The Application of Reproductive Technologies in the Management of Small Ruminants Genetic Resources

R. Machado, H.O. Salles, and A.A. Simplício

(EMBRAPA - Empresa Brasileira de Pesquisa Agropecuária
CNPC - Centro Nacional de Pesquisa de Caprinos
P.O. Box D-10, Sobral, CE. ZIP 62 011-970. Brazil.)

SUMMARY

This paper presents some applications of reproductive technologies in the management of small ruminants genetic resources. It is the importance of economics for large scale adoption of a given reproductive technique. Discusses the technical advances and hindrances.

1. INTRODUCTION

The management of genetic resources of small ruminants is associated with two aspects defending the kind of the target population. According to Lauvergne *et al.* (1992) traditional populations of goats, resulted from the first step of domestication were formed and preserved by the loosening of selection pressure. Standardized breeds were obtained as a second step of domestication, when mankind took a better control of livestock and imposed goals to achieve pre-determined performances. This process reduced genetic variability in domestic small ruminants population. Therefore, two different approaches must be taken to manage small ruminant genetic resources. First, traditional populations must be conserved, in order to rescue genes of interest, which were not properly assessed yet. It should be mention that several of such populations are endangered of extinction. Furthermore, indiscriminate crossbreeding may endanger the full expression of genes related with adaptability under harsh conditions, higher fertility and prolificacy etc. In contrast, standardized breeds are used to ensure high profitability to fulfill stricter demands of consumers. In this case, production of high quality marketable products must surpass the pace of the genetic trends in a population.

The application of reproductive technologies may play an important role in the management of small ruminants placed in both scenarios mentioned above. Long-term maintenance of genetic variability is possible not only by means of cryoconservation of gametes and embryos, but also by reducing the generation interval and increasing the intensity of selection. In addition, several reproductive technologies are available to progressively increase the rate of genetic improvement in standardized population of sheep and goats.

2. REPRODUCTIVE TECHNOLOGIES : TOOLS FOR GENETIC IMPROVEMENT

The large number of standardized purebred sheep and goats provides a fairly high diversity of animal types available. Therefore, it is feasible to mold the kind of sheep or goats to be produced. Actually, several crossbreeding programs have been successfully developed and eventually the crossbred end-products became described breed types, i.e., American Polypay, Columbia, Targhee and many other sheep breeds.

Genetic improvement in dairy goats, through milk selection programs has been successfully carried out in France and some other countries are launching their own programs. In turn, developing countries, such as Brazil, are making efforts to elevate goat milk yield or milk quality by means of crossbreeding. The strategy employed involves selection of a small inventory of purebred dairy goat, associated with the introduction of this exotic blood into the native population of goats. However, purebred seedstock is usually not enough to cope with the demand of this kind of program. As a result, a continuos flow of imported animals from other countries takes place and makes genetic

improvement expensive. In addition, sanitary concerns impose limitations on marketing live animals, thus claiming for the intensification of preserved germplasm exchange, i.e. frozen semen and embryos. Moreover, the adoption of reproductive technologies on a commercial basis is needed to achieve optimal reproductive rates, once high fertility is essential to profitability in livestock production systems.

Reproductive techniques as tools for genetic improvement are preceded by reliable performance recording and accurate estimation of breeding value (Ricordeau *et al.*, 1992). Therefore, improvements on reproductive techniques may not be adopted in the field without systematic screening and utilization of superior proven sires and dams. In a model developed by Serradilha *et al.* (1991), genetic gain would potentially be increased by 50% through the augment of adoption of artificial insemination up to 30% out of controlled does.

3. REPRODUCTIVE TECHNOLOGIES AVAILABLE

Several reproductive biotechniques have been developed and perfected in this century. However, only a few reached some commercial application due to some major constraints. Therefore, biological efficacy, safety of handling, ease of execution and economics are common concerns that shepherds and goat owners take into account before adopting an innovative procedure. The most important and promising reproductive techniques available to shepherds and goat producers are discussed below.

(i) Artificial Insemination

Artificial insemination (AI) can reach widespread utilization as more producers become acquainted with its benefits and research results reach the industry for improvements on its techniques of cryopreservation. Commercialization channels of small ruminants germplasm are still primitive, particularly in developing countries. Hence, buck or ram semen are quite expensive in comparison with the range of prices observed for bull semen and costs to produce frozen semen doses play a role in defining the price which the material will reach shepherds and goat owners.

France developed a successful example of the application of artificial insemination under commercial basis for small ruminants. Even though, overall fertility achieved through AI have not matched the levels obtained under natural mating. In 1992, while total fertility ranged from 83 to 92% for natural service, artificial insemination gave levels neighboring 60% (Grangere, 1993). Variations "between flocks" affected the efficacy of AI in France and, after estrus synchronization and AI using fresh semen, conception rate around 70% was achieved in 30% of flocks. On the other hand 23% of flocks showed conception rates lower than 50% (Leboeuf, 1993).

Some biological aspects of reproduction limit the wide-spread development of AI in small ruminants. As a result, estimated probabilities of having an estrus synchronized dairy goat pregnant after AI with fresh semen ranged between 0.13 and 0.39 (Dunner & Impastato, 1993). In this sense, to enhance AI performance, Grangere (1993) suggested to have defined criteria to choose females to be served and also, to know how the estrus synchronization procedures work. In France, the number of ewes artificially inseminated steadily increases since the seventies and reaches 700 to 800 thousand ewes/year. Almost 99% of these females are artificially inseminated through the use of fresh or chilled semen after transcervical artificial insemination. An important limitation for AI in sheep is the anatomical barrier represented by the small diameter of the cervical opening. To solve this problem, Grupp (1991) showed that intracervical treatment with PGE_2, with or without estradiol benzoate had a significant effect on the depth of cervical penetration with an AI gun and became possible transcervical access to the body of uterus in 80-90% of ewes and 100% of does. Mareco (1993) described a mechanical dilation of cervix to perform the deposition of semen in the uterus by means of a transcervical applicator gun. The method proved successful, even with maiden goats. Actually, young females may have lower parturition rates than mature ones (Martemucci *et al.*, 1992). Laparoscopic insemination with uterine deposition provides the best results. In a trial conducted by Ritar *et al.* (1990), cervical insemination provided kidding rate of 39.1% and laparoscopic reached 63.6%. On top of these limitations, seasonality of reproduction is by far the most important hindrance to be circumvented in small ruminants from temperate latitudes.

(ii) Semen Technology

(a) Reproductive Physiology. Photoperiodic variations throughout the year induce seasonal changes in libido, testicular weight, testosteroinemy, biochemical composition of seminal plasma, ejaculate concentration, ejaculate volume, sperm output, sperm quality and fertility in bucks and rams. Therefore, the utilization of stud males is restrict to a limited period of the year. On the other hand, seasonal variation on the reproductive behavior of small ruminants reared under Tropical (Machado *et al.*, 1992) or Mediterranean (Roca *et al.*, 1992) conditions are not enough to prevent reproduction over the year. In a tropical hot semi-arid environment, Machado *et al.* (1992) found that conservation of germplasm from Alpine, native Moxotó and the Alpine/Moxotó F_1 crossbred yearling stud bucks can be proceeded all year round. In contrast, males kept in temperate zones show significant decrease in semen quality and the production of semen doses available for artificial insemination also decreases during non-breeding season (Delgadilho *et al.*, 1992). Fortunately, some management alternatives overcome such hindrance. Monthly or bi-monthly artificial alternations between long (16L:8D) and short (8L:16D) days prevented the decay in semen quality over the non-breeding season (Delgadilho *et al.*, 1992). Moreover, bucks exposed to rapid artificial alternations between long and short days for three consecutive years maintained a high sperm production and, thus, the seasonality of hypothalamus-pituitary-testis activity was abolished (Delgadilho *et al.*, 1993).

Exogenous administration of melatonin has been used in an attempt to mimic the short-day signal at transductional level (Parry *et al.*, 1994). Advancing puberty in young rams was feasible with treatments using the succession of long days - decreasing days or long days-melatonin. This procedure permitted these animals to be submitted earlier for progeny testing, using artificial insemination. In adult rams, such treatments also caused increase in testicular weight and sperm production in spring (Chemineau *et al.*, 1992).

(b) Semen Processing. Most research efforts for application of fresh or chilled semen have been devoted to improve extender media. The association between egg-yolk and amine organic buffers to dilute buck semen showed to be effective and a Bes/KOH buffer provided the highest level of pregnancy rate (Dunner e Impastatato, 1993) However, the maintenance of fertilizing ability of chilled sperm of bucks for longer than 12 hours still has to be achieved.

Freezability of semen depends on sperm survival before freezing and after thawing. Season, genotype, individual and ejaculate are some of the sources of variation of freezability (Machado and Simplício, 1992a; Machado *et al.*, 1992) and freezing and thawing procedures also effect viability of frozen semen. Variations on procedures available to freeze buck sperm are concerned with seminal plasma washing, utilization of extenders rich in phospholipids and sperm numbers per doses. The activity of phospholipases and lisophospholipases from seminal plasma takes place in subzero temperatures, reducing after- thawing sperm motility, sperm viability and ultimately fertilizing ability of spermatozoa. The removal of seminal plasma through the Krebs-Ringer-Phosphate buffer (KRP) added with Sodium citrate was capable to almost double freezability. Sodium citrate probably acted to permeate the slower penetration of viscous cryoprotectant molecules into sperm cell. This property, helps to regulate osmotic equilibrium during freezing. In the same trial, only 89 out of 267 (33,3%) ejaculates washed with KRP provided batches approved for insemination (Machado & Simplício, 1992a). On the other hand, washing implies a higher reduction of sperm viability than cooling of semen (Pintado *et al.*,1992) and removal of seminal plasma prior to freezing spermatozoa in an extender containing egg yolk had an unfavorable effect on their post-thaw motility and integrity (Tuli and Holtz, 1994). Dilution of buck semen in amine-organic buffers involves washing of sperms and addition of egg-yolk in the extender (Dunner, 1993). This may represent a limitation of these media once some samples of goat sperm do not tolerate more than 1,5% of egg-yolk in the extender (Ritar and Salamon, 1991). For short-period storage, freezing without washing and employing egg-yolk can be successfully done. The optimizing action of washing and the absence of egg yolk in extender media on semen quality may not be noted once testosteronemy and enzymatic composition of seminal plasma are modulated by environmental cues, such as photoperiod. In this case, AI centers located in Mediterranean and Tropical zones should develop their own protocols to freeze semen according to their own resources, facilities and needs.

The actual relationship between sperm numbers inseminated and fertility is an exponential-like curve, meaning that there is a minimum amount of sperms, which posses proper characteristics to provide the highest likelihood of successful pregnancy (Saacke *et al.*, 1994). Compensable attributes are involved in spermatozoa functions prior to block of polyspermy. Improper expression of a compensable attribute results in failure of a given spermatozoon to reach the vicinity of the oocyte, or initiate the block of polyspermy, but does not prevent another spermatozoon from fertilizing the oocyte. Hence, for a sample containing a moderate proportion of spermatozoa with one or more compensable attributes defective, potential depressed fertility can be overcome by insemination with additional sperm and the ideal number of sperms in a inseminating dose may vary according to the male donor. Weitze and Petzoldt (1992) suggested to adopt individual approach to overcome problems related to compensable attributes of sperms once reduction in sperm numbers per doses has importance strictly under commercial basis. Additionally, ejaculate attributes have very low repeatability (Machado *et al.*, 1995a), implying in low heritabilities. Thus, selection of young semen donors based on quality of ejaculates deserves more consideration.

The number of motile spermatozoa did not affect ($P>0.05$) fertility after cervical (80, 120 and 160 $x10^6$) or laparoscopic (15, 30 and 60 $x10^6$) insemination Ritar *et al.* (1990). Similarly, Azevedo *et al.* (1995) did not verify differences in the conception rates of tropical goats artificially inseminated in natural estrus with inseminating doses of 50 $x10^6$ (CR=87.5%) and 100 $x10^6$ (CR=83.3%). Both groups were superior ($P<0.05$) than doses with 200 $x10^6$ sperms. In this case, efficiency of the production of frozen semen could be 2 or 4-fold increased by reducing sperm numbers per dose to 100 or 50 $x10^6$ sperms. This enhancement would dramatically reduce the costs to freeze goat semen, which had been estimated as US$ 0.94 (with 200$x10^6$ sperms/dose) by Machado and Simplício (1995).

Laparoscopic AI allows semen extension up to 24-fold before freezing without impair fertility of the processed sample and should be used always when is necessary sperm saving (Ritar *et al.*, 1990).

(iii) Control of Estrus and Ovulation

Out-of-season breeding, increase in natural prolificacy, anticipation of reproductive cycles in young females and reduction of number of services required for conception are some of the many attempting biological possibilities of controlling small ruminants reproductive cycles. However, technological gaps associated to high costs and risks put obstacles in the field utilization of estrous cycle control. Cost/benefit ratio must be considered as a guideline before choosing the method for reproductive control.

The male effect is a complex response involving the integration of a range of exteroceptive stimuli from the buck and, it is probably the cheapest method to induce ovulation in small ruminants. Responsiveness of females is higher during transitional period prior to the breeding season and management of bucks before introduction contributes for the variation in the ovulatory response of does. Moreover, separation between male and females should exceed 100m and estrus does are able to elicit ovulatory response in other females independent of bucks. (Waldken-Brown *et al.*, 1993a; 1993b).

Hormonal treatments to synchronize estrus give rise to a complete range of biological outcomes. Short-term (<12 day-long) progestogen-based treatments usually lead to higher fertility than long-term (≥12 days) protocols. Stimulation of follicular atresia, reduction of sperm transport, altered fertilization rate and retarded embryo cleavage are some possible effects of prolonged exposure to progestogens (Odde, 1990). Conception rates of long-term treatments range from 41.4% to 86.6% (Martemucci *et al.*, 1992; El-Amrami, 1993; Güven *et al.*, 1993). Improvements are achieved when treatment occurs during breeding season (El-Amrami *et al.*, 1993) while females have active ovaries (Llewelyn and Kadzere, 1992) and PMSG dosage is neither over nor underestimated (El-Amrami *et al.*, 1993). The use of fresh semen or natural service also increased fertility in some trials (Martemucci *et al.*, 1992; Azevedo *et al.*, 1993).

Short-term protocols require luteolytic agents. Among these, prostaglandin $F_{2\alpha}$ and its analogues are the most effective. Recently, recombinant sheep interferon-τ showed luteolytic effect in goats (Homeida & Al-Afaleq, 1994). Estradiol valerate was apparently efficient to demise corpus luteum in mature does. However, luteal dysfunction after treatment with 1.5mg norgestomet implants for 9 or 11 days severely depressed conception rate of goats treated (Azevedo, pers.comun.). FGA-impregnated sponges associated with PMSG injection synchronized estrus more tightly than with Melengestrol acetate (.11mg daily/9 days) given by feeding, with/or without PMSG shot . However, differences in estrus signals and fertility after mating were not significant (Chavez *et al.*, 1990). Similarly, intravaginal FGA-sponges and controlled internal drug release devices-CIDR, were equally effective for control of ovulation when combined with 200 IU of PMSG (Ritar *et al.*, 1990). CIDR also stimulated out-of-season breeding without PMSG when applied in conjunction with male effect (Wheaton *et al.*, 1993). On their turn, sub- cutaneous implants containing 6 or 3 or 1.5mg of norgestomet were equally effective as 45mg FGA or 60mg MAP impregnated sponges (Bretzlaff *et al.*, 1991; Azevedo, pers. comun.). Furthermore, reutilization of norgestomet implants can be done as long as daily secretion of steroid is 23μg (Machado *et al.*, 1995b).

The fertility response for progestogen-based short-term protocols also depends on the gonadotrophic challenge imposed. GnRH intramuscularly given to lactating goats primed either with MAP or FGA were not as effective as PMSG to control reproductive activity during seasonal anestrus (Robin *et al.*, 1994) and Bretzlaff *et al.* (1991) obtained high fertility after out-of-season induction of estrus with 250ng of GnRH, given through osmotic mini-pump, associated with 3mg norgestomet implants. PMSG, in a 500 IU doses, given during the non-breeding season in a short-term protocol increased fertility (66.7 - 73.3% vs. 53.5-60.0%) when compared to a 14 day sponge treatment (Greyling and Niekerk, 1991). Unfortunately, repeated administration of PMSG produces antibodies against itself, causing reduced ovarian stimulation after subsequent treatments. Moreover, low fertility rate after synchronization and AI may be related to high proportion of goats with delayed occurrence of estrus, which increases with animals that are repeatedly treated (Baril *et al.*, 1992a). Human menopausal gonadotrophin, in association with $PGF_{2\alpha}$ and MAP pessaries have been successfully used to induce ovulation in sheep (Machado & Simplício, 1992b) and goats (Oliveira and Rezende, 1990). Under similar experimental conditions, hCG was capable to promote ovulatory response in 68.9% of goats with ovulation rate of 1.45. This treatment associated with AI using frozen semen produced kidding rate of 32.6% (Machado, 1991).

In goats, conception rate ranged from 27.9% to 72.9% (Machado, 1991; Leboeuf *et al.*, 1994; Martemucci *et al.*, 1992) for treatments based on sponges. Conception rates after utilization of implants were placed between 35.7% and 100% (Bretzlaff *et al.*, 1991; Machado and Azevedo, 1995).

The $PGF_{2\alpha}$-based program is a more practical method to synchronize estrus in small ruminants. However, double PGF_{2a} regime proved ineffective outside of breeding season (Greyling and Niekerk, 1991). In addition, Carboprost tromethamine, a prostaglandin analogue, was more effective as luteolytic when goats were at the middle (days 9-12) of the luteal phase rather than at the beginning (d5-6) or at the end (d15-17) (Mahmood and Koul, 1990). The minimum efficient dose of Dinoprost Tromethamine for induction of estrus in Mexican Criollo goats was 4.0mg per doe. Goats injected intramuscularly take longer to display estrus than those receiving by intra-vulvo submucosal injection and estrus response ranged from 55% to 68% (Mellado *et al.*, 1994). On the other hand, Azevedo *et al.* (unpublished) obtained estrus response of 91.7% and non-return rate of 54.6% after using 50μg of cloprostenol, given by the intra-vulvo submucosal route into mature goats in the luteal phase. Restricted feed intake in goats tended to delay the onset of estrus and lowered the ovulation rate and pregnancy rate in dairy goats synchronized by means of $PGF_{2\alpha}$. (Mani *et al.*, 1992). Kidding rates varied from 45.0% to 100.0% (Machado, 1991; Ishwar & Pandey, 1990).

Machado *et al.* (unpublished) determined costs of adopting AI with frozen semen for goats associated or not with some estrus synchronizing procedures. Table 1 shows the results. Fertility rate and prolificacy are expressed as mean values of experiments carried out in Brazil by the research team of the Brazilian Agency of Agricultural Research- EMBRAPA. Prices were transformed into commercial values of dollar in Brazil during November of 1994. Implant method was the most cost effective disregarding the goal of operation, once dairy goat producers aim lower costs per kidding

while meat goat producers pursuit lower costs per kid, born or weaned. It deserves mention the fact that investment per treated doe may mislead the choice. As Table 1 shows, the cheapest combination AI/estrus synchronization protocol gave poor return.

From the flock management standpoint, timed AI after a synchronized estrus is a very attempting technique. However, conception rates after these programs are variable and frequently low (Table 1). Progestogen supplementation after an initial timed AI allows for a second opportunity to inseminate females at pre-determined moment. Machado *et al.* (unpublished) inserted a 60mg MAP-sponge five days after an initial timed AI in mature goats. Pessaries were withdrawn 16 days later and 46.7% of does returned to estrus and were inseminated. The overall cumulative kidding rate to the complete schedule, i.e. synchronization + resynchronization, allowed more than 50% of female goats became pregnant within the first 23 days of mating period through AI top-genetics quality males.

Melatonin implants with or without CIDR+PMSG were effective in promoting high estrus response and conception rate after natural service during anestrous season. CIDR with or without PMSG also was effective (Huang *et al.*, 1993). In the female goat, the succession of long days-melatonin treatment efficiently induces and maintains estrous and ovulatory activities in spring, leading to high fecundity after natural mating. This treatment seems to be less efficient with seasonal sheep breeds from Northern Europe (Chemineau *et al.*, 1992).

Table 1. **Biological performance and estimated costs of some estrus synchronizing protocols for goats due to AI.**

	Fertility		Costs (US$$_{comercial,\ nov\ 94}$) per		
	kidding (%)	prolificacy	kidding	kid born	treated goat
M_0	67.7	1.80	14.73	8.18	9.97
M_1	46.5	2.26	13.95	6.17	6.49
M_2	37.5	1.50	18.09	12.06	6.79
M_3	34.1	1.30	17.57	13.51	5.99
M_4	33.9	1.33	16.72	12.57	5.67

M0= natural estrus;
M1= 1.5mg norgestomet implants + 300 IU hCG + 50μg cloprostenol;
M2= 60mg MAP sponge + 300 IU hCG + 100μg cloprostenol;
M3= 60mg MAP sponge + 200 IU eCG + 100μg cloprostenol;
M4= 2x (100μg cloprostenol).

(iv) Pregnancy Diagnosis

The benefits of accurate pregnancy determination of small ruminant include: 1. Selection of replacement females on their ability to show estrus or conceive as yearlings; 2. Reduction of costs resulting from sale or differential management of open females; 3. Aid in early diagnosis of pregnancy failure; 4. Optimizing use of building, labor and equipment; 5. Ability to guarantee pregnant females for sale and 6. Feeding more accurately according to stage of production. Furthermore, the establishment of systems for accelerated lambing/kidding will also rely upon pregnancy diagnosis (SID, 1988).

From many biochemical approaches to diagnose pregnancy, early determination of plasma, serum or milk progesterone levels should be considered "non-pregnancy" tests rather than pregnancy status evaluation, due to their very high accuracy to find non-pregnant females (Wiel *et al.*, 1991). Determination of pregnancy specific Protein B (PSPB) is feasible in ewes at day 21 to term. Further testing and development is required for field application of this method. Several procedures have specific applications due to their low accuracy, costs, need for laboratory or equipment apparatus, or even their invasive approach to living animals. Listed as examples are: non-return rate; abdominal balotment; laparotomy; laparoscopy; radiography and vaginal cytology.

The most practical method of pregnancy testing is the use of ultrasound with an external transducer - ultrasound scanner or "echo" or "sonar" system. This procedure is based on the different sound-reflecting properties of fluid and tissue, thus the technique works satisfactorily only when the uterus contains a significant amount of fluid (between days 70 and 120). Another method, the Doppler principle of conversion into audible signals is based on the detection of fetal circulation, identified by a rectal probe.

Imaging real-time B-mode ultrasound is a variation of the use of sound waves to detect pregnancy. This method also employs an external transducer and allows to determine fetal numbers from 40 days to term. Determination of fetal gestation age can be done by experienced ultrasonographers. It can also be used to diagnose reproductive tract disease and monitor ovarian activity. The equipment is expensive (US$ 10,000 -15,000) and operator skill is important. These factors lead to suggest the practical application on a fee basis by individual contractor. The diagnosis in goat using an ultrasound transrectal scanner can be achieved since embryo vesicles can be seen (16/17th day) aided by a human prostate probe coupled to a portable echotomograph (Tainturier *et al.*, 1993). In addition, echotomography allows the direct visualization of the fetuses and of the amniotic liquid on a monitor. It can be used after 32 days of pregnancy with the probe placed either externally or in the rectum (Chemineau and Cognie, 1991). Lack of specificity is observed when diagnosis is proceeded as early as 25 days post-mating. This is due to the appearance of false negatives. The ultrasonic pregnancy evaluation of flocks requires planning to maximize information obtained from a single session (Haibel, 1990). Limited breeding period of 42 to 49 days followed by ultrasonography 35 days after studs removal will result in a large proportion of females being at a reproductive stage when both, pregnancy examination and determination of fetal numbers are quite accurate. Practices as flushing and estrus synchronization may enhance the efficacy of this scheme.

Does are often less co-operative than ewes when rectal probes are inserted. Fortunately, their abdominal suspension, lack of fat and the fact that most carry multiples makes a day 24-30 inguinal diagnosis easy and quite accurate, when using a 5 MHz head in the standing doe (Buckrell, 1988).

The ability to sort single from twin-bearing females holds as much or more interest for many producers than simple pregnancy diagnosis. The procedures which permit identification of those carrying twins certainly permit identification of non-pregnant females at the same time. The determination of fetal numbers allows to obtain increased birth weight and survivability of offspring born to females bearing multiples is increased. Accuracy in counting fetuses depends to a great degree on the stage of pregnancy, the ultrasound equipment available and the experience of the operator.

The commercial use of ultrasound technology in small ruminants will be accelerated as the equipment costs reduce. In addition, improving image quality and perfecting transducers will certainly increase the interest on using real-time ultrasound techniques in small ruminants operations. If pregnancy diagnosis could be accurately done at or before day 20, adoption of ultrasound echothomography would be exponentially increased in the field.

(v) Induction of Parturition

Assistance to neonates and parturient goats assumes paramount importance since the transmission of Mycoplasma and CAE Virus occurs through colostrum and milk. Presently, induction of parturition has been proceeded with prostaglandin $F_{2\alpha}$ and its analogues due to the consistency of results. Santos *et al.* (1992) reported that 75μg or 100μg of cloprostenol given between days 144 to 146 of gestation induced parturition in dairy goats within an interval of 28h54min to 33h36min after intramuscular injection. Intramuscular administration into the vulva muscle permitted to reduce the effective dosage of cloprostenol to 25μg, and promoted parturition within 30h08min after treatment (Salles *et al.*, 1995). No detrimental effect on offspring survival rate, placenta delivery or colostrum or milk production of dams occurred after cloprostenol treatments (Santos *et al.*, 1992). Association between cloprostenol and strategic withdrawn of supportive therapy with progestogens close to parturition supposedly would render more tightly synchrony of induced kiddings. However, poor results were obtained in preliminary attempts (Machado, 1994).

(vi) Embryo Transfer

Embryo Transfer (ET) improves the accuracy to screen breeding value of dams and accelerates the wide diffusion of superior genetics through the increase of offspring harvest. In addition, intensity of selection is augmented once embryos can be obtained from pre-pubertal or young females (Majumdar *et al.*, 1990). Disease transmission is reduced or abolished due to natural protection provided by the Zona Pellucida and maximized by embryo washing procedures. For instance, goats infected with CAE (Pinheiro-Andrioli *et al.*, 1995) or blue-tongue (Chemineau *et al.*, 1986) viruses and sheep infected with blue-tongue Virus (Hare *et al.*, 1988) may continue serving as embryo donors.

Recently, research efforts have tried to perfect less invasive approaches to collect and transfer embryos. Transcervical access to the uterus of sheep was possible pharmacologically by PGE_2 associated with estradiol and embryo recovery rate was 65% (Barry *et al.*, 1990). Flores-Foxworth *et al.* (1992) and Andrioli-Pinheiro (1993) described transcervical methods for successful embryo harvest in goats.

Idiosyncrasy in superovulatory response still lowers overall results after ET in small ruminants. Strategies to circumvent this limitation have proved ineffective. Hann (1992) estimated low repeatability and heritability for embryo numbers after FSH-based protocols. This finding discard the possibility of Bovine embryo donors selection upon responsiveness to superovulation. The same phenomenon may potentially affect small ruminants. Even though, Baril *et al.* (1992b) have found that repeated treatments with $_{porcine}$FSH led to a decay in the responsiveness in goat donors, due to the production of antibodies against $_{p}$FSH. In contrast, FSH obtained from caprine or ovine species did not evoke immunological response in goats (Baril *et al.*, 1992b). Additionally, melatonin-implant priming effects reduced seasonal variation in superovulatory response of ewes (Nibart *et al.*, 1994). PMSG superovulatory protocols result in high ovulation rate. However, numbers of transferable embryos is usually diminished in sheep (Schiewe *et al.*, 1990) and goats (Pendleton *et al.*, 1992). The inconvenient "serial shots with decreasing dosage" of FSH may be avoided through the dispersion of the gonadotrophin into PVP (polyvinyl pyrrolidine) vehicle (Dattena *et al.*, 1994) providing ovulation rate of 8.6 ± 4.8 and embryo recovery rate of 68.1% in sheep.

In order to reduce the dispersion between ovulations, Baril *et al.* (1994) used a GnRH antagonist 12 hours after FGA-sponges withdrawal followed by a $_{p}$LH intravenous shot. This method was equally effective as AI after estrus detection (Vallet & Baril, 1990), and permitted to perform only one timed AI.

Premature regression can affect 72% of corpora lutea in tropical reared goats (Andrioli-Pinheiro *et al.*, 1994) and may lead to low embryo recovery rates. Battye *et al.* (1988) hypothesized that superovulated goats prematurely release $PGF_{2\alpha}$ with consequent corpora lutea demise. Soares *et al.* (1995) used an cyclo-oxidase inhibitor - Flunixim Meglumine, while superovulatory scheme was in progress and corpora lutea regression dramatically dropped to 1.94%.

The slow-freezing method with ethylene-glycol as cryoprotectant gives satisfactory results to preserve embryos. Indeed, Le Gal *et al.* (1993) verified higher viability of embryos at the morula and blastocyst stages after freezing in ethylene-glycol than in glycerol. This finding held true for embryos cultured in both, "in vitro" or "in vivo" systems. Similar conclusions had been drawn to sheep embryos. However, slow-freezing is laborious and time-consuming. As a result, vitrification has gained more interest and eventually may replace slow-freezing. Ribeiro *et al.* (1989) obtained 57.1% of morphologically normal goat embryos after vitrification and thawing. Yuswiati and Holtz (1990) had achieved analogous outcome and obtained two kids after vitrification and ET. In sheep, embryo development to term was 11% for advanced morulae and blastocysts, while reached 32% for hatched blastocysts (Széll *et al.*, 1990).

The overall efficiency of ET is limited due to the small number of embryos obtained per donor. Baril *et al.* (1993) stated that ET can solely double the reproductive potential of ewes, once only 3 or 4 lambs can be obtained through ET. Embryo Bisection (EB) maximizes the number of embryos collected and also furnishes homozygotic twins for research purposes. In small ruminants, best

results are obtained when embryos are bisected as morulae or hatched blastocysts (Baril *et al.*, 1993) due to the damages caused by the EB procedure are less harmful to more developed structures (Skrzyszowska and Smorag, 1989).

(vii) In Vitro Fertilization

In vitro fertilization (IVF) represents not only a major advance in applied animal reproduction but also, a potent tool to study molecular and cellular interaction between gametes before, during and after syngamy. From animal scientists standpoint IVF reduces the costs to obtain embryos, thus allowing for rapid multiplication of offspring from selected genetics. In addition IVF gives the required support to develop more sophisticated biotechnologies such as production of transgenic animals, nuclear transfer, gene transfer, sexing, bioassays for fertilizing ability of sperms, etc. Slaughterhouse represents mainly the source of ovaries and oocytes for research purposes. However, a novel technique of laparoscopic folliculocentesis permitted 81.9% of oocyte recovery on the total number of punctured follicles (Baldassarre *et al.*, 1994). In this case, obtaining oocytes from top genetics living dams became possible.

Oocytes can be taken out of ovaries through follicle dissection, follicle puncturing, follicle aspiration or ovary slicing. The latter provides the highest number of recovered oocytes (Pawshe *et al.*, 1994). However, the obtained oocyte population is heterogeneous and large proportion of oocytes originated from small immature follicles decreases capacity for maturation. Furthermore, fertilization rate after ovary slicing may be lower (18.18%) than that after follicle dissection (29.07%) (Martino *et al.*, 1993). Integrity of the cumulus-oocyte complex is a requirement for successful oocyte maturation and ultimately IVF and embryo cleavage (Tan Lu, 1990: Madison *et al.*, 1992). Nonetheless, from one to three cumulus-cell layers neither maturation nor fertilization rates were affected. In contrast, polyspermy was more frequent in the complexes with one or two-cell layers (Palomo *et al.*, 1993). Similar findings were reported by Martino *et al.* (1995). Goat oocytes showed the higher percentages of nuclear maturation after a 27-hour period (Martino *et al.*, 1994) if compared to 24 to 25.5 hours. In sheep, oocyte maturation may take 20 to 26 hours (Bondioli *et al.*, 1982; Sun *et al.*, 1994).

Acrosome reaction of frozen thawed semen occurs faster than fresh semen. However, "in vitro" viability of frozen-thawed semen is lost sooner than fresh semen (Artiga *et al.*, 1993; Garde *et al.*, 1993). Acrosome reaction seems to be dependent on the presence of inactivated serum of ewe in estrus at a 20% concentration. Fertilization rate achieved 85% with serum compared to 0% without serum (Huneau *et al.*, 1994).

Conventional systems of culture have been shown to be inadequate to culture embryo at the initial stages of embryonic development. Co-culture media partially overcame the blockade to cell division during "in vitro" culture of embryos. That phenomenon takes place in the 8-16 cell stage in sheep (Gandolfi and Moor, 1987). Co-culture systems allow for the production of growth factors, promotion of embryo differentiation and medium detoxification (Kane *et al.*, 1992). Cleavage rates for goat (Sakkas *et al.*, 1989) and sheep (Holm *et al.*, 1991) are greater in co-culture systems with oviductal cells as compared with cultures without such cells. On the other hand, goat embryo development did not differ between the two systems (Sakkas *et al.*, 1989). In the same trial, Brackett's defined medium and synthetic oviductal fluid did not differ ($P>.05$) as in vitro fertilization media.

IVF can be strongly expanded with the development of oocyte cryopreservation. This fact would become IVF practices independent of superovulation programs or sporadic slaughters of sheep and goats.

(viii) Gene Transfer

The gene transfer technique aims to induce specific modifications in livestock by means of incorporation of particular genes into the genome of the target animal. Animals which possess a transferred gene, phenotipically express the coded characteristic of that gene and are able to transmit it to its offspring are named transgenic. In small ruminants, gene transfer has been attempted

through DNA mycroinjections into male pro-nucleus and Memon and Ebert (1992) aimed to incorporate a gene which expression would change milk composition of goats. From a pair of kids born, a transgenic female was obtained and it expressed the characteristic coded by the transferred gene. However, the offspring of this transgenic female did not incorporate the foreign gene. Walker *et al.* (1990) develop a special medium to culture transgenic sheep embryos, that allowed for higher viability of transgenic embryos before ET into synchronized recipients.

(ix) Nuclear Transfer

The principle of cloning production consists in splitting the blastomeres of a donor embryo and fusing them, one by one, to recipient cytoplasms. Nuclear transfer offers the possibility to produce sets of genetically identical animals, which contributes to accelerate genetic improvement and massive distribution of superior genetics material. Research efforts have been done to overcome the problems caused by the effect of oocyte cytoplasm on reprogramming of nucleus and also resetting of genes activities of donor nucleus. McLaughlin *et al.* (1991) reported that an easier fusion took place when recipient cytoplasm came from in vivo maturation and sheep embryos cloned supported better the in vitro development if compared with embryos derived from cytoplasms of in vitro matured oocytes. In goats, Yong *et al.* (1991) obtained the first offspring of cloned embryos derived from 4 to 32 cells and incorporated to in vivo matured oocytes.

(x) Sexing

Maximum genetic gain can be achieved by the combined used of pre-sexed spermatozoa for IVF, thus producing already sexed embryos for ET. Such a procedure will be cost-effective once this non-invasive approach would ensure embryo integrity. In addition, AI with pre-sexed semen would certainly become much more commercially attractive. However, to sort fertile sexed sperms is not a commercial reality yet. Spermatozoa sexing has been basically based upon differences in physical attributes of sperms that carry different sexual chromosomes. Separation through short-time centrifugation in Percoll gradients rendered a minimal damage to the morphology and the function of spermatozoa. However, the purity of samples, assessed through an "in situ" hybridization assay was 66,1 ± 2.5% under higher concentration gradient and 34.1 ± 10.3% under lower concentration gradient (Blottner *et al.*, 1993). Flow cytometry can provide samples with purity ranging from 80 to 85% (Johnson *et al.*, 1994). Unfortunately, flow cytometry is expensive and reduces the fertility of sexed spermatozoa. Some other techniques for sexing sperms are even more damageous to sperm cells, becoming of little usefulness for animal production. Several techniques are available for embryo sexing, such as 1- Cytogenetics evaluation, which permits to visualize the sexual chromosome pair, which gives accuracy of 50% for morula and 58.3% for blastocyst stages (Yoshizzawa *et al.*, 1989); 2- Detection of HY male specific antigen in the serum; 3- DNA molecular markers with Y_{DNA} specific sequences (Hossepian de Lima *et al.*, 1993). The sensitivity of DNA markers has exponentially increased through the amplification of DNA by means of the use of PCR - polymerase chain reaction, achieving high accuracy in sheep (Bredbacka and Peippo, 1992). In addition, the number of blastomeres required for the assessment is minimum and pregnancy rate after ET of sexed embryos is only 5-10% lower than entire not sexed structures (Herr *et al.*, 1990).

(xi) Preantral Follicles

Sheep and goat oocytes have thousands of preantral follicles. Nonetheless, the large majority of them are destined to atresia during growing and developing periods. Consequently, only a few viable oocytes are produced throughout reproductive life of the female. Recently, techniques to isolate, characterize and culture preantral follicles have become available for cattle (Figueiredo *et al.*, 1993; Nuttinck *et al.*, 1993). Certainly, protocols to small ruminants are about to be standardized. Improvements on the knowledge of the biology and biochemistry of follicle and oocyte developmental events will surely contribute along with the already available methods for using in animal production, filling up the gap ofoocytes from superior genetics dams.

5. RESEARCH NEEDS

Successful long-term maintenance and continuous improvement of small ruminants genetic resources depend on intensification of the adoption of the reproductive technologies available. Private and governmental efforts must define strategies of conservation, on a co-operative basis. In this sense, target populations, characteristics to be improved, financial priorities and technical approaches must be clearly set. Those exists on the abundance of knowledge of some technologies, which certainly can accelerate programs of conservation. Long-term maintenance of semen of small ruminants is a reality. Even developing countries (Brazil; India, Venezuela) posses high quality technology to support germplasm exchanging and/or genetic improvement programs. In addition, estrus control procedures are becoming cost-effective. Associated management practices were demonstrated to enhance the outcome of programs involving estrus synchronization and induction of ovulation. The incorporation of these techniques will certainly facilitate the large-scale application of AI. On its turn, ET can be practiced without major constraints. Apparently, Research efforts should address economics involved in modern biotechnologies. Doubtless, availability of accurately sexed embryos and sperms to be applied in females reliably estrus controlled would fulfill several technical gaps in the field. Actually, Research already provided results to perform that. However, costs and risks involved can not be afforded by husbandry operations. In this sense, economic viability of field application of reproductive technologies must be assessed and these types of study priorise. Future research should aim to improve the outcome of sorting sexed sperms and also, perfect IVF, having as substrates: sexed frozen semen and "in vitro" matured frozen oocytes obtained either through preantral follicles technology or folliculocentesis in living donors. In the future, procedures dealing directly with genome, as gene and nuclear transfer may have tremendous impact on animal production and more than ever conservation of genetic resources trough less sophisticated techniques will be proved worthwhile. Developed and developing countries must be together engaged to successfully accomplish the task of conservation of small ruminants genetic resources.

REFERENCES

Andrioli-Pinheiro, A. Métodos de colheita e de inovulação de embriões caprinos (Capra hircus, Linnaeus, 1758) e os efeitos de repetidas colheitas na vida reprodutiva de doadoras1993. M. Sc. Thesis. Univ.de São Paulo, São Paulo,Brazil., 100pp.

Andrioli-Pinheiro, A.; Simplício, A.A.; Salles, H.O. and Soares, A.T. (1994) Uso de diferentes doses do hormônio folículo estimulante suíno (pFSH) para induzir superovulação em caprinos. *Anais,* XXIII Congr. Brasileiro de Med. Vet.,. Olinda, Brazil. 1994, p.538

Artiga, C.G.; Gutierrez, A.; Garde, J.; Pintado, B.and Varquez, I. (1993) Physiological changes of fresh or frozen ram spermatozoa during capacitation "in vitro". *Theriogenology*, 39:182.

Azevedo, H.C.; Machado, R. and Simplício, A.A. 1995. Franqueamento cervical e taxa de concepção em cabras Moxotó inseminadas artificialmente com diferentes doses inseminantes. *Anais,* XI Congr.Brasileiro Reprod. Anim., Belo Horizonte, Brazil. p. 333.

Azevedo, P.; Baptista, M.C.; Simões Nunes, A. and Mascarenhas, R. (1993) Indução de ovulação e inseminação artificial em caprinos no início da época de reprodução. *Publicações* do V Simp.Intern. Reprod. Anim, 2: 231-239. Santarém, Portugal.

Baldassarre, H.; Matos, D.G. de; Furnus, C.C.; Castro, T.E.; Fischer, E.I.C. DE and Matos, D.G. 1994 Technique for efficient recovery of sheep oocytes by laparoscopic folliculocentesis. *Anim.l Reprod. Sci.*, 35:145-150,

Baril G. In: 1992a .VIII Colloque Scientifique, Association Scientifique Européenne de Transfert Embryonnaire,. Lyon. France. p. 126.

Baril, G,; Brebion, P. and Chésné, P.1993 Manuel de formation pretique pour la transplantation embryonnaire chez la brebis et la chèvre. Rome:FAO, 183p. (Étude FAO production et santé animales 115)

Baril, G.; Leboeuf, B.; Figueiredo Freitas, V.J.; Pougnard, J.L. and Saumande, J.1994 Control of the LH preovulatory surge by a gonadotropin releasing hormone antagonist (Antarelix®) in superovulation goats. In: X Colloque Scientifique, Association Scientifique Européenne de Transfert Embryonnaire, 1: 148. Lyon, France.

Baril G.; Remy, B.; Vallet, J.C.and Beckers, J.F. 1992b Effect of repeated use of progestagen-PMSG treatment for estrus control in dairy goats out of breeding season. *Reprod. Domest. Anim.*, 27: 161-168.

Barry, D.M.; van Niekerk, C.H.; Rust, J. and van der Walt, T. 1990 Cervical embryo collection in sheep after ripening of the cervix with prostaglandin E_2 and estradiol. *Theriogenology*, 33:190.

Battye, K.M.; Fairclough, R.L.; Cameron, A.W.N. and Trounson, A.O. 1988 Evidence for prostaglandin involvement in early luteal regression of the superovulated nanny goat (Capra hircus). *J. Reprod. Fert.*, 84:425-430.

Blottner, S.; Schwerin, M.; Böttcher, M. and Pitra, C.1993 Selective enrichment of bovine X- and Y-spermatozoa by Percoll density gradient centrifugation. *Arch. Tierz. Dummerstorf*, 36:153-162.

Bondioli, K.R.; Wright, Jr., and R.W. 1982 In vitro fertilization of ovine ovulated and ovarian oocytes. *Theriogenology*, 17:79.

Bretzlaff, K.N.; Nutti, L.C.; Scarfe, A.D.; Elmore, R.G.; Capehart, J.; Varner, D.D. and Weston, P.G. 1991 *Am.J.Vet.Res.*, 52 :1423-1426.

Buckrell, B.C. 1988 Applications of ultrasonography in reproduction in sheep and goats. *Theriogenology*, 29: 71-84.

Chavez, G.L.; Zarco, Q.L.; Ducoing, W.A. and Flores, P.G. (1990) Utilizácion del acetato de melengestrol y acetato de fluorogestona solos o combinados con gonadotropina sérica de yegua preñada para la synchronización de estros. *Memórias,* VII Congres. Nacional AZTECA, p. 147-158. Cualicán, México.

Chemineau, P. and Cagnié, Y. 1991 Training Manual on AI in sheep and goats. FAO Animal Production and Health . Paper No. 83, 224 pp

Chemineau, P.; Malpaux, B.; Delgadillo, J.A.; Guérin, Y.; Ravault, J.; Thimonier, J. and Pelletier, J. 1992 Control of sheep and goat reproduction: use of light and melatonin. *Anim. Reprod. Sci.*, 30 : 157-184.

Chemineau, P.; Procureur, R.; Cognie, Y.; Lefevre, P.C.; Locatelli, A. and Chupin, D. 1986 Production, freezing and transfer of bluetongue virus-free goat embryos. *Theriogenology*, 26:279-290.

Dattena, M.; Vespignani, S.; Branca, A.; Gallus, M.; Ledda, S.; Naitana,S.and Cappai,P. 1994 Superovulation response and quality of embryos recovered from anoestrus ewes after single injection of FSHp dissolved in poly vinylpyrrolidone. *Theriogenology*, 42: 235-239.

Delgadillo, J.A.; Leboeuf, B.and Chemineau, P. 1992 Abolition of seasonal variations insemen quality and maintenance of sperm fertilizing ability by photoperiodic cycles in goat bucks. *Small Rum. Res.*, 9 : 47-59.

Delgadillo, J.A.; Leboeuf, B. and Chemineau, P. 1993 Maintenance of sperm production in bucks during a third year of short photoperiodic cycles. *Reprod. Nutr. Devel.*, 33: 609-617.

Dunner, S. 1993 Freezing buck semen diluted in amine-organic buffers. *Anim. Prod.*, 56: 387-391.

Dunner, S. and Impastato, M. 1993 Effects of amine-organic buffers for semen dilution on fertility of does. *Small Rum. Res.*, 11: 57-64.

El-Amrami, G.A.; Hussein, F.M.and El-Bawab, I.E. 1993 Oestrus synchronisation and kidding rate in does treated with a vaginal sponge *Assiut Vet. Med.J.*, 29: 249-259.

Figueiredo, J.R.; Hulshof, S.C.J.; Van den Hurk, R.; Ectors, F.J.; Fontes, R.S.; Nusgens, B.; Bevers, M.M.and Becker, J.F. 1993 Development of a combined new mechanical and enzymatic method for the isolation of intact preantral follicles from fetal, calf and adult bovine ovaries. *Theriogenology*, 40:789-799.

Flores-Foxworth, G.; MsBride, B.M.; Kraemer, D.C. and Nuti, L.C. 1992 A comparison between laparoscopic and transcervical embryo collection and transfer in goats. *Theriogenology*, 37:213.

Gandolfi, F. and Moor, R.M. 1987 Stimulation of early embryoning development in the sheep by co-culture with oviduct epithelial cells. *J. Reprod.Fert.*, 81:23-28.

Garde, J.; Gutierrez, A.; Artiga, C.G.; Varquez, I. 1993 Influence of freezing process on "in vitro" capacitation of ram semen. *Theriogenology*, 39:225, 1993.

Gonzalez, C.I.M. ; Machado, R.; Simplício, A.A.; Cunha, M.G.G. and Bandeira, D.A. 1990 Ovarian activity after induced ovulation in goats. *Proceedings,* III Intern. Symp.on Goat Manag. in Arid Zones,. Coquimbo, Chile, p. 57.

Grangere, M. L'eleveur acteur et juge de la selection. 1993 *La chévre*, 40 p. 212.

Greyling, J.P.C. and Van Niekerk, C.H. 1991 Different synchronization techniques in Boer goat does outside the normal breeding season. *Small Rum. Res.*, 5: 233-243.

Grupp, T. 1991Untersuchungen zur Zervixdilation und transzervicalen Embryonengewinnung bei schaf und Ziege. Ph.D. Thesis. Ludwigg-Maximilians-Univ., Munchen. 144p.

Güven, B.; Özsar, S. Celebi, M.; Sungur, H. and Goncagül, T. 1993 Ankara keçilerinde anöstros dömende östrüs aktiviesinin indüklenmesi ve fertilite kontrolu. *Hayvancylik Arastirma Dergisi*, 3: 31-33.

Hahn, J. 1992 Attempts to explain and reduce variability of superovulation. *Theriogenology*, 38: 270-277.

Haibel, G.K. 1990 Use of ultrasonography in reproductive management of sheep ans goat herds. *Vet. Clin. of North America: Food Animal Practice*, 6 : 597-613.

Hare, W.C.D., Luedke, A.J.; Thomas, F.C.; Bowen, R.A.; Singh, E.L.; Eaglesome, M.D.; Randall, G.C.B. and Bielanski, A. 1988 Nontransmission of bluetongue virus by embryo transfer from bluetongue virus-infected sheep. *Am. J. Vet. Res.* 49:468-472.

Holm, P.; Irvine, B.; Armstrong, D.T. and Seamark, R.F. 1991 In vitro production of sheep blastocysts from ivm-oocytes using frozen semen and oviduct epithelial cell co-culture for IVF. *Theriogenology*, 35:214.

Homeida, A.M. and Al-Afaleq, A.I. 1994 Delayed luteolysis ans suppression of testosterone secretion after recombinant ovine interferon treatment in goats (Capra hircus). *J. Reprod.Fert.*, 102: 301-304.

Hossepian de Lima, V.F.M.; Moreira Filho, C.A.; De Bem, A.R. and Jorge, W. 1993 Sex determination of murine and bovine embryos using cytotoxicity and immunofluorescence assays. *Theriogenology*, 39:1343-1352.

Huang, J.C.; Lin, J.H.; Yuan, H.H.; Tseng, J.L. 1993 Induction of oestrus in dairy goats during the anoestrus season with melatonin and progesterone plus PMSG. *J.Taiwan Livest. Res.*, 26: 189-202.

Huneau, D.; Crozet, N. and Ahmed-ali, M. 1994 Estrous sheep serum as a potent agent for ovine IVF; effect on cholesterol efflux from spermatozoa and the acrosome reaction. *Theriogenology*, 42:1017-1028.

Ishwar, A.K. and Pandey, J.N. 1990 Estrus synchronization and fertility behavior in Black Bengal goats following either progesterone or prostaglandin treatment. *Theriogenology*, 34: 1015-1024.

Johnson, L.A.; Cran, D.G. and Polge, C. 1994 Recent advances in sex preselection of cattle: flow cytometric sorting of X- and Y-chromosome bearing sperm based on DNA to produce progeny. *Theriogenology*, 41: 51-56.

Kane, M.T.; Carney, E.W. and Ellington, J.E. 1992 The role of nutrients, peptide growth factors and co-culture cells in the development of preimplantation embryos in vitro. *Theriogenology*, 38: 297-313.

Lauvergne, J.J.; Renieri, C.; Machado, T.M.M. and Acharya, R.M. 1992 Goat Genetic Resources. *Proc.* V Int. Conf. on Goats,(Ed Acharya, R.M). New-Delhi, India. 2: 59-70.

Le Gal, F.; Baril, G.; Vallet, J.C. and Leboeuf, B. 1993 In vivo and in vitro survival of goat embryos after freezing with ethylene glycol or glicerol. *Theriogenology*, 40: 771-777.

Leboeuf, B. 1993 Maîtriser la reproduction. ,*Chevre,* 40: 13-16.

Leboeuf, B.; Nercy, C. and Ruyter, T. DE. 1994 L'insemination artificialle caprine in Rwanda. Adaptation à la chèvre rwandaise de la méthode utilisée pour le races latières europèennes. *Revue d'Elevage et de Med. Vét.Pays Trop.*, 47: 240-243.

Llewelyn, C.A. and Kadzere, C.J. 1992 Oestrus synchronization and fertility following treatment with fluogestone acetate (Chronolone) impregnated intra-vaginal sponges in indigenous goats of Zimbabwe *Zimbabwe Vet. J.*, 23: 159-165.

Machado, R. 1994 Combinação Prostaglandina-progestágeno na indução e sincronização do parto em cabras leiteiras. *Anais,* VIII Seminário de Pesquisa Agropecuária do Piauí, Teresina, Brazil, 83 pp.

Machado, R. and Azevedo, H.C. 1995 Resposta biológica de caprinos ä sincronização do estro. *Anais*, XI Congres. Brasileiro Reprod. Anim., Belo Horizonte, Brazil, 355 pp.

Machado, R.; Azevedo, H.C.; Salles, H.O.; Soares, A.T. and Santos, D.O. 1995b Supressão do estro em caprinos pela reutilização de implantes de norgestomet: estimativa da secreção do esteróide. *Anais,* XI Congres. Brasileiro de Reprod. Anim., Belo Horizonte, Brazil, 357 pp.

Machado, R. 1991 Inseminação artificial com sêmen congelado em caprinos. *Anais,* IX Congres.Brasileiro Reprod. Anim., p.265-276. Belo Horizonte, Brazil, p. 265-276.

Machado, R.; Simplício, A.A. and Andrioli, 1992 A. Body wight, scrotal-testicular measurements and semen charactheristics in three different goat genotypes. *Rev. Terra Arida*, 11: 127-132.

Machado, R. and Simplício, A.A. 1992a Effects of two washing solutions on sperm survival of bucks. *Proceedings,* V Intern. Conf. on Goats. New Delhi, India. In: Recent Advances in Goat Production.(Ed). Acharya, R.M. p.1089-1094.

Machado, R. and Simplício, A.A. 1992b Ovulation in female hair sheep after hormone treatment using hCG or hMG as gonadotrophis sources. *Procedings,*XII Int. Congr Anim. Reprod., The Hague, Netherlands. p. 1151-1153.

Machado, R. and Simplício, A.A. 1995 Inseminação artificial em caprinos no Brasil: estádio atual. *Rev. Brasil.Reprod. Anim.*,19: 61-72.

Machado, R.; Simplício, A.A. and Araújo, M.R.A. 1995a Repetibilidade de algumas características reprodutivas de caprinos. *Procedings,* XIV Reunion Latinoamericana de Produccion Anim.. MardelPlata, Argentina. (In press).

Madison, V.; Avery, B. and Greve, T. 1992 Seletion of immature bovine oocytes for developmental potential in vitro. *Anim. Reprod. Sci.*, 27/n:1-11.

Mahmood, S. and Koul, G.L. 1990 Synchronization of oestrus in Pashima goats by prostaglandin analogue. *Ind. Vet.J.*, 67: 326-328.

Majumdar, A.C.; Mogha, I.V. and Ansarj, M.R. 1990 Successful superovulation in prepubertal Barbari goats. *Ind. J. Anim.Sci.*, 60: 1304-1306.

Mani, A.U.; McKelvey, W.A.C. and Watson, E.D. 1992 Effects of low level of feeding on response to synchronization of estrus, ovulation rate and embryo loss in goats. Theriogenology, 38: 1013-1022,1992.

Mareco, G. 1993 Inseminacion intrauterina transcervical de cabras. *Vet.Arg.*, 10: 455-457.

Martemucci, G.; Toteda, F.; Dálessandro, A. and Lacetera, G. 1992 Fecondità in capre trattate con FGA, $PGF_{2\alpha}$, PMSG durante il periodo di ipoattivatà sessuale. *Zoot.. Nutr.. Anim.*, 18: 139-145.

Martino, A.; Mogas, T.; Palomo, M.J. and Paramio, M.T. 1993 Effect of method of recovery on the in vitro maturation and fertilization of pre-pubertal goat oocytes. In: IX Réunion Assoc. Europ. Transfert Embryonnaire, Lyon, France, p- 238.

Martino, A.; Mogas, T.; Palomo, M.J. and Paramio, M.T. 1995 In maturation and fertilization of prepubertal goat oocytes. *Theriogenology*, 43:473-485.

Martino, A.; Palomo, M.J.; Mogas, T. and Paramio, M.T. Influence of the collection technique of prepubertal goat oocytes on in vitro maturation and fertilization. *Theriogenology*, 42:859-873.

McLaughlin, K.J.; Pugh, A.P.; Logan, K. and Tervit, H.R. 1991 Assessment of oocyte source for ovine nuclear transfer. *Theriogenology*, 35:240.

Mellado, M.; Alemán, R.; Orozco, F.J. and Uribe, G. 1994 Effect of prostaglandin $F_{2\alpha}$ dosage and route of administration on estrus response in Criollo goats under range conditions. *Small Rum. Res.*, 14: 205-208.

Memon, M.A. and Ebert, K.M. 1992 Gene manipulations in goats through biotechnology. *Proc.* V Int. Conf.on Goats, (Ed. Acharya, R.M). New-Delhi, India. Vol.2: 37-50.

Nibart, J.M.; Chemineau, P. and Thibier, M. 1992 Use of melatonin implant to enhance ovarian response of superovulated ewes. In: VIII Colloque Scient., Assoc. Scientif. Europ. Transfert Embryonnaire, Lyon. France. p. 216.

Nuttinck, F.; Mermillod, P.; Massip, A. and Dessy, F. 1993 Characterization of in vitro growth of bovine preantral follicles: A preliminary study. *Theriogenology*,39:811-821.

Oliveira, J.V.L. and Rezende, J. 1990 índices de fecundação em cabras com estros sincronizados e coadjuvadas com gonadotropina. Arq.Esc.Med. Vet. UFBA, 13 : 3-12.

Palomo, M.J.; Martino, A.; Mogas, T. and Paramio, M.T. Effect of oocyte type on the in vitro maturation and fertilization of pre-pubertal goats oocytes. In: IX Réunion Assoc.Europ. Transfert Embryonnaire, Lyon, France. p.252.

Parry, A.L.; Paterson, D.J.; Litherland, A.J., Death, A.F. and Staples, L.D. 1990 Concentration of melatonin in plasma of goats treated with regulin. *Proc. New Zealand Soc. Anim. Prod.* 50: 297-300.

Pawshe, C.H.; Totey, S.M. and Jain, S.K. 1994 A comparison of three methods of recovery of goat oocytes for in vitro maturation and fertilization. *Theriogenology*, 42:117-125.

Pendleton, R.J.; Youngs, C.R.; Rorie, R.W.; Pool, S.H.; Memon, M.A. and Godke, R.A. 1992 Follicle stimulating hormone versus pregnant mare serum gonadotropin for superovulation of dairy goats. *Small Rum. Res.*, 8:217-224.

Pinheiro-Andrioli, A.; Salles, H.O.; Pinheiro, R.R.; Simplício, A.A.; Soares, A.T.; Sobrinho, P.A.M.; Soares, B.A. and Santa-Rosa, M.R. 1995 Uso da transferência de embriões como método de controle da artrite encefalite caprina a vírus (CAEV) - resultados parciais. *Anais,* X Reunião Anual Soc.Bras. Transf. Emb. Águas de Lindóia, Brazil, (In press)

Pintado, B.; Perez Llano, B.; Gil, L. and Gutierrez, A. 1992 Seasonal evolution of *in vitro* viability from goat ejaculates during freezing. Procedings,XII Intern. Congr. Anim. Reprod., The Hague, Netherlands. p. 1478-1480.

Ribeiro, V.M.F.; Oliveira, M.A.L.; Lima, P.F. and Wischral, A. 1989 Vitrificação de embriões caprinos. *Rev. Centro Ciênc. Rur. UFSM,* supl: 19-20.

Ricordeau, G.; Wiggans, G.R.; Serradilha, J.M.; Khan, B.U.; Sanchez, G.F.; Mavrogenis, A.P.; Misra, R.K. and Manfredi, E.J. 1992 Breeding plans for milk and meat production. *Procedings*, V Int. Conf. on Goats, (Ed. Acharya, R.M). New-Delhi, India. Vol 2 : 85-99.

Ritar, A.J.; Ball, P.D. and O'May, P.J. 1990 Artificial insemination of Cashemere Goats: Effects on fertiliy and fecundity of intravaginal treatment, method and time of insemination, semen freezing proces, number of motile spermatozoa and age of females. *Reprod. Fertil.Dev.*, 2: 377-384.

Ritar, A.J. and Salamon, J. 1991 Effects of month collection, method of processing, concentration of egg yolk and duration of frozen storage on viability of Angora goat spermatozoa. *Small Rum. Res.*, 4 : 29-37.

Robin, N.; Laforest, J.P.; Lussier, J.G. and Guibault, L.A. 1994 Induction of estrus with intramuscular injections of GnRH or PMSG lactating goats (Capra hyrcus) primed with a progestagen during seasonal anestrus. *Theriogenology,* 42: 107-116.

Roca, P.G.;. Martinez, E. and Vásquez. J.M. 1992 Characteristics and seasonal variations in the semen of Murciano-Granadina goats in the Mediterranean area. *Anim. Reprod. Sci.*, 29: 255-262.

Saacke, R.G.; Nadir, S. and Nebel. R.L. 1994 Relationship of semen quality to sperm transport, fertilization, and embryo quality in ruminants. *Theriogenology*, 41: 45-50.

Sakkas, D.; Batt, P.A. and Cameron, A.W.N. 1989 Development of preimplantation goat (Capra hircus) embryos in vivo and invitro. *J. Reprod.Fert.*, 87:359-365.

Salles, H.O.; Azevedo, H.C.; Soares, A.T.; Santos, D.O. and Machado, R. 1995 Cloprostenol na indução do parto em cabras: eficiência da aplicação intramuscular na vulva. *Anais*, XI Congres. Brasileiro Reprod. Anim., Belo Horizonte, Brazil. 1995. pp. 385.

Santos, D.O.; Simplício, A.A. and Machado, R. 1992 Indução do parto em cabras pela aplicação intramuscular de cloprostenol. *Rev. Bras.Reprod. Anim.*, 16: 41-53. 1992.

Schiewe, M.C.; Howard, J.G.; Goodrowe, K.L.; Stuart, L.D. and Wildt, D.E. 1990 Human menopausal gonadotropin induces ovulation in sheep, but embryo recovery after prostaglandin $F_{2\alpha}$ synchronization is compromised by premature luteal regression. *Theriogenology*, 34:469-486.

Serradilha, J.M.; Elsen, J.M. and Piacere, A. 1991. Modelisation de schema de selection des caprines in France., Doc. INRA-SAGA, 16 pp.

SID - Sheep Industry Handbook. Reproduction Chapter. 1988. p.701-738.

Skrzyszowska, M.; Smorag, Z. 1989 Cell loss in bissected mouse, sheep and cow embryos. *Theriogenology*, 32:115-122.

Soares, A.T.; Simplício, A.A.; Pinheiro-Andrioli, A.; Salles, H.O.; Sobrinho, P.A.M.; Soares, B.A.; Moraes, J.B. and Wanderley, K.O. 1995 Uso de flunixin meglumine para impedir a regressão prematura de corpos lúteos após a superovulação em caprinos. *Anais*, X Reunião Anual Soc.Bras. Transf. Emb. Águas de Lindóia, Brazil, (In press)

Sun, F.J.; Holm, P.; Irvine, B. and Seamark, R.F. 1994 Effect of sheep and human follicular fluid on the maturation of sheep oocytes in vitro. *Theriogenology*, 41:981-988.

Széll, A.; Zhang, J. and Hudson, R. 1990 Rapid cryopreservation of sheep embryos by direct transfer into liquid nitrogen vapour at -180°C. *Reprod. Fert.Develop.*, 2: 613-618.

Tainturier, D.; Fieni, F.; Bruyas, J.F.; Chemli, J.; Allaire, F. and Zaiem, F. 1993 Diagnostic de gestation chez la chevre par echotomographie par la voie transrectale a l'aide d'une sonde prostatique humaine. *Sci.Vét.Méd.Comp.*, 95: 81-87.

Tan, S.J. and Lu, K.H. 1990 Effect of different oestrus stages of ovaries and sizes follicles on generation of bovine embryos in vitro. *Theriogenology*, 33:335.

Tuli, R.K. and Holtz, W. and Effect of glycerolization procedure and removal of seminal plasma on post-thaw survival and GOT-release from Boer goat spermatozoa. *Theriogenology*, 42: 547-555.

Vallet, J.C. and Baril, G. 1992 Effect of laparoscopic intra-uterine insemination in superovulated dairy goats. In: VI Colloque Scient., Assoc.Scient. Europ.Transfert Embryonnaire, 1: 188. Lyon, France.

Waldken-Brown, S.W. & Restall, B.J. and Nenniawati. 1993a the male effect in Australian Cashemere goat. I- Ovarian and behavioural response of seasonally anovulatory does following the introduction of bucks. *Anim. Reprod. Sci.*, 32: 41-53.

Waldken-Brown, S.W. & Restall, B.J. and Nenniawati. The male effect in Australian Cashemere goat. 3- Enhancement with buck nutrition and use of oestrus females. *Anim. Reprod. Sci.*, 32: 69-84.

Walker, S.K.; Heard, T.M.; Verma, P.J.; Bawden, C.S.; Sivaprasad, A.V.; Rogers, G.E. and Seamark, R.F. (1994) In vitro culture of sheep zygotes following pronuclear microinjection. *Theriogenology*, 33:342.

Wheaton, J.E.; Carlson, K.M.; Windels, H.F. and Johnson, L.J. CIDR: 1993 a new progesterone-releasing intravaginal device for induction of estrus and cycle control in sheep and goats. *Anim. Reprod. Sci.*, 33 : 127-141.

Wiel, D.F.M. Van De; Koops, W. Vos, E.; Shah, S.N.H.; Barrios, V.; Vendrig, A.A.A. and Adrichem Boogaert, D.H. 1991 Use of enzymeimmunoassay to measure progesterone and oestrone sulphate in milk and/or plasma for monitoring of fertility in goats. In: Isotope aided studies on sheep and goats production in the tropics. *Proc.*, Final Coord. Meet./1989.. Perth, Australia. p.107-124

Yong, Z.; Jianchen, W.; Jufen, Q. and Zhiming, H. 1991 Nuclear transplantation in goats. *Theriogenology*, 35:299.

Yuswiati, E. and Holtz, W. 1990 Successful transfer of vitrified goat embryos. *Theriogenology*, 34:629-632.