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Implications of recent advances in reproductive physiology for reproductive management of goats

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The control of reproduction in goats is interesting for technical reasons (synchronization of kiddings, adjustment to forage availability or to economy), and for genetic reasons (identification and dissemination of improved genotypes). The use of short-light rhythms leads to markedly increased production of semen per buck and prevents occurrence of a 'resting' season. Recent identification of a bulbourethral lipase in goat spermatozoa opens new perspectives in sperm preservation. Light plus 'short day' treatments also allow induction of out-of-season oestrous cycles and ovulations leading to enhanced fertility. Repeated use of eCG provokes the production of antibodies, delays the timing of ovulation and causes a reduction in fertility after fixed-time artificial insemination. All steps of embryo production, freezing and transfer are now controlled and allow the attainment of satisfactory numbers of kids born per donor female, which are compatible with the development of the technique for exchanging genotypes between countries. *In vitro* production of embryos allows high development rates to be achieved after *in vitro* maturation and fertilization of oocytes, and will ensure the production of synchronous populations of one-cell zygotes at the stage required by new biotechnologies.

Introduction

As in other domestic species, the control of reproduction in goats offers advantages at the farm and at the level of the population where genetic improvements can be made. The first advantage is the choice of a kidding period at a given time of the year (adjustment to favourable external conditions imposed by the season of forage growth or by marketing of the products). The second advantage is synchronization of kiddings over a reduced period leading to a reduction in kid mortality, constitution of homogeneous groups of mothers and allowing kids to be fed more adequately to their requirements, and optimization of labour for care of the animals. The third advantage of controlling reproduction in goats is that it allows manipulation and storage of the genetic material. Artificial insemination (AI), even used on a small scale, allows links between herds and this increases the efficiency of indexation of sires. Early and accurate estimation of the genetic value of young bucks is feasible. Once identified, the improved males can be used in a large number of herds. Embryo transfer increases the number of progeny from a genetically superior female and is a method for exchanging genotypes without transmitting diseases. Finally, *in vitro* production of embryos, in the near future will give access to the genome of the one-cell embryo.

In this review only a limited number of techniques that have undergone marked progress in recent years are discussed. These techniques are recommended for use in intensive systems in which the income per goat per year is very high, generally because of the price of goat milk.

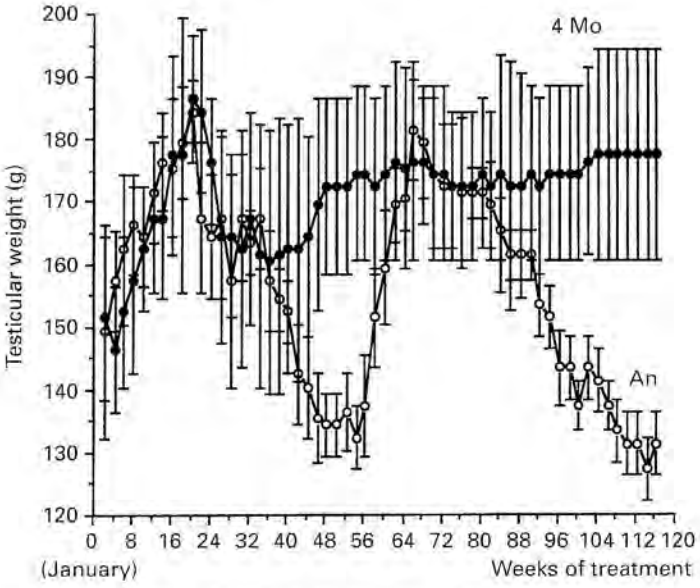

Fig. 1. Testicular weight of Alpine and Saanen bucks treated or not with an accelerated light rhythm of 4 months. Values are monthly means ± SEM. An: natural photoperiodic variations at 46° N latitude (○, $n = 6$). 4 Mo: alternation between two months of long days (16 h light: 8h dark) and two months of short days (8 h light:16 h dark) (●, $n = 6$) (B. Leboeuf and P. Chemineau, unpublished)

Sperm Production and Processing

The application of photoperiodic treatments to bucks of seasonal breeds alleviates the problem of seasonality of sperm production. Initially developed in rams, short light rhythms (that is alternations between 1 or 2 months long days (16 h light: 8 h dark; 16L:8D; LD) and 1 or 2 months short days (8L:16D; SD)) overcome seasonal variations in testis size and sperm production. Alpine and Saanen bucks, subjected for 3 consecutive years to photoperiodic treatments showed a marked increase in all parameters of sperm production, compared with control bucks under natural photoperiod (Delgadillo *et al.*, 1993). When collected twice a week, the total number of spermatozoa produced was improved by 61% (Delgadillo *et al.*, 1991). Semen quality after deep-freezing no longer exhibited the marked seasonal changes observed in untreated bucks. The total number of AI doses produced during the first 2 years of the treatment was much higher (62%) than that produced by control males. Fertility of the semen was not significantly altered by such treatments, in spite of a slight decrease in fertility rate in one group of bucks (Delgadillo *et al.*, 1992).

From these results, it was also apparent that the collection rate (twice a week) could be increased in treated males. It was therefore decided to compare overall sperm production of treated bucks collected four times a week all year round, with sperm production of control bucks collected four times a week from September to February only, as is normal practice. During the 24 months of treatment, as expected, testicular weight of bucks remained constant, at the maximal weight of the full sexual season, while testicular weight of control males underwent the normal seasonal variations (Fig. 1).

As a consequence, sperm production either in terms of total number of spermatozoa produced, or in terms of AI doses, was significantly improved by the treatment (2212 versus 3111 doses per buck). Fertility of AI doses was slightly, although not significantly, lower for light-treated bucks (Table 1; B. Leboeuf and P. Chemineau, unpublished).

Such a high production probably originates from unexpected changes in spermatogenic

Table 1. Number of bucks, collection rhythm, number of AI doses produced per year and fertility after artificial insemination of control bucks (subjected to natural lighting), or treated with short light rhythms of alternation of long days (16 h of light per day) and short days (8 h of light per day); artificial inseminations are done in each flock after distribution of the females to be inseminated in each group of control or treated bucks (Data from Delgadillo *et al.* 1992; and B. Leboeuf and P. Chemineau unpublished results).

	Experimental groups		
	Control groups (natural lighting)	1 month LD/ 1 month SD	2 months LD/ 2 months SD
Experiment 1 (Delgadillo *et al.*, 1992) (1599 goats in 58 herds)			
Number of bucks	6	6	6
Collection rhythm	2 ejaculates/week	2 ejaculates/week	2 ejaculates/week
Number of AI doses (at 200 × 10^6 sperm/doses) produced per year and per buck	253	427	391
Fertility (% producing kids)	62.5	57.9	57.8
Experiment 2 (B. Leboeuf and O. Chemineau, unpublished) (785 goats in 25 herds)			
Number of bucks	6	6	
Collection rhythm	4 ejaculates per week from Sept to Feb	4 ejaculates per week, all the year round	
Number of AI doses (at 100 × 10^6 sperm/doses) produced per year and per buck	1106	1556	
Fertility (% producing kids)	69.5	61.2	

processes. Light-treated bucks had significantly increased numbers of spermatogonia (the stem cell of the spermatogenic line) while maintaining spermatogenic divisions at the high rate of the full sexual season (Delgadillo *et al.*, 1995). By allowing sperm collection all the year round rather than for 6 out of 12 months, these photoperiodic treatments may accelerate the production of AI doses in young bucks during the 2.5 years of progeny testing. This photoperiodic treatment is now used to improve sperm production of one-year-old bucks in the French national selection programme.

The most recent data obtained in the field of semen technology have been the identification of a seminal plasma enzyme that decreases sperm survival *in vitro*. Egg yolk or skim milk is widely used in extenders for mammalian semen because of their protective role against cold shock of spermatozoa. However, the cryopreservation of goat semen in these media requires that most of the seminal plasma be removed before sperm dilution (washing method) to improve the survival of spermatozoa after freezing and thawing. The bulbourethral gland secretion (BUS) is the fraction of goat seminal plasma responsible for deterioration of sperm viability in egg yolk (Roy, 1957) and milk-based diluents (Nunes *et al.*, 1982). The egg-yolk coagulating enzyme (EYCE) from goat BUS displays phospholipase A activity and may hydrolyse egg yolk lecithin into fatty acids and lysolecithin (Roy, 1957; Iritani and Nishikawa, 1972) which are toxic to goat spermatozoa (Aamdal *et al.*, 1965). The BUS component has been recently purified and identified as a 55–60 kDa glycoprotein (BUSgp60) with triglyceride lipase activity (Pellicer-Rubio *et al.*, 1997). Indeed, BUSgp60 provokes a decrease in the percentage of motile spermatozoa, a deterioration in the quality of movement, breakage of acrosomes and cellular death of goat epididymal spermatozoa diluted in skim milk (Fig. 2).

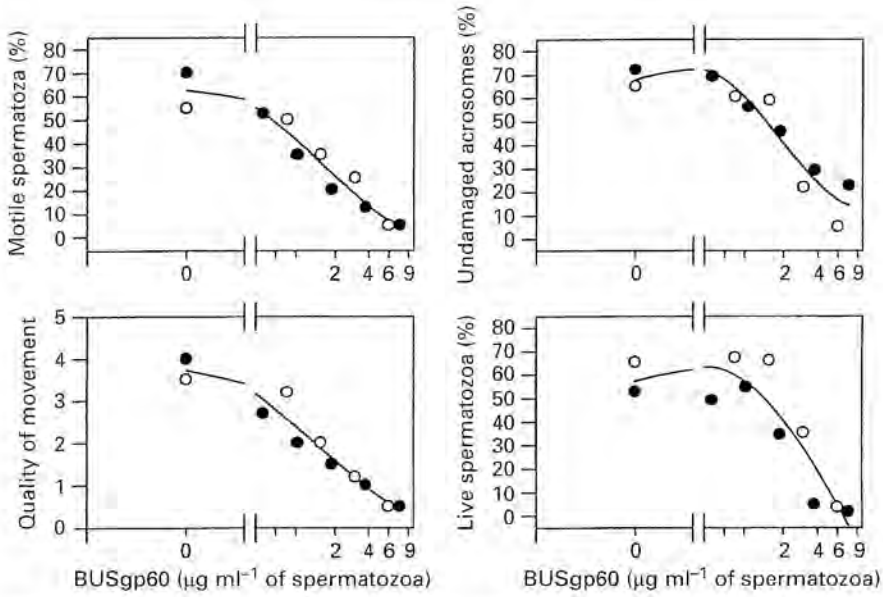

Fig. 2. Comparative dose-dependent effects of purified BUSgp60 (○) and BUS (●) on the quality parameters of goat epididymal spermatozoa diluted in skim milk after incubation for 60 min at 37°C. Values are the mean of two observations. Doses of BUS are expressed as equivalent doses of BUSgp60 (Adapted from Pellicer-Rubio *et al.*, 1997).

The catalysis of oleic acid formation from residual milk triglycerides by BUSgp60 appears responsible for these effects (Pellicer-Rubio and Combarnous, 1998). Interestingly, BUSgp60 has been classified as a novel lipase most probably belonging to the pancreatic lipase-related protein 2 (PLRP2) family (Pellicer-Rubio *et al.*, 1997). Since PLRP2 enzymes are known to display both phospholipase A and lipase activities (Carriere *et al.*, 1994), it has been suggested that BUSgp60 and EYCE are related or even identical enzymes (Pellicer-Rubio and Combarnous, 1998). These results allow the possibility of specifically inhibiting BUSgp60 lipase in milk-based extenders, and avoiding the harmful step of washing goat semen before deep-freezing. Moreover, the use of BUSgp60 inhibitors for better cryopreservation of unwashed goat semen in egg-yolk diluents should be considered.

Induction of Out-of-Season Cyclicity in the Female Goat by Using Photoperiodic Treatments

Appropriate treatment of animals with melatonin could be used to mimic short days while their visual system perceived long days (Chemineau *et al.*, 1992; Deveson *et al.*,1992a; Malpaux *et al.*, 1993), to induce an advance of ovulatory and oestrous activities. However, when used alone in highly seasonal breeds, melatonin treatment provides a maximum advance of only 1.5 months. This is not satisfactory for many farmers, especially in the dairy goat industry in France, who wish to induce a complete out-of-season breeding (that is from April to July). Under such conditions, melatonin treatment should be preceded by at least 2 months of a light treatment composed daily either of long days (Deveson *et al.*, 1992a), or of two periods of supplementary light (Fig. 3; Chemineau *et al.*, 1992). Such long day (LD) treatment probably provides the photoperiodic signal for the onset of the annual breeding season and also restores sensitivity to melatonin (Chemineau *et al.*, 1992; Malpaux *et al.*, 1993). In French dairy goats maintained in open barns, the use of this succession LD + melatonin followed by a 'buck effect' with 'light'-treated bucks, induces ovulatory and oestrous activities that

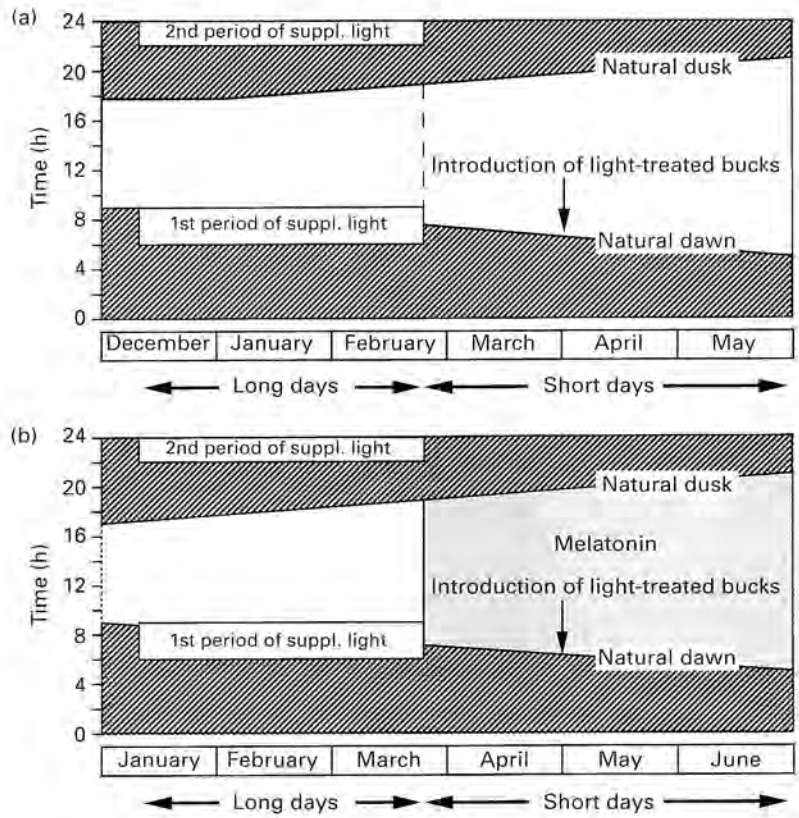

Fig. 3. Photoperiodic treatments applied in open barns and using the succession of long day and short day treatment followed by a 'buck-effect'. The upper treatment (a) is applied early in the year when natural day lenght is short, and the lower treatment (b), using melatonin after the end of the long day period is applied later in the year.

are sufficient to achieve a fertility and prolificacy close to those of the normal annual breeding season (Chemineau *et al.*, 1996).

The LD treatment must be for longer than 2 months; the melatonin concentration provided by the implants should be optimized; and bucks treated with LD + melatonin should be introduced for natural mating 35–70 days after the onset of melatonin treatment (Chemineau *et al.*, 1996). If these conditions are met, peak rates of conception generally occur about 10 days after introduction of bucks and some females conceive at the return to oestrus one cycle later. Such photoperiodic treatments may change the speed of hair growth (Gebbie, 1993) and light-treatment during pregnancy was shown to delay the onset of puberty by about 4 weeks in young female goats born from light-treated mothers (Deveson *et al.*, 1992b).

More recently, it was demonstrated that when applied early in the season (ending before the end of March), the melatonin treatment was not necessary and the return to natural lighting after LD allowed satisfactory fertility rates (Table 2).

Hormonal Synchronization of Oestrus

Hormonal treatment of female goats to induce a synchronous onset of oestrous behaviour and ovulation within a limited time after the end of the treatment is a prerequisite to the use of AI. The

Table 2. Fertility of goats after photoperiodic treatment alone in various flocks and comparison within flock of the treatment with and without melatonin.

	No females	Fertility (No. producing kids)	Litter size (No. kids born/ lambing
Photoperiodic treatment alone Natural mating, 20 herds (GRC, 1996)	3236	76.8%	1.83
Photoperiodic treatment with and without melatonin Natural mating 1996+1997, 1 single herd (B. Lebeouf and P. Chemineau, unpublished)			
With melatonin	126	75.3%	2.07
Without melatonin	115	73.0%	2.00

association between a progestagen (delivered by a vaginal sponge or by a subcutaneous implant), a prostaglandin analogue and PMSG (now called equine chorionic gonadotrophin, eCG) remains the most efficient tool to achieve this objective. These treatments are now widely and successfully used all over the world to control reproduction in female goats. Their use in association with AI on a fixed-time basis in thousands of goats has led to high levels of fertility (Leboeuf *et al.*, 1998). This treatment could also be applied to young goats if specific conditions are respected.

Recent experiments were performed to test modifications to reduce the variability in the interval between the end of the sponge–eCG treatment and onset of oestrus. Neither increase in the quantity of fluorogestone acetate (FGA) delivered by the sponge, nor the use of subcutaneous ear implants reduced this variability (Freitas *et al.*, 1996a, 1997a). Neither the number of corpora lutea, nor the number and size of the follicles observed on the ovary before and during the FGA treatment strongly influenced the response (Freitas *et al.*, 1996b). Finally, it was observed that during natural cycles, the variability in the interval between luteolysis and the onset of oestrus or onset of the LH surge was higher than after FGA–prostaglandin treatment (Freitas *et al.*, 1997b). Thus, it was concluded that further improvements of the 'classic' hormonal treatment would be difficult to obtain.

Paradoxically, when eCG is used repeatedly on the same females, its efficiency decreased. In a single Saanen herd of 169 females in which breeding takes place each year out of season after FGA and eCG treatment, the percentage of goats showing oestrus and producing kids was significantly lower for multiparous than for nulli- and primiparous goats (64 versus 99, and 34 versus 67%, respectively). When goats were treated for the second time during the same year, the percentage showing oestrus was lower than after the first treatment (45 versus 71%; Baril *et al.*, 1992). This situation is due to the appearance of antibodies against eCG (Roy *et al.*, 1995; see later). When eCG binding of the serum was calculated by radioimmunoassay, and expressed as percentage of bound radioactive eCG with plasma (Baril *et al.*, 1992), this percentage was associated with fertility results. Before the treatment, it was higher in multiparous than in nulli- and primiparous goats (18 versus < 1%), and higher in non-pregnant than in pregnant goats (26 versus 7%) (Baril *et al.*, 1992). These results obtained in a single herd have prompted large-scale surveys in private flocks, using FGA/eCG treatments, associated with 'classic' AI with deep-frozen semen. In the first survey, oestrous behaviour was induced in almost all treated goats (98.1% of the 368 Alpines and 272 Saanens goats of 19 private herds) between 24 and 72 h after sponge removal. The distribution of the onset of oestrus after sponge removal did not differ between breeds or with age but was affected by the number of treatments previously received by the females and seemed to increase markedly after the second treatment (Fig. 4). Fertility but not prolificacy after AI was negatively correlated with the interval between sponge removal and onset of oestrus ($R = 0.92$). Fertility of goats that came into

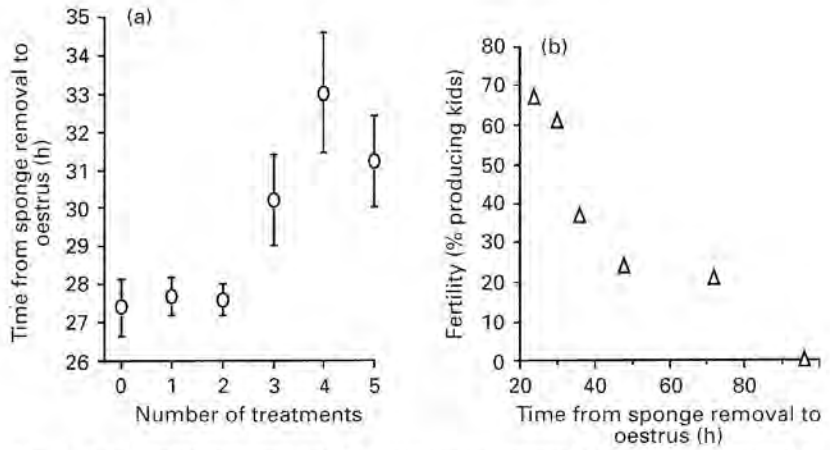

Fig. 4. Relationship between (a) the mean (±SEM) interval from sponge removal to onset of oestrus and the number of treatments previously received by the female goat and (b) between the interval from sponge removal to onset of oestrus and fertility (Adapted from Baril *et al.*, 1993).

oestrus later than 30 h after sponge removal was significantly lower than for those that were first observed in oestrus 24 or 30 h after sponge removal (33 versus 65% respectively, Fig. 4; Baril *et al.*, 1993). This delay in the onset of oestrous behaviour is associated with a delay in the LH preovulatory surge (Maurel *et al.*, 1992) and with a delay in the time of ovulation (Leboeuf *et al.*, 1993, 1996). In the second survey, eCG binding (measured in 524 dairy goats of 17 private herds) before the onset of treatment was significantly lower in herds in which treatments were never used than that measured in samples of the other goats and was not dependent on the age of the female. Binding was increased in the females that had previously received from two to five treatments, compared with that in females that had received no treatment or one treatment (3 versus 10%). On an individual basis, the percentage of goats showing onset of oestrus behaviour more than 30 h after sponge removal was higher (38 versus 7%) and fertility was decreased (51 versus 66% on 166 versus 353 females) when eCG binding was higher (more than 10% of radioactive eCG binding versus less than 5%). When measured 25 days after eCG injection, eCG binding was increased (7% before injection versus 28% after injection), and correlated with binding detected before treatment (Baril *et al.*, 1996b).

Complementary studies were conducted to evaluate the induction of an anti-eCG humoral immune response after a 500 iu eCG injection. An ELISA was developed to quantify the plasma concentration of anti-eCG antibodies and compare kinetics of antibody secretion between individuals (F. Roy *et al.*, 1999). For this experiment 15 goats were treated for the first time with eCG and exhibited an increasing concentration of anti-eCG antibody 10 days (day 10) after eCG injection. Maximum values were reached between day 10 and day 17; thereafter antibody concentration showed a progressive decline over 2 months. Goats previously treated (one or more times) with eCG ($n = 29$) displayed similar kinetics of humoral immune response, except that they exhibited an earlier increase in antibody concentration at day 7 and a longer decreasing phase of the antibody secretion (Fig. 5). Within both treatment groups, all goats had an identical immune response but differed markedly in their anti-eCG concentrations, regardless of the number of previous treatments. Indeed, maximal anti-eCG antibody concentrations varied from 0.7 to 102 μg ml^{-1} in goats treated for the first time and from 3.0 to 219 μg ml^{-1} in goats treated several times. Nevertheless, in spite of the heterogeneity of antibody secretion, results showed that mean antibody concentration measured before treatment increased significantly ($P < 0.05$) as a function of the number of previous eCG treatments. The antibody concentration measured before treatment was defined as residual anti-eCG antibodies. These antibodies resulted from the previous immune response induced by the last eCG injection (about one year before). High residual antibody concentration resulted in decreased

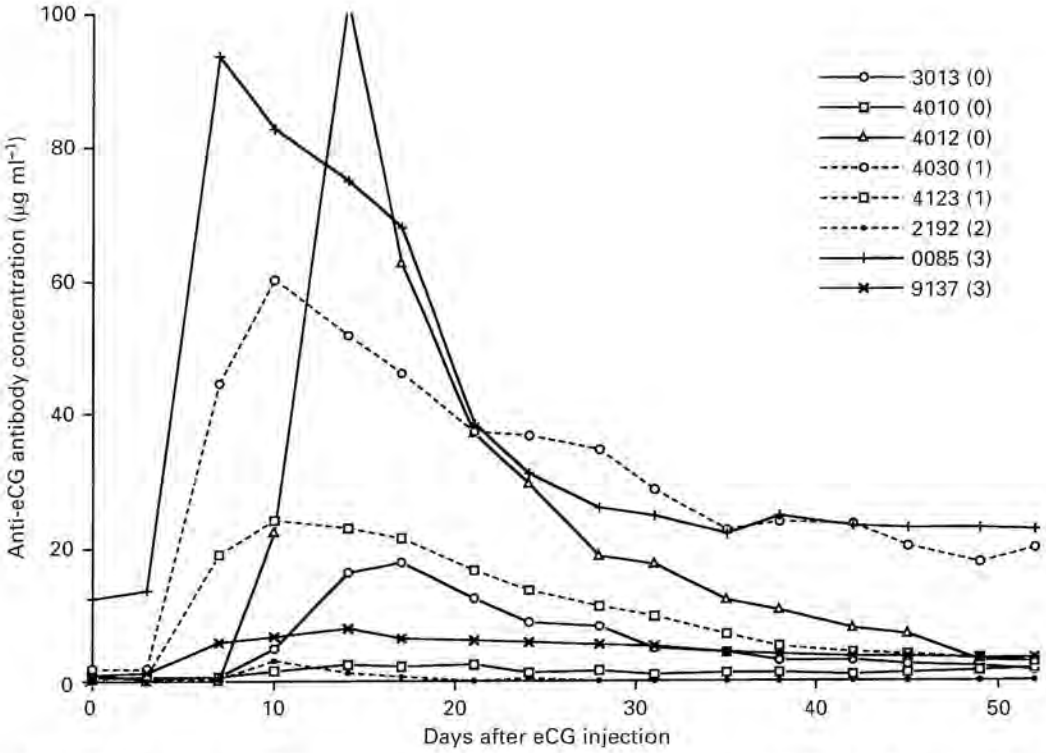

Fig. 5. Evolution of anti-eCG immune response in Alpine goats that received 500 iu of eCG at day 0. The eight goats were considered as representative of the entire group. Anti-eCG antibody concentration was determined by ELISA in plasma samples. Number of previous eCG treatments are indicated in parenthesis. Each point is the mean of duplicate determinations (Adapted from from Roy *et al.*, 1995).

fertility on subsequent treatment for inseminated females, in contrast to antibody concentration measured during the following immune response (at day 10 and day 25).

Fertility of female goats that exhibited oestrous behaviour more than 30 h after sponge removal (representing only 18% of the sample in the previous experiments) is low probably because of their delayed ovulation and because of the use of deep-frozen semen which has a limited lifespan. When these females are artificially inseminated later, adequately with oestrous detection, their fertility was not altered (B. Leboeuf and G. Baril, unpublished). Thus, we recommend artificial insemination only of the females that are detected to be in oestrus 30 h after sponge removal.

Pseudopregnancies

The fertility of goats after artificial insemination can be reduced by pseudopregnancy at the time of induction of oestrus by progestagen or eCG or by other means. Several field trials using ultrasonography have shown that pseudopregnancy appeared in 3–4% of does, sometimes in 20% in some herds (Mialot *et al.*, 1991; Hesselink, 1993; Leboeuf *et al.*, 1994). Pseudopregnancy was related to breed in some trials (Leboeuf *et al.*, 1994) but not in others (Mialot *et al.*, 1991), with reproduction method (3.8% in 1 493 FGA/eCG treated goats versus 2.5% in 3 774 naturally mated goats; Mialot *et al.*, 1991), with sire (20% of 125 daughters from five sires versus 0% of 326 daughters from 12 sires in the same herd; Soulière 1991), with parity (1% of nulliparous versus 18% of primiparous or

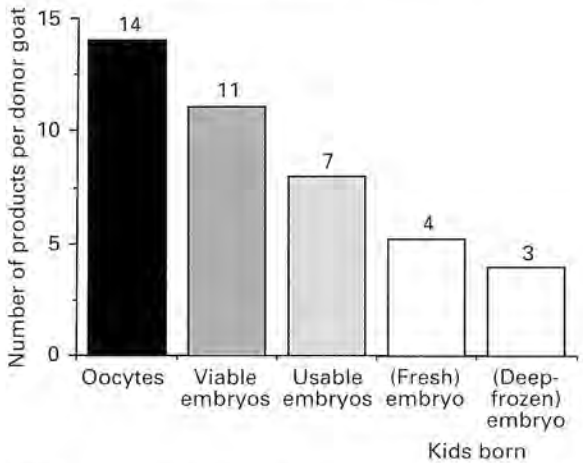

Fig. 6. Number of products per donor female goat at each step of *in vivo* embryo production, collection and transfer, using fresh or deep-frozen embryos (Adapted from Baril, 1995).

multiparous; Hesselink 1993), and with age (10% of 280 does < 5 years old versus 32% of 34 does > 6 years old; Hesselink, 1993).

A recent study perfomed in artifically inseminated dairy goats has demonstrated that more than 50% of the pseudopregnancies identified by ultrasonography at about 45 days after AI followed a late embryonic mortality. Among 67 dairy goats that were diagnosed pseudopregnant 40–60 days after AI, 54% had detectable concentrations of PSPB, a placental hormone, demonstrating the presence of fetal tissue, 30–36 days after AI (Humblot *et al.*, 1995). Thus, at least 50% of so-called pseudopregnancies were the consequence of late embryonic mortality. The reason for this is unknown.

Treatment with a prostaglandin analogue stopped pseudopregnancy (Hesselink 1993), and restored fertility. However, after one or two prostaglandin analogue injections (100 μg), followed 10 days later by progestagen/eCG treatment, the fertility after AI was only 45% (n = 286, Leboeuf *et al.*, 1998).

Embryo Production, Collection, Freezing and Transfer

Embryo transfer is used less frequently in goats than it is in bovine species and is used in goats mainly for the international exchange of genetic material between countries with a concomittant marked reduction in the risk of disease transmission, when international protocols for embryo manipulations are respected. Embryo transfer is also used in transgenic goat programmes to maximize the number of day 3 embryos to be micro-injected (Gootwine *et al.*, 1997).

Donor female goats receive a progestagen treatment ending with gonadotrophic preparation injections to stimulate follicular growth and induce superovulation. The use of FSH is now widely accepted (in fact more or less purified pituitary extracts) rather than eCG to achieve high rates of superovulation. If collection is to be repeated on the same donor females, ovine or caprine FSH (o or cFSH) should be used instead of porcine FSH (pFSH) because of the rapid appearance of antibodies against pFSH which limits the superovulatory response of the females (Remy *et al.*,1991). oFSH can be injected 6–8 times, at intervals of 12 h during the last 3–4 days of the progestagen treatment, with a total dose (for Alpine and Saanen goats) from 16 to 21 mg (standard Armour units). This dose should be adapted to genotype. The use of constant versus decreasing doses may be dependent on the origin of the preparation (Baril, 1995). An FSH:LH ratio increased with 40% LH seems adequate

(Nowshari *et al.*, 1995). On average, the number of ovulations induced by such treatments ranges from 12 to 16 ovulations per goat. However, it should be noted that there is a large variability between females (from 0 to 40, Baril *et al.*, 1995) and that a seasonal effect was described in seasonal breeds (Gootwine *et al.*, 1997).

One of the limitations of these superovulatory treatments comes from the early regression of corpora lutea in 10–35% of the treated females, about 6–8 days after oestrus. The associated decrease in plasma progesterone led to a marked decrease in collection rate (Borque *et al.*, 1993). Even accounting for low body condition score, which could be one of the reasons for such luteal regression, the main causes remain unknown. Use of an antiluteolytic compound or progesterone injections has been described with varying success rates (review Baril,1995).

Successful fertilization of donor females depends on synchronization of ovulation and on the method used to inseminate the females. A reduction in the range in ovulation timing (that is, the time elapsed between the first and the last ovulation) and an increase in ovulation rate was obtained using GnRH injections at a fixed time after the end of the progestagen treatment (Akinlosotu and Wilder, 1993; Krisher *et al.*, 1994). Another alternative is the use of a GnRH antagonist, 12 h after sponge removal, followed by an intravenous injection of 3 mg pLH 24 h later, which mimics the preovulatory LH surge and allows the artificial insemination of the females only once, 16 h after LH injection (Baril *et al.*, 1996a). Natural insemination (mating) can be used satisfactorily (fertility about 80%), but fertility can be reduced during the anoestrous season. If AI with deep-frozen or liquid semen from improved males is used, classic deposition of the semen via the cervix leads to reduced fertilization rates, especially for high ovulation rates. Intra-uterine deposition of the semen after laparoscopy allows the achievement of fertilization rates equivalent to those obtained after natural mating (Vallet *et al.*, 1991).

Embryos can be collected at days 6, 7 and 8 by laparotomy which allows for high collection rates but only once or twice per animal. Collection under laparoscopic control should be used for repetitive collections on the same females (up to 7; Baril 1995). Collection via the cervix should be discounted because penetration into the uterine horns is difficult and collection rates remain low (Soonen *et al.*, 1991; Flores-Foxworth *et al.*, 1992).

Deep freezing of goat embryos is feasible using classic techniques derived from those used in the bovine species. *In vitro* development of frozen–thawed blastocysts was higher than that of frozen morulae whatever the cryoprotectant, glycerol or ethylene glycol, (blastocyst 40.8% $n = 129$, and morula 14.3% $n = 161$; $P < 0.01$). However, *in vivo*, frozen–thawed morulae developed as well as blastocysts did. But for both stages, more embryos developed to term when embryos were frozen with ethylene glycol (51%, $n = 100$), than with glycerol (30%, $n = 83$; $P < 0.08$, Le Gal *et al.*, 1993). Successful vitrification of goat embryos has also been described (Yuswiati and Holtz, 1990). A preliminary result indicated that a similar pregnancy rate was obtained after embryo transfer (ultrasound diagnosis on day 43) for the two methods of cryopreservation, vitrified versus deep-frozen embryos (vitrified 5 pregnancies/7 recipient goats versus deep-frozen 7/8; Traldi *et al.*, 1997).

Transfer should be carried out via laparoscopy which gives equivalent or higher fertilization rates than laparotomy (Baril, 1995) and higher fertility than via the cervix (Flores-Foxworth *et al.*, 1992). Nutrition of recipient goats before and after transfer should be adequate to reach a high fertility (25 versus 67% of kiddings in restricted versus normal-fed Angora goats; Mani *et al.*, 1994).

The number of kids born per donor goat (collected once) varied from three to four, depending on whether embryos were deep frozen or not (Fig. 5; Baril, 1995).

In Vitro Production of Embryos

It is now possible to achieve development to term after transfer of blastocysts produced completely *in vitro* to recipient females(Crozet *et al.*, 1993; Keskintepe *et al.*, 1994). For generation of blastocysts, different steps must be achieved *in vitro*: the maturation of ovarian oocytes (IVM), the capacitation of spermatozoa and fertilization events (IVF) and early cleavage and development to the blastocyst stage in culture (IVC). However, for producing oocytes *in vitro* with full developmental capacity, it is

necessary to select oocytes at the end of their growth phase when they became competent for supporting meiotic maturation and embryonic development. Oocytes from small (2–3 mm diameter) and medium follicles (3.1–5.0 mm diameter) yielded a significantly lower proportion of blastocysts than those from large follicles (> 5 mm diameter) (24 versus 39 versus 53%, respectively; Cognié *et al.*, 1996). Ovulated oocytes, fertilized and cultured *in vitro* under the same conditions, yielded 70% blastocysts (G. Baril, N. Poulin and Y. Cognie, unpublished) indicating that the conditions of maturation (*in vivo* or *in vitro*) may also influence the developmental potential of the oocyte. Important progress has been made regarding the development of the optimal medium for maturation of oocytes which consists of caprine follicular fluid (10%) and FSH (100 ng ml^{-1}) in medium M199 under 5% CO_2 allowing a simplification (omitting co-culture with granulosa cells) and better efficiency of the IVM method (Poulin *et al.*, 1996; Cognié *et al.*, 1996).

The age of the donor female may also influence the quality of the oocyte. Oocytes collected from prepubertal goats demonstrated a lower percentage of normal fertilization after IVM than oocytes from adult goats (Martino *et al.*, 1995).

Collection at the abattoir of oocytes from ovaries by aspiration or dissection of follicles provides 1.5–2.1 oocytes per ovary (Martino *et al.*, 1994; Pawshe *et al.*, 1994). Slicing the goat ovary was found to be more efficient for recovering a large number of cumulus–oocyte complexes (six complexes per ovary; Martino *et al.*, 1994), but the extra oocytes, obtained essentially from small follicles, are less competent to develop after IVF (Keskintepe *et al.*, 1994). Ultimately, these three collection techniques seem to be equivalent in terms of embryo yield (Pawshe *et al.*, 1994).

An average of nine cumulus–oocyte complexes per ovary (including four complexes from follicles larger than 5 mm) can be obtained with FSH-primed goats (Crozet *et al.* 1995). When recovery is to be done on improved females, oocytes can also be collected repeatedly(once a week) by laparoscopic aspiration which allows the recovery after FSH priming of 3– 4 cumulus–oocyte complexes per ovary (Todini *et al.*, 1994; Graff *et al.*,1995, respectively). High fertilization rates (about 85%) are achieved using culture media supplemented with serum from oestrous sheep to induce capacitation in spermatozoa (De Smedt *et al.*, 1992). These conditions are also efficient for frozen semen (Cognié *et al.*, 1992). After discarding polyspermic eggs (10–20%), an average of 70% *in vitro* fertilized eggs can be routinely obtained. Procedures for sperm capacitation and IVF conditions will ensure synchronized sperm–egg penetration and, consequently, the production of synchronous populations of one-cell embryos at the stage required for gene injection in pronuclei, performed 14–18 h after insemination. Heparin was shown to increase sperm–egg penetration when added to IVF medium containing sheep serum (Cox *et al.*, 1994) but the quality of the embryos produced with heparin treatment is questionable (Poulin *et al.*, 1996).

For IV development, culture of early embryos (2- to 4-cell embryos) in the presence of oviduct cells leads to significantly more blastocysts and hatched blastocysts than culture with uterine cells or culture in medium alone (Prichard *et al.*, 1992). With the continued refinement of culture techniques, an alternative system, a simple balanced salt solution (SOF: synthetic oviduct fluid) supplemented with amino acids and serum and incubated under an atmosphere of 5% 0_2, 5% $C0_2$, 90% N_2, is being used. Under these conditions, the developmental ability of blastocysts to term after transfer is close to the developmental rate of their *in vivo* counterparts (61% of *in vitro* produced blastocysts gave birth to live young kids; Poulin *et al.*, 1996). Promising results have now been obtained in survival rate of vitrified–thawed and transferred embryos produced *in vitro* (A. Traldi *et al.*, 1998).

Conclusion

Rapid and significant progress has been made in the control of reproduction in goats, in the study of various treatments applied to animals at farm and AI centres, as well as in the field of *in vitro* treatment of caprine gametes. However, in each of these areas, new research results are needed.

In the study of semen production and processing and of AI, additional progress is needed for improving the efficiency of deep-freezing techniques . The use of BUSgp60 lipase inhibitors in seminal plasma for improving sperm viability in milk-based or egg-yolk diluents should be tested.

One of the major problems to be addressed regarding hormonal control of oestrus is reducing the effects of repeated use of eCG, which reduces the fertility of artificially inseminated females. The reasons for the large inter-individual variability in animal response and the development of new products to be administered to female goats in replacement of eCG are the two main directions that should be followed.

Major advances have been made in the area of *in vivo* embryo production, collection, freezing and transfer, and this is now a technique that can be used for exchanging improved breeds with a reduced risk of disease transmission.

In vitro production of embryos has undergone major and rapid progress in recent years, but significant progress in the yields of the different steps still need to be made, to increase the commercial value of the technique. It is reasonable to expect that we will soon obtain the same yield as for *in vivo* production, but at a lower price than current *in vitro* production costs.

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