall and Seidel, 1992).

A major expense in the generation of transgenic cattle lies in the acquisition and maintenance of recipient animals, most of which will never carry a transgenic fetus to term. Attempts to discriminate between transgenic and non-transgenic bovine embryos prior to transfer have included PCR of embryo biopsies (Bowen *et al*., 1994; Krisher *et al*., 1994) and inclusion of fluorescent reporter genes into the injected constructs (Menck *et al*., 1997). However, all of these methods have given rise to substantial proportions of false positives, rendering them unsuitable for routine screening. Analysis of fetal or placental cells recovered by allanto- or amniocentesis (Garcia and Salaheddine, 1997) could provide an alternative means of identifying transgenic pregnancies, though this approach does not eliminate the initial requirement for large recipient herds. Apart from some breakthrough in transgene integration technology, or in development of PCR or other methods that would eliminate false positives (e.g. fluorescence *in situ* hybridization (FISH): Lewis-Williams *et al*., 1997; constitutively expressed green fluorescent protein: Takada *et al*., 1997) improvements in the efficiency of microinjection-based methods for making transgenic cattle are likely to be incremental for the foreseeable future.

The presence of FCS in embryo culture medium from days 5 to 7 or 8 did not affect development to the compact morula or blastocyst stage, nor did it affect initial pregnancy rate, calving rate or calf birthweight (Table 13.2). These observations are in contrast to reports in sheep (Thompson *et al*., 1995) where the presence of FCS was shown to result in larger offspring. However, in that report, embryos were cultured in the presence of serum from the one-cell to blastocyst stage. Several investigators have reported accelerated fetal growth (Farin and Farin, 1995) and the birth of large calves after *in vitro* embryo culture (Sinclair *et al*., 1994; Behboodi *et al*., 1995; Kruip and Den Daas, 1997) compared with embryos generated *in vivo* by artificial insemination or conventional embryo transfer. Though no formal experiment has addressed the question of when during embryo development FCS might influence downstream parameters like birthweight, the data cited above suggest that such effects may occur during exposure of embryos to serum prior to day 5 p.i.

Table 13.2. Effect of FCS from day 5 to 7 or 8 p.i. on embryo development, pregnancy and calving rates and birthweight.

FCS	No. developed/ no. injected[a] (%)	No. pregnant/ no. recips[a] (%) day 30	No. calves/ no. recips[a] (%)	Mean BW[a] (SD), kg	BW range, kg
+	139/952 (15)	23/84 (27)	8/84 (10)	42.08 (4.00)	38.18–49.09
−	132/882 (15)	25/61 (41)	11/61 (18)	38.38 (5.22)	31.82–47.73

[a] No difference between treatments ($P>0.05$).

Recent advances in nuclear transfer technology have made possible the generation of adult sheep from cultured somatic cells. In an unprecedented report, Wilmut *et al.* (1997) transferred nuclei from sheep fetal fibroblasts and even mammary epithelial cells from a lactating adult ewe into enucleated oocytes, then transferred the 'reconstructed' oocytes to recipient ewes that later gave birth to lambs. Thus, fully differentiated somatic cell nuclei were reprogrammed to a state of totipotency upon transfer to an oocyte cytoplasm. This feat has now been repeated using fetal fibroblasts bearing a transgene (Schneike *et al.*, 1997) indicating that differentiated cells can be genetically modified *in vitro* and give rise to viable, cloned transgenic offspring. If this technology proves feasible in cattle, it will be possible to generate clonal somatic cell lines bearing either randomly inserted genes or more importantly, site-specific gene insertions and/or deletions derived by homologous recombination. A major advantage to this approach is that all calves born after transfer of nuclei from a genetically modified cell line would bear the desired modification(s), thus eliminating the burden of recipients harbouring non-transgenic fetuses. Moreover, the variety of genetic modifications possible with homologous recombination ('knock-outs', 'knock-ins', 'knock-out/replacements') expands the range of novel genotypes possible. For example, it has been proposed that the ultimate milk modification for infant formula applications would be to replace all of the bovine milk protein genes with their human counterparts and thus create cows capable of producing milk containing a complete human milk protein profile (Yom and Bremel, 1993). Theoretically at least, infant formulae based on such milk would be more suitable for human infant consumption than formula manufactured from ordinary cows' milk.

Homologous recombination, combined with somatic cell nuclear transfer, creates a real possibility of generating agriculturally important transgenic modifications. For example, it may be possible to 'knock out' genes that encode receptors for specific viruses and thus knock out their susceptibility to economically important viral diseases, e.g. foot-and-mouth disease, bluetongue and bovine viral diarrhoea. In view of the reproductive similarities between the sheep and cow, the transfer of somatic cell nuclear transfer technology to the cow should be relatively straightforward; we may thus look to this new technology as the next major improvement for the generation of transgenic cattle, and one that may play an important role in creating useful modifications for agricultural purposes.

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