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# Biotechnological Advances in Goat Reproduction<sup>1,2</sup>

E. A. Amoah<sup>3</sup> and S. Gelaye

Agricultural Research Station, Fort Valley State University, Fort Valley, GA 31030-3298

**ABSTRACT:** Goat selection and reproduction have resulted from using conventional methods of natural mating and artificial insemination. Genetic improvements resulting from these are usually slow. Innovative developments in biotechnology rapidly propagate superior genes, offering hope for modeling and designing animals to fit market and environmental requirements. Use of Tris, citric acid, fructose, egg yolk, and glycerol extender has enabled goat sperm to be stored successfully for several years before being used in cervical or laparoscopic insemination. Laparoscopic recovery of goat embryos to reduce adhesions from repeat surgeries has great potential in improving embryo production for direct transfer or for future transfer after cryopreservation. Goat kids have been

produced, as a result of experiments to refine techniques of in vitro maturation and fertilization of recovered oocytes, with successful culture and transfer of embryos. In vitro fertilization technology is also essential for cloning goat embryos and for gene transfer. Transgenic goats have already been produced due to new genes being expressed from biologically active molecules altering the phenotype of the transferred goat. The introduced gene is capable of transmission between generations. The goat's diversified commercial value and convenient size make it a benefactor to new technology for rapid genetic improvement as a supplement to conventional selection methods.

Key Words: Goats, Biotechnological Advances, Reproduction

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## Introduction

Gene cloning and transfer technology has provided methods for engineering alterations into the genomes of livestock to improve the quality of animal products. These techniques are providing new tools for genetic improvement as a supplement to conventional selective breeding. The goat is a convenient domestic species for current biological investigation and application because it has diversified products of commercial value and relatively short gestation period (5 vs 9 mo for a cow). Biotechnological alterations could make significant impact on the quality of goat meat; broiled goat meat has lower total lipid, phosphorus, and vitamin B<sub>12</sub> but higher calcium, potassium, thiamin, and cholesterol than composite values reported for beef (Johnson et al., 1995). Ebert et al. (1991) produced transgenic goats that expressed a heterolo-

gous protein (a glycosylation variant of human tissue plasminogen activator protein) in their milk, with potentially tremendous pharmaceutical value to cardiac patients. Genetic transfer from exotic breeds could accelerate improvements in fiber quality of local cashmere-bearing goats or improve the fertility of mohair-producing Angora goats. The rare skin-type trait in some goats (Red Sokoto or Morocco) could be introduced by genetic transfer into endermic goats with resistance to local diseases and capable of survival in extreme environmental conditions. The production of transgenic goats leads to unique solutions to problems that may not be encountered when mice are used as models (Steele and Pursel, 1990). This paper discusses various biotechnological advances, focusing on artificial insemination, embryo cloning and transfer, in vitro goat embryo production, and gene transfer.

## Artificial Insemination

The success of AI is based on the ability to efficiently collect and cryopreserve spermatozoa from quality bucks for use on does over generations. Using a few selected superior male spermatozoa resources and conducting an efficient AI system rapidly im-

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<sup>3</sup>To whom reprint requests should be addressed.

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Table 1. Pre- and post-freezing percentage of progressive motility and percentage of live spermatozoa in Boer goat semen at different seasons of the year<sup>a</sup>

Season <sup>b</sup>	No. of does	Progressive spermatozoa motility, %			Percentage live spermatozoa		
		Pre-freezing ± SEM	Post-freezing ± SEM	Freezing loss	Pre-freezing ± SEM	Post-freezing ± SEM	Freezing loss
Spring	35	62 ± 2 <sup>c</sup>	29 ± 2 <sup>c</sup>	54	61 ± 2 <sup>c</sup>	32 ± 3 <sup>c</sup>	47
Summer	45	65 ± 1 <sup>c</sup>	35 ± 2 <sup>c</sup>	46	71 ± 2 <sup>d</sup>	40 ± 3 <sup>d</sup>	44
Autumn	60	71 ± 2 <sup>e</sup>	39 ± 2 <sup>d</sup>	45	75 ± 3 <sup>de</sup>	44 ± 2 <sup>d</sup>	41
Winter	37	73 ± 2 <sup>e</sup>	45 ± 3 <sup>e</sup>	38	76 ± 3 <sup>e</sup>	49 ± 3 <sup>e</sup>	36

<sup>a</sup>Tuli and Holtz (1995).

<sup>b</sup>Spring = Mar. 21–June 20; summer = June 21–Sept. 20; autumn = Sept. 21–Dec. 20; winter = Dec. 21–March 20.

<sup>c,d,e</sup>Within columns, different superscripts indicate differences ( $P < .05$ , least significant difference test).

proves the selection differential of a goat population (Amoah and Gelaye, 1990).

In the 1940s and 1950s it was realized that bull spermatozoa could be stored frozen and two to four “ejaculates” a week could provide sufficient spermatozoa after frozen-storage to inseminate at least 2,000 cows. This accelerated selection of sires doubled the genetic ability for milk production in dairy cows (First, 1992). For years, successful techniques used for preservation of bovine semen were the only available model used for goats (Corteel, 1992). Egg yolk buffers were used to extend goat semen for AI for decades with limited success, despite the early finding by Roy (1957) that the goat Cowper’s gland secretion has an egg yolk coagulating (EYC) enzyme. In the presence of calcium the EYC enzyme hydrolyzes egg yolk lecithin to fatty acids and lysolecithin. With the proportions of egg yolk being used in buffers, lysolecithins are released in quantities that are highly toxic to spermatozoa (Corteel, 1992). The enzyme acts on the ester linkage with the acyl groups of egg yolk phospholipid to release saturated (palmitic and stearic acids) as well as unsaturated (oleic and linoleic acids) fatty acids, resulting in a sudden drop in pH to 6.0. Also, there was evidence of a depressed respiratory rate of ejaculated goat spermatozoa under in vitro conditions. Iritani et al. (1961) indicated that bull, ram, boar, and rabbit semen did not have the EYC enzyme, which contains a phospholipase A similar to that in snake venom (Iritani and Nishikawa, 1963). Fakuahara and Nishikawa (1973) recommended that ejaculated goat spermatozoa should be washed twice before preservation to remove exogenous substrates that are detrimental to sperm motility and respiration. Techniques developed to remove seminal plasma from ejaculated sperm cells resulted in successful in vitro survival of sperm before and after freezing and thawing. This resulted in extending the fertile storage of goat sperm by several years (Corteel, 1974; Ritar and Salamon, 1982). An approximately 62% kidding rate was achieved by using hormones to induce and inseminate 17,438 Alpine and Saanen goats using semen doses stored from .5 to 3 yr (Leboeuf, 1989). Skim milk heated at

92°C for 10 min inactivates detrimental factors such as lacterin, making it a slightly better spermatozoa diluent than egg yolk (Leboeuf, 1992). Long-term preservation of goat sperm could, however, be simplified by using diluents that do not necessitate the elimination of seminal plasma (Corteel, 1992). Sahni (1987) recommended the use of citrate, oxalate, and also phosphate to inhibit the enzyme activity. Ten to twenty percent of the egg yolk is the optimal level for the preservation. Earlier, Roy and Gupta (1959) had pointed out that duration of spermatozoa viability in egg yolk citrate was best with unwashed sperm. However, with washed sperm, glycine egg yolk and glycerophosphate egg yolk were the better extenders.

It seems that the best goat sperm extender is Tris, citric acid, fructose, egg yolk, and glycerol. Pirohit et al. (1992) did not find any difference between direct dilution and washing of sperm before dilution with this extender. These authors found out that 5% glycerol is superior to 3 to 4%, and Singh et al. (1995) recommended similar extender containing 6% glycerol and 1% dimethyl sulfoxide (DMSO). Also, a combination of glycerol with lactose is a better cryoprotectant than glycerol alone (percentage live sperm, 56.8 vs 51.1).

Two methods are used in AI for goat production. Cervical insemination involves deposition of sperm on the uterine side of the cervix. Conception rate (CR) using this method ranges from 50 to 70% depending on the season of insemination. The CR is low during spring and summer due to lower sperm motility than in the autumn and winter (Table 1, Tuli and Holtz 1995). However, photoperiod treatment of bucks (Delgadillo et al., 1995), which enabled quality sperm collection all year round, may alleviate such seasonal variation in sperm quality. Laparoscopic insemination, the second method, involves the use of laparoscope and manipulating probe to aid in depositing fresh or frozen-thawed sperm directly into the uterine horns. Laparoscopic insemination procedures are described for sheep and goats by Ritar and Ball (1991) and by Jackson (1993). More than 80% success rate of conception is realized (Amoah and Gelaye, 1990).

Despite higher skills required in laparoscopic insemination, a producer may have to make an economic decision if AI is to be used for breeding. About half of the does will be expected to breed if very good fertile spermatozoa are used on a large number of females by cervical insemination, and laparoscopic insemination with > 80% CR could be used for the quality does. Reports indicate that the effective dose of fresh semen is about  $2 \times 10^7$  for laparoscopic insemination. For fresh semen, the recommended dose is about  $1 \times 10^8$  sperm concentration for cervical insemination; a similar success rate could be achieved with about half that sperm concentration for laparoscopic insemination (Amoah and Gelaye, 1990; Berger et al., 1994).

### Embryo Recovery, Storage, and Transfer

Bilton and Moore (1976) reported the first successful cryopreservation of goat embryos. Since then, larger numbers of goats have been successfully produced after transferring embryos that have been recovered and cryopreserved (Chemineau et al., 1986; Tsunoda, et al. 1987; Baril et al., 1989). Leibo's (1988) comprehensive review concluded that techniques in permeability, cryopreservation, and freezing characteristics of embryo cells of one species cannot be extrapolated to another. Thus, more specific caprine embryo preservation data are required.

Detrimental effects of repeated surgical embryo recoveries cause adhesions and reduce fertility of donor females, limiting the usefulness of the embryo transfer technique for genetic improvement. Two effective methods of embryo recoveries that require less surgical trauma are laparoscopic and other nonsurgical procedures. BonDurant et al. (1984) recovered blastocysts with the aid of a laminaria japonica unit for dilation of the cervix. Embryos were collected with animals standing, using epidural anesthesia. Collection of 15 of 26 attempts resulted in obtaining 34 embryos from Angora goats with 38 corpora lutea (Bessoudo, 1988). Van Niekerk et al. (1990) administered prostaglandin E<sub>2</sub> and estradiol for priming the cervix in goats for a successful embryo recovery.

Puls-Keingeld et al. (1992) used a freezing medium of PBS + 20% heat-inactivated goat serum + 1.4 M glycerol to cryopreserve goat embryos by one-step or three-step equilibration with glycerol cryoprotectants. Transferring morulae produced an 11 vs 22% pregnancy rate and 3 vs 15% embryo survival using one-step compared to three-step, respectively. Blastocysts had 90 vs 57% pregnancy rate and 45 vs 19% embryo survival rates for the one-step and the three-step, respectively. It was thus reported that blastocysts cryopreserve better than morulae and the one-step (1.4 M glycerol) technique was better than the three-step (.47, .94, and 1.40 M glycerol) technique. Embryos were usually immersed in liquid nitrogen for

storage after equilibration, subsequent to controlled cooling with a cryopreservation unit. Caprine embryos were successfully traded internationally using ethylene glycol, which is a better cryoprotectant agent than glycerol (35 vs 22% kids born from embryos thawed, respectively; Le Gal et al., 1993). Holm et al. (1990) used ethylene glycol and had 54% embryo survival for 270 Angora goat embryos transported from Denmark to New Zealand; McKelvey and Bhat-tacharyya (1992) had a 67% pregnancy rate and 53% embryo survival for cashmere goat embryos transported from Russia to Scotland.

Vitrification involves the exposure of goat embryos to high concentration of cryoprotectants followed by direct immersion in liquid nitrogen. Yuswiati and Holtz (1990) placed goat embryos in a solution of modified PBS with 10% (1.4 mol) glycerol and 20% (2.7 mol) propanediol. After 10 min of equilibration, each embryo was drawn into .04 mL of a solution consisting of modified PBS with 25% (3.4 mol) glycerol and 25% (3.4 mol) propanediol contained in a .25-mL straw. Separated with an air bubble, the straw also contained .2 mL of a 1.0 mol sucrose solution. Straws were then sealed and submerged in liquid nitrogen directly for storage. The authors recovered 88% good embryos subsequent to vitrification and thawing. There were low rates of development after thawing (7.7% for morulae and 6.2% for blastocysts). Only 2 of the 16 embryos transferred produced successful pregnancies.

### In Vitro Fertilization of Goat Embryos

The initial two reports of in vitro fertilization (IVF) of goat oocytes were produced by Rao et al. (1984), who used in vitro capacitated sperm (xenogenous fertilization in rabbit oviduct), and Hanado and Pao (1984), who achieved fertilization using ionophore-treated spermatozoa. The work of Rao and his associates resulted in the birth of kids from the transfer of the embryos into goats. Caprine embryos have also been cultured in vitro in ligated oviducts or in chicken eggs (Blakewood et al., 1990). McLaughlin et al. (1989) cultured goat embryos in vitro using synthetic oviductal fluid medium, whereas Prichard et al. (1990) used oviduct and uterine cell monolayer culture systems. However, Hanada (1985) produced the first goat kids using the IVF procedure on ovulated oocytes in the goat. Younis et al. (1991) initiated pregnancy in goats by embryo transfer after in vitro maturation and fertilization of goat oocytes. It was not until 1992 that the first birth of a kid from an oocyte matured and fertilized in vitro took place (Crozet et al., 1993).

A number of factors have been shown to affect in vitro production of blastocysts for successful transfer. The process includes oocyte maturation, sperm capacitation, and fertilization and culture of the early

cleaving embryos and their development to blastocysts.

Oocytes with the capacity to develop could be selected from goat ovaries obtained from slaughterhouses. Between 1.5 and 2.1 oocytes per ovary were obtained by aspiration or dissection of follicles (Martino et al., 1994; Pawshe et al., 1994). They concluded that the recovery of cumulus oocyte complexes (COC) by slicing (average of six COC recovered) on the goat ovary was a simpler and a more efficient technique than aspiration and puncturing methods. Because prepubertal goats are more frequently slaughtered, Martino et al. (1994) used these goats for their investigation. They observed no significant difference in percentages of maturation between adult and prepubertal goat oocytes (81.8 vs 72.4%, respectively, recovered from follicles between 2.5 and 6.0 mm diameter). However, they concluded in their study that as the follicular diameter increased, oocytes complete their growth and reach meiotic competence. Follicles larger than 3.0 mm have more cumulus layers and have better *in vitro* maturation (IVM) results (Martino et al., 1994). Thus, it is necessary to select oocytes at the end of their growth phase. Oocytes from large follicles (> 5 mm diameter) yield a significantly higher number of blastocysts than those from small to medium follicles (< 5 mm diameter; Crozet et al., 1995 observed 26 vs 9%, respectively).

An average of nine COC per ovary was obtained by Crozet et al. (1995) with FSH-primed goats. Graff et al. (1995) collected oocytes by laparoscopic aspiration from genetically superior goats after FSH priming and obtained three to four COC per ovary. Maturation of prepubertal goat oocytes in co-culture + granulosa cells did not show any significant difference from percentage maturation from IVM in 50- $\mu$ L microdrops of medium (72 vs 77%, respectively; Martino et al., 1995). Keskintepe et al. (1994) investigated the effects of medium supplementation using glycoproteins (LH, FSH, hCG, and thyroid-stimulation hormone, TSH) during IVM of goat oocytes in IVF and embryo production. It was concluded that such glycoprotein hormones are required in maturation media to develop oocytes for successful IVF. Inclusion of these gonadotrophins for IVM enhances oocyte quality and developmental potential by possibly altering metabolic processes (Brackett and Zuelke, 1993). Significant progress made with development of a good medium for goat oocyte maturation consists of caprine follicular fluid (10%) and ovine FSH (100 ng/mL) in medium M199 cultured under 5% CO<sub>2</sub> at 39°C. This adequately replaces co-culturing with granulosa cells, providing a simplified, efficient IVM method (Poulin et al., 1996).

Spermatozoa undergo a maturation process called capacitation, which leads to "acrosome reaction," causing the release of proteolytic enzymes that may assist sperm penetration into the oocyte. Any agent that causes Ca<sup>++</sup> entry into the sperm acrosome and

an increase of pH within the sperm enables capacitation to be accomplished. Capacitation-stimulating agent, heparin, or caffeine may be added to the medium. Younis et al. (1991) described the frozen-thawed, "swim-up" heparin/caffeine-treated sperm in modified Tyrode solution with 3% BSA, lactate, and pyruvate (TALP) medium. A penetration rate of 70 to 85% was achieved. Heparin was shown to increase sperm-egg penetration when added to IVF medium containing sheep serum (Cox, 1994). However, Poulin et al. (1996) attributed the limited success of IVM/IVF technique in producing live goat offspring to date to the use of heparin as a capacitating agent for buck spermatozoa. Thompson et al. (1992) showed that a capacitation period may not be required for goat sperm. A high fertilization rate (85%) was achieved using culture media supplemented with estrous sheep serum to induce capacitation in spermatozoa (De Smedt et al., 1992).

Co-culturing of two- or four-cell embryos to blastocysts has been more successful with oviduct cells than with uterine or culturing in medium alone (Prichard et al., 1992). There is continued refinement of culture techniques, and a simple salt solution (synthetic oviduct fluid) supplemented with amino acids and serum with incubation under 5% O<sub>2</sub>, 5% CO<sub>2</sub>, 90% N<sub>2</sub> has been very successful. Poulin et al. (1996) showed that under these conditions the ability of embryos to develop to term after transfer of blastocysts is similar to the rates of those developed *in vivo* (61% of *in vitro* produced blastocysts resulted in live kids).

### Bisection and Cloning of Goat Embryos

Production of monozygotic twin goats offers various possibilities of studying embryo development. Rorie et al. (1987) bisected morulae and expanded blastocysts. When each half was placed into a zona pellucida, 34% became pregnant after transfer and produced monozygotic normal kids. Two does produced twins and one produced triplets.

Udy (1987) reported that goat embryos seem to be more difficult to bisect than other livestock embryos; the cell-to-cell tight junctions seem to be far weaker, and thus manipulations cause the disintegration of most embryos; the caprine zona pellucida seems to be more flexible than that of other livestock, and thus a greater degree of squashing occurs when cutting the zona with a micro-knife; and the cell debris resulting from bisecting of goat embryos causes more adherence to the instrument. Nevertheless, an embryo survival rate of 29% of demi-embryos transferred to recipient goats resulted, compared to 37% for whole embryos. Vivanco et al. (1991) used new techniques of electrostatically anchoring embryos to the base of the culture dish, thus eliminating the need for a holding pipette, and therefore cutting down the time of

Table 2. Pregnancy results after transfer of fresh and frozen-thawed demi-embryos<sup>a</sup>

Status of demi-embryos	No. of recipients	Pregnant at 21 d <sup>b</sup>		Carried to term		Live kids born		Percentage of demiembryos surviving
		n	%	n	%	Twins	Single	
Fresh	11	9	82	8	73	5	3	59*
Frozen-thawed	11	6	55	2	18	0	2	9

<sup>a</sup>Nowshari and Holtz (1993).

<sup>b</sup>Serum progesterone > 1.5 mg/ml.

\*Superior to frozen-thawed demi-embryos ( $P < .01$ , chi-square test).

micromanipulation. More than 50% of demi-embryos resulted in live offspring.

Fusion of blastomeres to enucleated oocytes in goats (i.e., nuclear transplantation) produced about a 25% success rate for blastocysts developing to offspring (First, 1992). Embryos at morula, blastocyst, and hatched blastocyst stages were obtained from superovulated and naturally ovulated Japanese native goats (Tsunoda et al., 1985). They were bisected into halves with a glass needle and transferred immediately or after culture (for morulae) to recipients. The study demonstrated that hatched blastocysts were most suitable for bisection goats. For freezing and preservation, goat demi-embryos have successfully been placed into zona pellucidae and embedded in agar (Tsunoda et al., 1987). Nowshari and Holtz (1993) split goat embryos with a simple and efficient procedure and froze demi-embryos without zona pellucidae. Table 2 indicates that of the nine does becoming pregnant from transfers of fresh demi-embryos, eight produced offspring (five twins and three singles). For frozen-thawed demi-embryo, six became pregnant and two produced offspring, both singles.

### Goat-Sheep Chimera

One of the initial chimeras was the product of a bold experiment in embryo manipulation. The interspecies chimera produced was not a hybrid, but rather a composite, in which some of the cells were derived from sheep and others from goats. The "geep," as it was called, produced by Fehilly et al. (1984) was developed from a combination of cells of sheep and goat embryos. These chimeras of sheep and goat showed phenotypic characteristics of both parental genes. One geep when mated to a male goat produced a normal kid. The geep resulted from the aggregation of female sheep and goat embryos from which the ovary produced a goat oocyte that was fertilized by goat sperm to produce the goat.

Chimeras have been used as a model for research in developmental biology. Inner cell mass (ICM) transfer may be useful in overcoming the interspecies embryo transfer barrier. Polzin et al. (1987) produced sheep-goat chimeras by immunosurgical isolation of

caprine ICM, injecting it into ovine blastocysts and transferring the resulting chimeric embryos to ovine recipients. By maintaining the trophoblastic integrity of the recipient species, it is apparently possible to "mask" the antigens of a foreign fetus from the mother's immune system, thereby increasing its chances of survival. Twenty-two manipulated blastocysts were surgically transferred into 12 ovine recipients. Nine ewes gave birth to a total of 13 young (59% embryo survival). Ten were classified by serum electrophoretic assays or karyotypes as lambs, one as a kid, and two as interspecific chimeras. Roth et al. (1989) also produced 11 overt goat-sheep chimeras. Interspecific chimeras are useful biological models for the study of cell development during fetal development because each cell contains species-specific markers that distinguish it from cells of other species. Mammalian chimeras are generally considered to have allogenic tolerance of the component lines, and interspecific chimeras also provide a model for the study of maternal-fetal incompatibilities (Rossant et al., 1982).

### Gene Transfer

A few genes that control production traits have been identified, and mapping of the genes of economic importance in farm animals is an area of research that will prove very useful if adequately addressed in the future. The ability to target gene expression exclusively to the mammary gland will allow modification of milk composition to make novel cheeses, reduce milk fat, remove lactose or allergenic proteins, and increase protein content in goat milk. It is expected also that pharmaceutical products will be produced from milk of goats expressing pharmaceutical transgenes in their mammary glands. Transgenic goats and other livestock have been produced and express either the pharmaceutical proteins of tissue plasminogen activator (tPA) clotting factor 9, alpha-1-antitrypsin, lactoferrin, eurokinase, FSH, protein C, human growth hormone or interleukin 2 in their milk (Ebert et al., 1991; First et al., 1991; Rexroad, 1992).

Gene transfer by microinjection of DNA into a pronucleus of a one-cell zygote has been successful in goat studies. Five hundred to one thousand copies of

relevant genes are injected into the pronucleus of IVF embryos or recovered embryos from superovulated donors. Injected embryos are either directly transferred into an oviduct of recipient goats or cultured for some days to assess viability before transfer into the uterus. The rates of incorporation of the foreign DNA and the survivability of injected embryos are low, with only .84% of injected sheep embryos resulting in transgenic sheep (Wilmot et al., 1990). The survival rate in goat embryos is better than in sheep (1%, Selgrath et al., 1990; Ebert et al., 1991).

Ebert et al. (1991) produced an enzymatically active heterologous protein in milk (tPA) of transgenic goats. Thirteen recipients that had oviduct embryo-injected transfers (43%) became pregnant and delivered 22 offspring (13%). Five of six recipients that received cultured one-cell to morula stage embryos had seven offspring (18%). Two transgenic goats were identified, indicating 1% efficiency in developing transgenic goats from microinjected embryos. The transgenic female was mated and it delivered two non-transgenic offspring. Analysis of milk indicated a glycosylation variant of human longer-acting tPA (**LA**tPA). This was extensively purified, and although it is structurally not identical to the C 127 cell-derived human protein, tPA, it has comparable enzymatic activity, and the transgenic expression system is capable of adding carbohydrate residues to a heterologous protein that is normally glycosylated. A concentration of 3 mg/mL of LA tPA is produced in transgenic goats' milk. Improved modeling of a desired outcome of the transfer gene will occur as gene mapping projects provide genome knowledge sufficient to allow accurate modeling of the genome and gene construct. Perhaps the greatest challenge will be the development of consumer confidence that specific genetically engineered animal transgenics are safe (First, 1992).

### Marker-Assisted Selection

Mapping of the genomes of goats and other livestock has resulted in DNA markers that are beginning to be associated with desired or undesired productivity traits. For example, the study of Buitcamp et al. (1991) on DNA fingerprinting of goats showed at least four polymorphic banding patterns per individual using oligonucleotide probes (GACA)<sub>4</sub>, (GT)<sub>8</sub>, and Hinf-1 for DNA digestion. At Kyoto University another nucleotide sequence 880 bp cDNA encoding goat growth hormone gene was revealed (Yamano et al., 1988). Later in the same laboratory, a clone containing growth hormone (gGH) gene was isolated from a goat genomic library using bovine growth hormone cDNA as a probe. The gGH gene was located on a 5.8-kb *EcoRI-HindIII* fragment using southern hybridization analysis and the gGH gene and its flanking regions were completely sequenced.

Currently, limited gene mapping, cloning, and sequencing work involve the goat genome and characterization of various capripoxviruses, but these genes will not be directly involved in animal production (Mukherjee, 1992). Folch et al. (1994) reported a goat  $\beta$ -lactoglobulin ( $\beta$ -LG) encoding gene that has been isolated from caprine  $\lambda$ EMBL 3 genomic library (Clontech, Palo Alto, CA) probed with a caprine  $\beta$ -lactoglobulin cDNA. It is important to continue characterizing homologous sequences such as  $\beta$ -LG among closely related nutrients to ascertain the distribution and significance of repetitive elements in the goat genome. Differences in regulatory elements conservation may also provide clues to help understand mammary gene regulation (Folch et al., 1994).

Markers are important in screening genetic defects, and when genes are introduced from other populations markers can be used to track their segregation in the population. Marker-assisted selection imposes little to no risk to the animal donating blood, sperm, or embryos for an assay and therefore imposes no risk to the consumer. Marker-assisted selection is, therefore, expected in the short term to be most commonly used in association with the above other goat techniques. The caprine arthritis encephalitis virus (**CAEV**) causes a devastating disease in goats. The virus is a member of the family retroviridae and subfamily lentivirinae, slow viruses. This group of cell-associated viruses is characterized by the presence of RNA-dependent DNA polymerase, and the CAEV is functional only when the viral RNA is transformed to viral DNA and is integrated into the host cell DNA (Froenkel-Courat and Kimbul, 1982). One serological survey representing 24 states indicated that up to 81% of the domestic goat population was seropositive for CAEV (Wolfe et al., 1987). The use of DNA fingerprinting for diagnosis of such viruses will enable a development of new vaccines using recombinant technology.

### Implications

Biotechnology potentially reduces the need for goats required in research to improve food and quality products. Embryos can be frozen and split to double offspring numbers with minimal risk to goats. This has tremendous interest to the consumer, since quality meat, milk, and other goat products could be produced cheaply. The benefits of nuclear and gene transfer could accelerate genetic improvements in production and the use of products of pharmaceutical value with environmental adaptation. Before multiplication and release for use, genetic material could be characterized for predictable performance, nutrient requirements, disease resistance, and extensive screening of clonal lines for genetic defects for a profitable goat industry.

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