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Exploring the potential applications of advanced reproductive technologies in deer farming

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ABSTRACT

Advanced reproductive technologies include *in vitro* embryo production, cloning and genetic modification. However, only the former has been developed in deer. Considerable experimentation is required to develop cloning and transgenesis which is only justified if the deer industry foresees future potential and if animal welfare and public acceptance issues are addressed. Efficient *in vitro* embryo production and cloning potentially enables rapid dissemination of desired genotypes but relies on accurate identification of genetically superior individuals, which is limiting. *In vitro* embryo production with oocytes from elite females increases genetic gain and is further enhanced with pre-pubertal animals by shortening generation interval. Utilising sexed sperm to pre-determine gender increases reproductive efficiency. Cloning by nuclear transfer pre-determines the genotype of the animal and could effectively produce cloned stags from progeny-tested sires for natural breeding. Transgenesis introduces novel genetic modifications into the genome but is limited by the present poor understanding of which genes to manipulate to enhance desirable traits. The advancement of animal genomics will identify both individual genes and entire genotypes to multiply. Nevertheless, these technologies are considerably more difficult to cost-effectively implement than even poorly adopted artificial insemination in an extensive deer industry and will only be relevant for specialised markets.

Keywords: deer; reproductive technologies; *in vitro* produced embryos; sexing; cloning; nuclear transfer; genetic modification; transgenics.

INTRODUCTION

Assisted reproductive technologies are tools used singularly or in combination to accelerate the rate of genetic gain and/or to disseminate desirable genes into the general population. Some of these tools, such as artificial insemination (AI) and multiple ovulation and embryo transfer (MOET) are commercially available to the deer industry and used to disseminate European and North American genetics throughout the New Zealand deer industry (Asher, 1998). More advanced reproductive technologies, such as *in vitro* embryo production (IVP), ultrasound guided or laparoscopic oocyte pickup (OPU), sexing of embryos, sperm and foetuses are used in the dairy and beef industries. These tools have been developed or modified for farmed deer and can be made available to the industry within a short time frame. The potential of advanced reproductive technologies such as cloning and transgenics coupled with genomics can be predicted, but their application in any livestock industry is still dependent upon economics, consumer acceptance and regulatory approval addressing environmental concerns, animal welfare and food safety issues (Faber *et al.*, 2003).

Most advanced reproductive technologies are expensive. They involve specialised equipment that is not designed for on-farm use, require skilled technicians and rely heavily on intensive animal management to achieve results. Furthermore, technologies such as cloning and transgenics are considered high-risk ventures in terms of a successful outcome and are reserved for the most valuable genes usually associated with high value-added niche market products (Smeaton *et al.*, 2003).

Does the deer industry need advanced reproductive technologies? One could argue that an animal industry which lacks an industry-led sire selection scheme to identify present and future genetic traits is unlikely to gain from using reproductive technologies; although a few specialised deer breeders do profit from using currently available tools, e.g., AI/MOET.

Two factors working in combination have the potential to advance a sire-selection breeding scheme. One is the technological breakthrough of palpation per rectum of red deer for cervical AI, ultrasound-guided ovum pickup, and non-surgical embryo transfer (Berg & Asher, 2003; Rhodes, 2003). This, in conjunction with the rapidly expanding field of animal genomics has the potential to drive innovative animal breeding schemes such as marker-assisted selection, in association with a small nucleus herd of females for elite sire propagation and quantitative trait loci (QTL) mapping of the deer genome to identify genes important in growth, puberty and seasonality. Assisted reproductive technologies are the tools used to deliver these genes of interest into the industry.

In vitro embryo production

In vitro embryo production provides the opportunity to increase the number of offspring from superior genotypes by maximising semen and oocyte usage, and is the backbone for advanced reproductive technologies. This process is an alternative to MOET, producing embryos from oocytes collected from the ovaries of donor females of various ages and reproductive status, either repeated collections or post-mortem.

IVP is gaining wider acceptance as an alternative to MOET because it does not rely on exogenous pituitary hormones (follicle stimulating hormone, FSH) for follicular growth and, hence, does not interfere with the animals' immediate future reproductive cycles. More importantly, there is mounting hostility against the use of pituitary extracts in Europe because of the BSE threat, since the pituitary gland is now considered "specific risk material". Germany has outlawed the use of pituitary

extracts for superovulatory treatments in cattle leaving only equine chorionic gonadotropin (eCG) as an economically viable alternative (Galli *et al.*, 2003). The use of eCG alone in red deer MOET programmes and for the collection of *in vivo* matured oocytes for IVF is ineffective (Fennessy *et al.*, 1994; Bainbridge *et al.*, 1999)

In vitro embryo production is a combination of three biological steps: oocyte *in vitro* maturation (IVM), *in vitro* fertilisation (IVF) and *in vitro* embryo culture (IVC). Reliable red deer and wapiti systems have been developed that allow maturation and fertilisation *in vitro* and culture systems that can be used to grow the embryos to a stage where they can be transferred (non-surgically or laparoscopically), although the efficiencies of deer IVP are lower than those for cattle and sheep (Pollard *et al.*, 1995; Comizzoli *et al.*, 2001; Berg & Asher, 2003).

Ovum pickup

Oocytes for IVM can be collected from different types of donors and by different methods. The most flexible and repeatable technique to collect oocytes from live donors is ultrasound-guided ovum pickup (OPU). Follicles within the deer ovary grow in waves and this enables follicles to be harvested at regular intervals from the same donor on a twice-weekly or weekly schedule throughout the breeding season and from pregnant hinds up to day 60 of pregnancy (Asher *et al.*,1997; Berg & Asher, 2003). The technique is minimally invasive and involves introducing a transducer, equipped with a needle, into the vagina of lightly sedated hinds. The ovary is positioned against the transducer by manipulation per rectum. Aided by the image on the ultrasound screen, the needle is pushed through the vaginal wall into the follicle, and the oocyte is aspirated into a collection tube. The entire procedure takes approximately seven minutes to complete and the hind walks off the crush. On average, four follicles are aspirated, two oocytes are suitable for IVM and one embryo is produced from each hind per OPU session (Berg & Asher, 2003).

OPU and IVP are replacing MOET to produce elite dairy sires. A small nucleus herd of selected heifers is inseminated followed by twice-weekly OPU-IVP until the pregnant heifers reach Day 90 of gestation. This technique increases the number of embryos produced per donor and shortens the generation interval (den Daas, 1997). OPU-IVP opens the way for factorial mating designs which result in greater genetic gain without increasing the level of inbreeding (van Arendonk & Bijma, 2003). To achieve even greater genetic gain by decreasing the generation interval, oocytes are recovered from pre-pubertal females (juvenile *in vitro* embryo production, JIVET). Female bovine calves (2-4 months of age) have been used as donors with the oocytes recovered by surgical laparotomy. To achieve consistent results, the ovaries need to be stimulated with gonadotrophins (FSH and LH), however, the efficiencies are much lower with calf oocytes compared with adult oocytes; 10% compared to 35% blastocyst rate, respectively. Pregnancy rates vary amongst laboratories but most authors report a higher incidence of embryonic loss after embryo transfer when the embryos are produced from calf oocytes (Taneja *et al.*, 2000; Armstrong, 2001). This lower developmental competence and embryonic loss is partly attributed to incomplete or delayed cytoplasmic maturation of the calf oocyte (Armstrong, 2001)

OPU is variable because of individual differences in follicle numbers and oocyte quality. Although OPU can be conducted on-farm, it requires specialised equipment and access to an embryo laboratory.

Abattoir recovered oocytes

"Genetic rescue" involves recovering ovaries from individual females of high genetic value that are destined for slaughter because of terminal illness, age, or infertility. The success of embryo production is dependent on the reason for slaughter. Cattle embryo production is poor for terminal donors (those already dead, in critical general condition or with progressed foot or leg injuries). Results improve with healthy donors (infertile, end-of-career or eradication of infectious diseases). This technique has been used to rescue the genetics of dairy and beef herds infected with leucosis, brucellosis, tuberculosis and BSE (Galli *et al.*, 2003).

Large numbers of embryos can be produced from the ovaries of commercially slaughtered donors when the female genetic contribution is unimportant. The ovaries of similar breeds are pooled, the oocytes are fertilised with semen from a high-genetic-value sire and frozen for future transfer. The cattle industry has used this "bulk IVP" system to generate low-cost embryos for commercial production of beef calves from dairy herds and to produce dairy embryos of average genetics for developing countries (Galli *et al.*, 2003).

***In vitro* fertilisation**

Unlike the dairy and beef industries, deer semen is a limiting resource. Semen is collected seasonally and only 100 to 200 straws are collected per stag. These stags are also used for natural mating and in MOET programmes, which curtails the length of time semen can be collected. Using IVF, one straw of deer semen can fertilise 200 oocytes recovered from 50 pairs of ovaries at slaughter or oocytes recovered from 20 OPU donors. Multiple dam and sire combinations are an important advantage of IVF.

***In vitro* embryo culture**

The early cleavage stage embryo is usually cultured for 6 to 7 days until it reaches the blastocyst stage at which it can be either sexed, frozen or transferred non-surgically; fresh or thawed. Pregnancy rates with IVP embryos can be variable especially with the frozen embryo (Galli *et al.*, 2003). Culturing embryos to the blastocyst stage can produce offspring with extended gestation length, higher birth weight, and peri-natal mortality, collectively referred to as "large offspring syndrome" (Holm & Callesen, 1998; Young *et al.*, 1998). This problem has been overcome largely by using Synthetic Oviduct Fluid medium (SOF) with 95% of the offspring born being normal (Thompson *et al.*, 1995; Galli *et al.*, 2003). A red deer-specific culture medium has been developed, termed Deer Synthetic Oviduct Fluid (DSOF). Blastocyst development rate is 20% and preliminary results demonstrate 50% of the

transferred embryos produce calves of normal birth weight after a normal gestation length (Berg, unpublished data).

Potential opportunities for the deer industry from IVP

The OPU-IVP dairy model is well suited to the deer industry's current pyramidical breeding structure where <5% of deer farming enterprises contribute to genetic gain (Asher, 1998). The technology could be implemented within a short time (two years), but infrastructural support would be needed. JIVET is difficult to implement in the deer industry because of the extreme seasonal nature of breeding and calving, which currently constrains oocyte collection to the months of January and February. Fresh embryos could not be transferred and, instead, embryos would need to be frozen for later transfer. Suitable freezing methods for deer IVP embryos need to be developed. Genetic rescue has the potential to recover female genetics that would otherwise be lost because of disease, accident or age. Bulk IVP could be used to export low cost commercial deer embryos to emerging deer farming countries. With the outbreak of chronic wasting disease in North America, this bulk-embryo approach could be used to repopulate commercial deer herds in that region.

Determination of sex

The possibility of sex pre-selection has always generated extreme interest in livestock industries and the deer industry is no exception. Three types of sex selection are available to the cattle industry: sorted sperm, embryo biopsy and foetal sexing. Bovine sperm are sorted based on a 4% difference in DNA content between the X and Y bearing sperm using a high-speed cell-sorting machine (Johnson & Welch, 1999). Sperm can be used (fresh or frozen/thawed) for AI, MOET or IVP programmes. Artificial insemination of elk cows with frozen sexed sperm resulted in a 61% (11/18) pregnancy rate, with 82% of the calves being the predicted sex (Schenk & DeGrofft, 2003). However, the high cost of obtaining a license for this technology impedes its availability in New Zealand (Smeaton *et al.*, 2003).

Embryos can be sexed at the blastocyst stage by taking a biopsy of the embryo and amplifying Y-chromosome specific DNA using the polymerase chain reaction (PCR). Biopsy is effective for >90% of the embryos and PCR is >95% accurate. Biopsy and PCR are time-consuming and the technique is difficult to perform under field conditions. Embryos can be biospied and frozen/thawed although the pregnancy rate is decreased by half (Faber *et al.*, 2003). The *in vitro* development rate of the embryo can also be used as a predictor of sex. The proportion of males is significantly higher among embryos that cleave to the two-cell stage within the first 30 hours after IVF and the sex ratio is further influenced by the IVC system (Yadav *et al.*,1993; Holm & Callesen, 1998; Kochhar *et al.*, 2001). Ultrasound foetal sexing can be accurately performed at approximately day 60 of gestation in cattle. The undesired pregnancies are problematic because the limited length of the breeding season reduces the opportunity for rebreeding and red deer do not respond well to prostaglandins this late in gestation (Asher *et al.*,1996). Somatic cell cloning is perhaps the ultimate sexing technique.

Cloning deer by nuclear transfer

Nuclear transfer is essentially a method to produce offspring cloned from single cells. Although not yet accomplished in cervids, it is expected that deer will eventually be included to the ever-increasing list of mammalian species cloned by nuclear transfer (Wilmut *et al.*, 2002). Success in red deer is likely because the understanding of cervid reproductive physiology has lead to methods for oestrus synchronisation, *in vitro* embryo production and embryo transfer to a level now satisfactory to underpin cloning technology. Two key obstacles, however, are complete reprogramming of donor nuclei (common to all species; see below) and an effective embryo activation strategy specific for deer species, since bovine protocols perform poorly (Berg, personal observations).

Although there are many variations in protocols and timing of events for particular species, the generic approach for nuclear transfer common to most mammals and envisioned for deer is outlined below. First, the chromosomes from mature, unfertilised oocytes, arrested at metaphase of the second meiotic division, are aspirated with the aid of fine micro-surgical instruments to produce a cytoplast. In livestock, immature oocytes are typically recovered from abattoir-derived ovaries of culled females and matured *in vitro*. This provides a cheap, bulk source but one which, unlike cattle, is constrained by the seasonality of folliculogenesis in deer, limiting the numbers of cloned embryos that could be produced. In those deer species in which suitable IVM conditions have not been defined an alternative, albeit expensive, option is to surgically recover *in vivo* oocytes just after ovulation.

The nucleus from a donor cell obtained from the animal being cloned is then introduced into the cytoplast either by direct injection or, more commonly, following electrically-mediated cell fusion. Interactions between the donor chromatin and factors present within the oocyte cytoplasm provide opportunity for reprogramming. The reconstructed one-cell embryos are then artificially activated to initiate embryonic development. Activation regimes that mimic the repetitive intracellular calcium oscillations that occur during normal fertilisation result in significantly improved development (Ozil & Huneau, 2001). In species for which media have been developed to meet the specific metabolic requirements of pre-implantation stage embryos, reconstructed embryos may be cultured *in vitro* up to the blastocyst stage. If suitable media are unavailable, cloned embryos may be transferred to a temporary recipient for initial development. Suitable embryos are transferred non-surgically to the reproductive tract of synchronised recipient females where some may implant and develop to term for the eventual birth of cloned offspring.

Current cloning efficiencies

The present nuclear transfer methods are inefficient in all species. The range is generally between 1-7% of

reconstructed one-cell cloned embryos resulting in viable offspring (Wilmut *et al.*, 2002). AgResearch's experience in cattle, with the best methodology, shows that 40% of reconstructed embryos result in transferable quality blastocysts, similar to IVP, but only 17% of transferred embryos currently result in viable cloned calves at weaning. This is approximately one-third the efficiency of IVP in cattle. Whilst current efficiency remains too low for large-scale opportunities in livestock industries, significant increases have been achieved through the prolonged exposure of donor nuclei to oocyte cytoplasm, controlling the cell cycle stage of the donor nucleus and cell type (Wells *et al.*, 2003).

Donor cells

The cells used for nuclear transfer may come from a variety of sources including early embryos or somatic cells, obtained from a foetus or small tissue biopsy taken from a chosen adult (Oback & Wells, 2002). Cell type affects cloning efficiency, with less-differentiated cells apparently more amenable to, or requiring less, reprogramming. Furthermore, the cells may be cultured to establish a primary cell line with ready access to millions of cells. Cultured cells are easily cryobanked and, thus, used to preserve valuable farm animal genetic resources complementing the storage of gametes and embryos. Alternatively, donor cells may be genetically modified and used to produce cloned-transgenic animals. Thus, the core technique of nuclear transfer can produce either clones or transgenics depending upon the choice of donor cell.

Reprogramming donor cells

Many factors affect the success of nuclear transfer. One critical factor is reprogramming. For normal development, the organisation of the chromatin and the pattern of gene expression in a specialised somatic cell must be completely reprogrammed to a zygotic state to enable embryonic genes to be reactivated in the correct tissues, in the correct abundance and at the correct times. Clearly this is a highly orchestrated process and is understood poorly. There is, however, increasing evidence of epigenetic errors in reprogramming following nuclear transfer leading to abnormal patterns of gene expression (Rideout *et al.*, 2001; Fairburn *et al.*, 2002) and these are considered the major cause of the developmental failures. Understanding the molecular mechanisms involved in reprogramming will ultimately improve cloning efficiency. Many of the advances made in other species will be directly relevant to deer species also.

Cloned pregnancies

Complete reprogramming is apparently rare, with the majority of cloned embryos failing at various stages of development. There is every reason to expect cloned deer embryos to show similar problems as other species. An extreme exemplification of the animal welfare issues is illustrated in cattle. Although the rate of pregnancy establishment is similar to the transfer of *in vivo* and *in vitro* fertilised embryos, with 50% embryo survival on Day 50, unlike fertilised embryos, 60% of the cloned bovine foetuses are subsequently lost throughout the remainder of gestation.

The main consequence of incomplete nuclear reprogramming is a failure of the placental membranes to develop and function normally. In cattle, the number of placentomes is approximately halved, compared to normal, with compensatory overgrowth. Of concern is hydroallantois because 25% of established cloned bovine pregnancies may succumb to this syndrome. The volume of allantoic fluid may be four times greater than normal, necessitating elective abortion in mid-gestation to minimise distress to the recipient. Research aims to detect hydroallantois in the first trimester to lessen the welfare burden and, ultimately, prevent the syndrome through improved reprogramming.

Parturition and neo-natal survival

Generally, recipients pregnant with clones show poor preparation for parturition, prolonged gestation and increased risk of dystocia, often prompting elective caesarean section. However, corticosteroid therapy to induce parturition one week before expected full term has successfully aided foetal maturation, (assisted) vaginal delivery and improved the maternal response towards rearing offspring (Wells *et al*., 2003). Nevertheless, birth weight may be 30% greater with clones.

Post-natal mortality is also greater in clones, especially in cattle and sheep but less so in pigs and goats (Wilmut *et al.*, 2002). The stage of the donor cell cycle at the time of nuclear transfer has a significant effect, with a higher proportion of calves at term, derived from quiescent G_0 donor cells, surviving to weaning (76%) compared to clones derived from G_1 cells (57%) (Wells *et al*., unpublished). Newborn clones have an altered metabolism, possibly due to the *in utero* placental abnormalities (Garry *et al*., 1996), and require time to adjust to a normal physiology (Chavatte-Palmer *et al.*, 2002). Most deaths are due to either abnormalities of the cardiovascular system, the skeletal system, brain, or kidney, along with umbilical and lung infections and digestive disorders (Wilmut *et al.*, 2002).

Health of clones in adulthood

Although there are reports of physiologically normal cloned animals (Lanza *et al.*, 2001; Renard *et al.*, 2002) displaying normal behaviour, growth rates, reproduction, livestock production characteristics and lifespans (Wells, 2003), other reports point to longer-term health concerns. These have included obesity (Tamashiro *et al.*, 2002) and shortened lifespan (Ogonuki *et al.*, 2002) in mice and compromised immune systems in cattle (Renard *et al.*, 1999). The incidence of these clone-associated phenotypes varies according to the particular species, genotype, sex, cell type or specific aspects of the nuclear transfer and culture protocols used. In cattle, the proportion of cloned calves born that are long-term survivors ranges between 47-80% (Lanza *et al.*, 2001; Heyman *et al.*, 2002). The cloned offspring syndrome is a continuum, in that lethality or abnormal phenotypes may occur at any phase of development depending upon the degree of dysregulation of key genes. Even apparently

normal clones may have abnormal regulation of many genes that are too subtle to result in an obvious phenotype (Humpherys *et al.,* 2002).

Trans-generational effects

Although there are problems in the cloned generation stemming from incomplete reprogramming, the offspring of surviving clones produced following sexual reproduction appear completely normal, even when male and female clones have been mated together (Tamashiro *et al.,* 2002; Wells, 2003). This suggests that any epigenetic errors in the clones are corrected during gametogenesis. However, additional studies need to exclude the possibility of transmission of differential recessive genetic or epigenetic traits between the two cloned parents.

Safety of food products from clones

International food regulatory agencies are presently addressing issues surrounding the safety of food products derived from clones and their offspring. Although subtle epigenetic errors in surviving clones will contribute to phenotypic variability, it is difficult to foresee that milk or meat from cloned livestock would be outside the normal range of food products consumed by humans.

Scientific data is extremely limited at present; however, initial results from one small set of cloned dairy cows indicate that milk composition is within the broad range of milk produced from conventional cows (Wells, 2003).

Potential opportunities for the deer industry from cloning

It is assumed that deer cloning is biologically feasible but initially will be fraught with the same developmental problems and animal-welfare issues as other species. If the ethical costs associated with producing the few surviving healthy clones can be justified, then limited practical applications may be realised. Other opportunities, however, would not be feasible nor tolerated until complete reprogramming resulted in an efficient and acceptable deer-cloning technology.

Efficient cloning could enable the rapid dissemination of superior genotypes from nucleus breeding herds, directly to commercial farmers. Genotypes could be provided that are ideally suited for specific product characteristics or possess traits conferring resistance to particular pests or diseases. These genotypes could be disseminated by the controlled release of selected lines of elite live animals for breeding or the transfer of frozen/thawed cloned embryos. The latter would be an alternative to AI but needs to be equally successful and cost-effective. Farmers could select different lines of cloned embryos to maintain genetic diversity in their herds.

Possibly the best opportunity involves the production of cloned stags from progeny-tested sires for wide-spread dissemination of their elite genetics following natural breeding or alternatively, increased semen production for AI. If cloned sires are faithful genomic copies of the original donor, this application avoids confounding issues with the transmission of mitochrondrial DNA (which is only maternally inherited) and phenotypic differences arising from environmental influences and subtle differences in gene expression patterns, as these epigenetic variations appear to be corrected via gametogenesis. The equivalent opportunity in the dairy cattle industry is being developed through AgResearch's involvement with Clone International.

Efficient cloning potentially enables the rapid production of large herds generating economic volumes of specific products tailored for niche markets. This relies on identifying animals that meet these criteria and then their subsequent multiplication. It is possible to identify carcasses with superior meat characteristics and to clone animals either for breeding purposes, and so capture these genetics after slaughter, or to generate herds with more consistent meat production. This has been exemplified by the resurrection of a steer following post-slaughter meat assessment to generate a set of cloned bulls for breeding (Wells, 2003). Marker-assisted selection strategies to identify favourable genes that correlate with production will aid in selecting desirable genotypes in the future. Coupled with embryo cloning this would also increase the rate of genetic gain.

Genetic modification of deer

Genetically modified animals are commonly referred to as "transgenic", however, this term is biologically imprecise. It does not accurately reflect the nature of the introduced genetic changes, for these may be either within a species or from different species of varying evolutionary divergence. Given that conventional animal breeding aims to improve the genetic makeup of future offspring, the intention of genetic modification using modern molecular biology is no different. Combined with nuclear transfer, the technology is being developed in livestock to enable specific genetic enhancements to an existing genetically superior background from an animal of chosen performance and sex. These genetic modifications have the potential to be far more precise (and extensive) than what can be achieved with the lottery of traditional breeding. The genetic modifications may include: (1) the specific addition of desirable genes (from the same species or a different species); (2) the deletion of undesirable genes on an otherwise favourable genetic background and (3) precise base alterations to a specific gene improving a particular function in the resulting protein. The rate of genetic progress can be considerably more rapid than conventional breeding. The introduction of additional copies of bovine b- and k-casein genes into dairy heifers resulted in a 30% increase in casein protein in milk within one generation (Brophy *et al.,* 2003). The most efficient means of disseminating a genetic modification into the population will be through low cost AI (or natural mating) from males homozygous for the desired trait.

Practical opportunities for genetic modification in the deer industry are presently limited. Possible transgenic deer projects in the future may investigate improvement to the quantity and quality of valuable meat or velvet products and environmental sustainability by altering specific genes that affect feed conversion efficiency, meat composition, or disease and pest resistance. There is,

however, a lack of current knowledge of the deer genes that influence these (and other) production traits to easily justify such projects at present. Moreover, animal industries are reluctant to fund research to develop the capability and to explore the potential benefits and risks from genetic modification. Additionally, the public are sceptical, especially towards food-producing animals. Thus, we do not envisage any practical opportunities for the genetic modification of deer until animal genomics first identifies potential candidate genes for deer traits of sound commercial relevance, with economic benefit for either farmers or processors, or additional health benefits for consumers and which receives support from the deer industry. Gene discovery programmes in deer will identify potential pharmaceutical and nutraceutical products. In some cases, it may be profitable to over-express the relevant genes in deer. However, for large-scale production it is likely that over-expression of the relevant deer genes in the mammary gland of dairy cows will result in easier harvest and purification of the protein from milk.

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