Recent advances in the control of goat reproduction

Chemineau P., Baril G., Maurel M.C., Cognie Y., Leboeuf B.

in

Morand-Fehr P. (ed.).
Recent advances in goat research

Zaragoza : CIHEAM
Cahiers Options Méditerranéennes; n. 25

1997
pages 29-37

RECENT ADVANCES IN THE CONTROL OF GOAT REPRODUCTION

P. CHEMINEAU[A1], G. BARIL[A], B. LEBOEUF[B], M.C.MAUREL[A] and Y. COGNIE[A]

[A]*INRA Physiologie de la Reproduction des Mammifères Domestiques 37380 Nouzilly, France*
[B]*INRA Station Experimentale d'Insémination Artificielle 86480 Rouillé, France*

SUMMARY

The control of goat reproduction is interesting for technical reasons (synchronization of kiddings, adjustment to forage availability or to economy), and for genetical reasons (identification and dissemination of improved genotypes). The use of short-light rhythms leads to dramatically improved production of AI doses per buck and the reduction of the period of production by preventing the appearance of a "resting" season. Attempts to improve semen technology had been rare and unsuccessful until the establishment of the hamster oocyte test as a predictor of semen fertility. Light+melatonin treatments allow the induction of out-of-season cyclicity of estrus and ovulation leading to improved fertility. Repeated use of PMSG 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 rates of kids born per donor female, which are compatible with the development of the technique for exchanging genotypes across countries. In vitro production of embryos is the field in which major progress has taken place in the recent years, allowing high development rates to be achieved after in vitro maturation and fertilization of oocytes, and development of embryos.
Keywords: Reproduction, photoperiod, AI, embryos, fertilization

INTRODUCTION

As in other domestic species, the control of goat reproduction offers different advantages at farm level and at the level of a population where genetic progress could be developed. The first advantage is the choice of a kidding period at a precise season 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 the synchronization of kiddings over a limited period of time leading to a reduction in kid mortality, the constitution of homogeneous groups of mothers and kids to be fed more adequately to their requirements, and the optimization of labor in the care of the animals. The third advantage of controlling goat reproduction is that it allows manipulation and storage of the genetic material. Artificial insemination (AI), even used on a small scale, allows links between flocks which 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 spread rapidly in a large number of flocks. Embryo transfer (ET) increases the number of progeny of a genetically improved female and is a good way 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 the present paper we deliberately choose to present only a limited number of techniques which have undergone marked progress in the last five years and were developed in our laboratory. They should be used in intensive systems where the income per goat and per year is very high, generally because of the price of goat milk.

I - SPERM PRODUCTION AND PROCESSING

The application of photoperiodic treatments to bucks of seasonal breeds alleviates the problem seasonality of sperm production. Initially developed in rams, short light rhythms (i.e. alternations between 1 or 2 months long days (16 h light: 8 h darkness; 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 during 3 consecutive years to photoperiodic treatments presented a dramatic increase in all parameters of sperm production, compared to control bucks subjected to natural photoperiodic changes. When collected twice weekly, the total number of sperm produced was improved by 61% (Delgadillo *et al.* 1991). Quality of semen after deep-freezing no longer exhibited the dramatic seasonal changes observed in untreated bucks. The total number of AI doses produced during the two first years of the treatment was much higher (62%) than that

[1] Correspondance: fax 33 47 42 77 43, email: Philippe.Chemineau@tours.inra.fr

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).

After completion of this large experiment, it was also apparent that the collection rate (twice weekly) could be increased in treated males. It was therefore decided to compare the overall sperm production of treated bucks collected four times a week all year round to 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 application, as expected, testicular weight of bucks was maintained constant, at the maximal level of the full sexual season, while testicular weight of control males underwent the normal seasonal variations observed earlier (Figure 1).

As a consequence, sperm production either in terms of total number of spermatozoa produced, or in terms of AI doses, was dramatically improved by the treatment (2 212 vs 3 111 doses per buck). Fertility of AI doses was slightly, although non significantly, lower for light-treated bucks (Leboeuf and Chemineau, unpublished results).

Such a high production probably originates from unexpected changes in spermatogenic processes. Light-treated bucks had significantly increased numbers of spermatogonia (the stem cell of the spermatogenic line) while maintaining the efficiency of their spermatogenic divisions at the high rate of the full sexual season (Delgadillo *et al.* 1995). Such photoperiodic treatments, by allowing sperm collection all the year round instead of six months out of 12, may accelerate the constitution of a stock 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 scheme of selection.

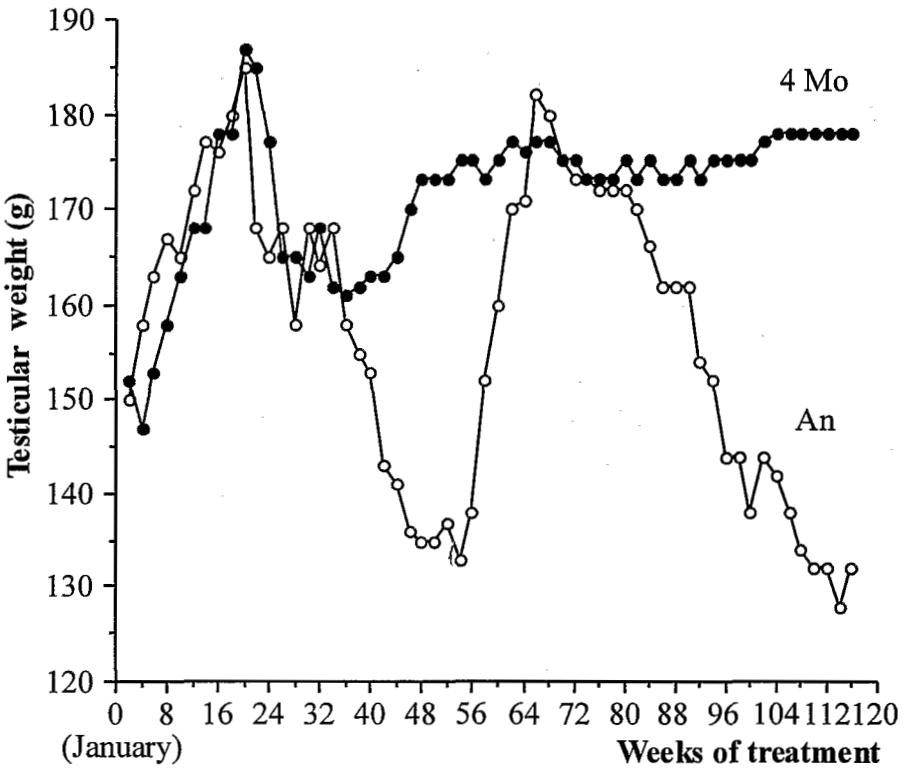

Figure 1. Testicular weight of Alpine and Saanen bucks treated or not with an accelerated light rhythm of 4 Mo period. Monthly mean ± SEM. An: natural photoperiodic variations at 46° N latitude (open circles, N=6). 4Mo: alternation between two months of long days (16L:8D) and two months of short days (8L:16D)(closed circles, N=6) (From Leboeuf and Chemineau, unpublished)

In the field of semen technology, attempts were made to freeze-thaw Cashmere buck semen in pellet form at high densities (1:0.5 to 1:2; semen:diluent). However, under these conditions, fertility after cervical AI remained low when compared to that obtained after laparoscopic AI of 1:23 diluted semen (33 vs 43% of kiddings; Ritar and Ball 1993). By contrasts, an interesting set of data was obtained in the area of the establishement of in vitro predictors of semen fertility. Evaluating relative fertility of cryopreserved goat

sperm by using heterospermic AI, Berger *et al.* (1994) have determined that the ability of spermatozoa to fuse with zona-free hamster ova after one hour of capacitation was highly correlated with their relative in vivo fertility (R=0.88). Acrosomal integrity, ability of spermatozoa to undergo an acrosomic reaction, and "classical" parameters of sperm motility were not correlated with differences in relative fertility.

II - INDUCTION OF OUT-OF-SEASON CYCLICITY WITH PHOTOPERIODIC TREATMENTS

Appropriate melatonin treatments to animals could be used to mimic short days while their visual system perceived long days (Chemineau *et al.* 1992, Malpaux et al., 1993; Deveson *et al.* 1992; von Brackel-Bodenhausen *et al.* 1994), in order to induce an advance of ovulatory and estrous 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 (i.e. from April to July). Under such conditions, melatonin treatment should be preceeded by at least 2 months of a light treatment composed daily either of real long days (Deveson *et al.* 1992a), or of 2 periods of supplementary light (Figure 2; 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" induces ovulatory and estrus activities that are sufficient to achieve a fertility and prolificacy close to those of the normal annual breeding season.

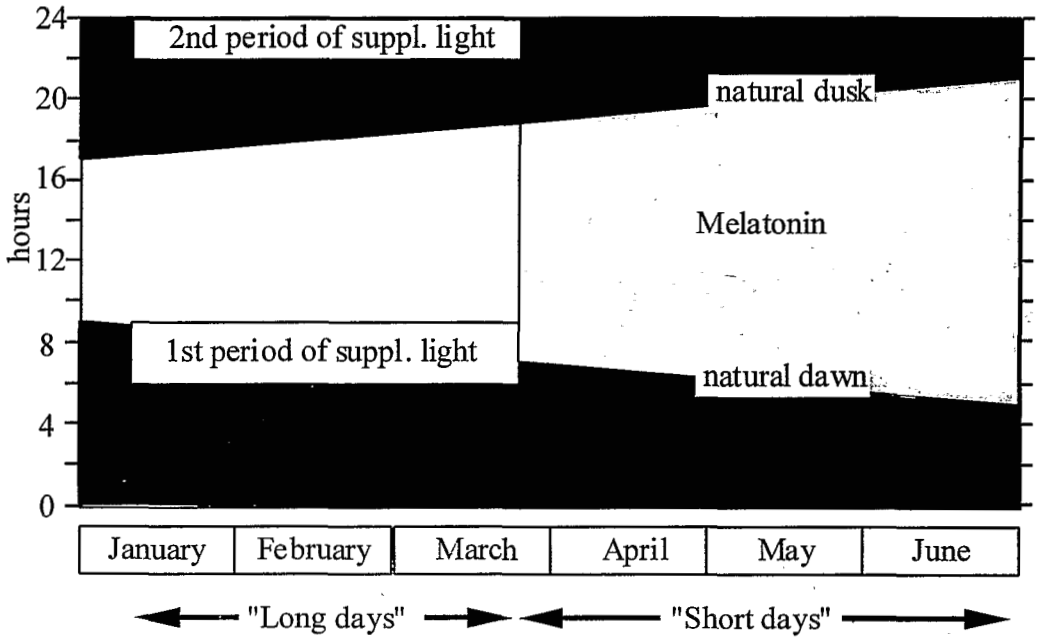

Figure 2. "Photoperiodic" treatments to be applied in open barns and using the succession of "long days" and melatonin treatment followed by a"buck-effect"

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

III - HORMONAL SYNCHRONISATION OF ESTRUS

Hormonal treatment of female goats to induce a synchronous onset of estrous behaviour and ovulation within a limited duration of hours after the end of the treatment is a prerequisite to the use of AI. The association between a progestagen (delivered by a vaginal sponge or by a subcutaneous implant; Bretzlaff *et al.* 1992), a prostaglandin analogue and PMSG (now called equine Chorionic Gonadotrophin, eCG) remains

the most efficient tool to achieve such an objective. These treatments are now widely and successfully used all over the world to control female goat reproduction.

Paradoxically, recent studies using this technique have involved trying to better understand the reasons for its decreased efficiency when PMSG is used repeatedly on the same females.

In a single Saanen flock of 169 females where breeding takes place each year out of season after fluorogestone acetate (FGA) and PMSG treatment, the percentage of goats showing estrus and kidding was significantly lower for multiparous than for nulli- and primiparous goats (64 vs 99 and 34 vs 67%, respectively). When goats were treated for the second time during the same year, the percentage showing estrus was lower than after the first treatment (45 vs 71%; Baril *et al.* 1992). This situation is due to the appearance of antibodies against PMSG (Roy *et al.* 1995). PMSG binding of the serum was calculated by RIA method, and expressed as percentage of bound radioactive PMSG with plasma (Baril *et al.* 1992). Before the treatment, it was higher in multiparous than in nulli- and primiparous goats (18 vs <1%), and higher in non-pregnant than in pregnant goats (26 vs 7%) (Baril *et al.* 1992). Such drastic results obtained in a single flock have prompted large-scale surveys in private flocks, using FGA/PMSG treatments, associated with "classical" AI with deep-frozen semen. In the first survey, estrous behaviour was induced in almost all treated goats (98.1% of the 368 Alpines and 272 Saanens goats of 19 private flocks) between 24 and 72 hours after sponge removal. The distribution of the onset of estrus 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 dramatically increase after the second treatment (Figure 3). Fertility but not prolificacy after AI was negatively correlated to the interval between sponge removal and onset of estrus (R=0.92). Fertility of goats which came into estrus later than 30 hours after sponge removal was significantly lower than those which were first observed in estrus 24 or 30 hours after sponge removal (33 vs 65% respectively, Figure 3; Baril *et al.* 1993). This delay in the onset of estrous behaviour is associated with a delay in the LH preovulatory surge (Maurel *et al.* 1992) and in a delay in the time of ovulation (Leboeuf *et al.* 1993, Leboeuf *et al.* 1996). In the second survey, PMSG binding (measured in 524 dairy goats of 17 private flocks) before the onset of treatment was significantly lower in flocks where 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 which had previously received from 2 to 5 treatments, compared to those of females which had received only 0 or 1 treatments (3 vs 10%). On an individual basis, the percentage of goats showing onset of estrus behaviour more than 30 h after sponge removal was higher (38 vs 7%) and fertility was decreased (51 vs 66% on 166 vs 353 females) when PMSG binding was higher (10% vs <5%). When measured 25 days after PMSG injection, the level of PMSG binding was increased (7% before injection vs 28% after injection), and correlated with binding level detected before treatment (Baril *et al.* 1995b).

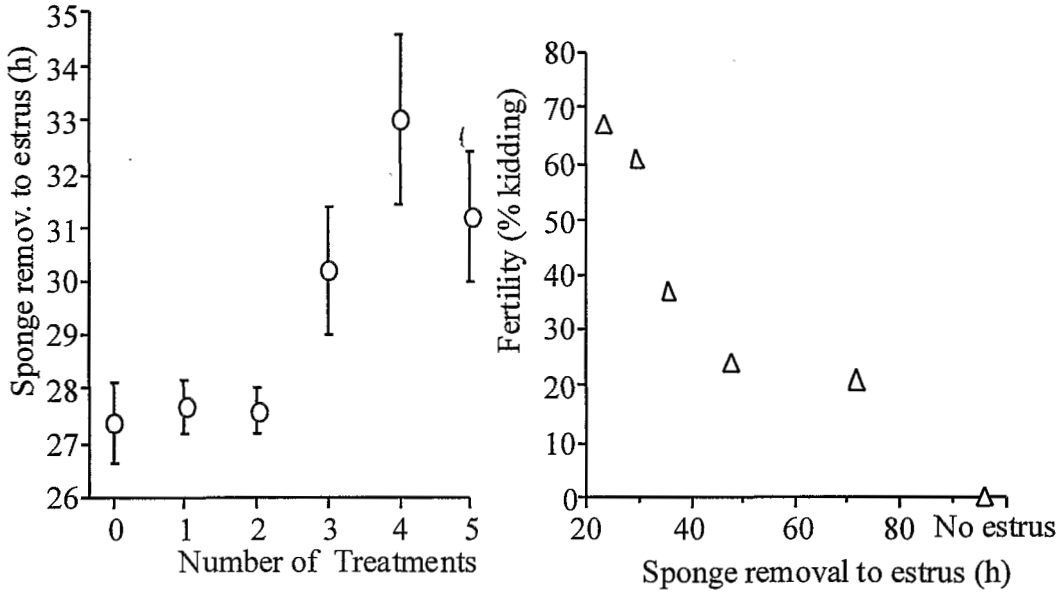

Figure 3. Relationship between the interval "sponge removal to onset of estrus" and the number of treatments previously received by the female goat (left graph) and between the interval "sponge removal to onset of estrus" and fertility (right graph) (from Baril *et al.*, 1993)

Fertility of female goats which exhibited estrous 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 of the use of deep-frozen semen which has a limited life-time. When these females are artificially inseminated (later on), their fertility is not altered (Leboeuf *et al.* 1995, unpublished results).

Antibodies against PMSG are not transmitted to the kids before birth via the placenta, but are transmitted after birth by the colostrum, then absorbed in the plasma of kids. They are detected 2 days after colostrum consumption for several weeks (Beckers *et al.* 1995). It would be interesting to know if this consumption of antibodies may modify the future immune response of adult goats.

In the first experiments performed to synchronise estrus and artifically inseminate nulliparous goats, the fertility rate was equivalent to those of primi and multiparous goats. For some years now, fertility rates have decreased to about 50% (Leboeuf 1993). Breed, age and body weight at sponge insertion appeared as the main reasons for fertility variations. Fertility was lower in Saanen than in Alpine does (33.4% in 134 goats vs 58.8% in 211 goats), and for animals less than 240 days old and 32 kg body weight (44% in 277 goats vs 68% in 68 goats; Corteel *et al.* 1993). Fertility was lower in Angora than in Cashmere goats (19% in 91 goats vs 57% in 27 goats). A lower proportion of 8 month-old than 20 month-old does kidded following A.I. (36% in 36 goats vs 68% in 38 goats). For both genotypes, the body weight of non-pregnant goats after A.I. followed by mating was lower than that of pregnant does (Ritar *et al.* 1994).

The fertility of goats after artificial insemination can be penalized by pseudopregnancy at the time of induction of estrus by progestagen/PMSG or by other means. Several field trials using ultrasound echography have shown that pseudopregnancy appeared in 3-4% of does, sometimes 20% in some breeds (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/PMSG treated goats vs 2.5% in 3 774 naturally mated goats; Mialot *et al.* 1991), with sire (20% of 125 daughters from 5 sires vs 0% of 326 daughters from 12 sires in the same flock; Soulière 1991), with parity (1% of nulliparous vs 18% of primi or multiparous; Hesselink 1993), and with age (10% of 280 less than 5 years old does vs 32% of 34 does more than 6 years old; Hesselink 1993).

Treatment with prostaglandin analogue stopped pseudopregnancy (Hesselink 1993), and restored fertility. After 1 or 2 prostaglandin analogue injection (100μg), followed 10 days later by progestagen/PMSG treatment, the fertility following A.I. was 56% (n=97, Leboeuf *et al.* 1994). This fertility was not different from unaffected does, but a recent study showed that fertility after A.I. under the same conditions was only 45% (n=286, Leboeuf *et al.* unpublished).

IV - EMBRYO PRODUCTION, COLLECTION, FREEZING AND TRANSFER

Less used than in the bovine species, goat embryo transfer (ET) is now mainly used for the international exchange of genetic material across the countries with a concomittant dramatic reduction in the risk of disease transmission, in so much that international rules for embryo manipulations are respected (Wrathall 1995).

Donor female goats received a progestagen treatment at the end of which gonadotrophic preparations were injected to stimulate follicular growth and induce superovulation. The use of FSH is now widely accepted (in fact more or less purified pituitary extracts) in place of PMSG to achieve high rates of superovulation. If collection should be repeated on the same donor females, FSH of ovine or caprine origin (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. oFSH can be injected 6 to 8 times, at 12 h intervals 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 the genotype and the use of constant vs decreasing doses may be dependant on the origin of the preparation (Baril 1995). An FSH/LH ratio elevated 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 a large variability exists between females (from 0 to 40, Brebion *et al.* 1992).

One of the limitations of these superovulatory treatments comes from the early regression of corpora lutea in 10 to 35% of the treated females, about 6-8 days after estrus. The associated decrease in plasma progesterone led to a dramatic decrease in collection rate (Borque *et al.* 1993). Even if low body condition score 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 in the literature with varying success rates (Baril 1995).

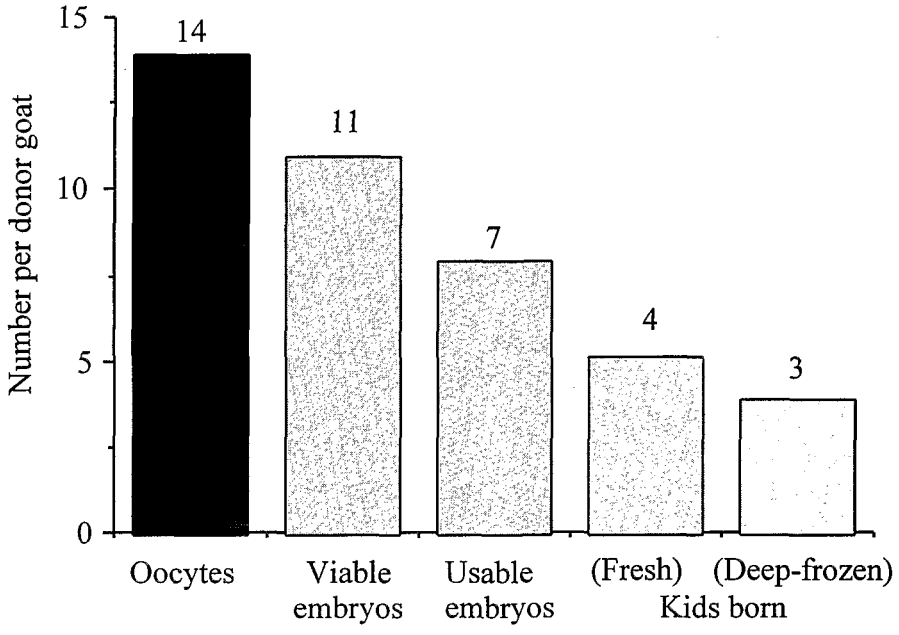

Figure 4. Number of products per donor female at each step of embryo production, collection and transfer, using fresh or deep-frozen embryos (adapted from Brebion *et al.*, 1992)

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 (i.e. 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 *et al.* 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 of pLH 24 hours later, which mimics preovulatory LH surge and allows the artificial insemination of the females only once, 16 h after LH injection (Baril *et al.* 1995a). Natural insemination (i.e. mating) can be used satisfactorally (fertility about 80%), but fertility can be reduced during the anestrus season. If AI with deep-frozen or liquid semen from genetically improved males is to be used, "classical" deposition of the semen via 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 6-7-8 by laparotomy which allows high collection rates but only once or twice. 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 discarded 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 "classical" techniques derived from those used in the bovine species. Ethylene-glycol appears to be a better cryoprotectant than glycerol (35 vs 22% of kids born relative to embryos thawed) and blastocysts better support freeze-thawing processes than morulae, irrespective of the cryoprotectant (40 vs 14%; Le Gal *et al.* 1993; Fiéni *et al.* 1995). Successful vitrification of goat embryos has also been described (Yusiwiati and Holtz 1994).

Transfer should be carried out via laparoscopy which gives equivalent or higher fertilization rates than laparotomy (Brebion *et al.* 1992) 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 vs 67% of kiddings in restricted vs normal-fed Angora goats; Mani *et al.* 1994).

As a whole, the number of kids born per donor goat (collected once) varied from 3 to 4, depending on whether embryos were deep frozen or not (Figure 4; Brebion *et al.* 1992).

V - IN VITRO PRODUCTION OF EMBRYOS

It is now possible to achieve development to term after transfer to recipient females, of blastocysts produced completely *in vitro* (Crozet *et al.* 1993; Keskintepe *et al.* 1994). To generate blastocysts, different steps must be achieved *in vitro* : the maturation of ovarian oocytes (IVM), the capacitation of spermatozoa and fertilization events (IVF) and early cleavages and development to the blastocyst stage in culture (IVC). However, for producing *in vitro* oocytes 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 and medium follicles (<5 mm diameter) yielded a significantly lower proportion of blastocysts than those from large follicles (>5 mm diameter) (9 vs 26% respectively; Crozet *et al.* 1995). Ovulated oocytes fertilized and cultured *in vitro* under the same conditions, yielded 41% blastocysts 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) in medium M199 under 5% CO2 allowing a simplification (omitting co-culture with granulosa cells) and better efficiency of the IVM method (Poulin *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 of oocytes from slaughterhouse ovaries by aspiration or dissection of follicles provides 1.5 to 2.1 oocytes per ovary (Martino *et al.* 1994; Pawshe *et al.* 1994). Slicing of the goat ovary was found to be a more efficient tool for recovering a high number of cumulus-oocyte complexes (6 coc/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 seems to be equivalent in term of embryo yield (Pawshe *et al.* 1994).

An average of 9 coc per ovary (including 4 coc from follicles larger than 5 mm) can be obtained with FSH-primed goats (Crozet *et al.* 1995). When recovery is to be done on genetically improved female, oocytes can be also collected by laparoscopic aspiration which allows the recovery after FSH priming of 3 to 4 coc per ovary (Todini *et al.* 1994; Graff *et al.* 1995, respectively). High fertilization rates (around 85%) are achieved using culture media supplemented with estrus sheep serum to induce capacitation in spermatozoa (De Smedt *et al.* 1992). These conditions are also efficient for frozen semen (Cognié *et al.* 1992). Heparin was shown to increase sperm-egg penetration when added to IVF medium containing sheep serum (Cox 1994) but the quality of the embryos produced with heparin treatment is questionable (Poulin *et al.* 1996).

For IVD, culture of early embryos (2- to 4-cell embryo) 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). Today, with the continued refinement of culture techniques, an alternative system is being used and comprises of a simple balanced salt solution (SOF: synthetic oviduct fluid) supplemented with amino acids and serum and incubated under an atmosphere of 5% 02, 5% C02, 90% N2. Under these conditions, the developmental ability to term after transfer of blastocysts 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).

CONCLUSION

Significant progress has been made in the last five years regarding the control of goat reproduction. In the field of semen production and processing and of AI, progress remains to be done for improving the efficiency of deep-freezing techniques. The prediction of fertility using the hamster zona-free oocyte test should be confirmed on a larger number of males and a larger number of artificially inseminated female goats. One of the major problems to be addressed regarding hormonal estrous control is to try to reduce the effects of repeated use of PMSG which reduce the fertility of artificially inseminated females. Major advances have been made in the area of in vivo embryo production, collection, freezing and transfer, which is now a technique which can be used for exchanging genotypes with a reduced risk of disease transmission. In vitro production of embryos has also known major and very rapid progress in recent years and it is reasonable to expect that we will soon obtain the same yield as for in vivo production, but at a lower price.

ACKNOWLEDGEMENTS

A part of the experiments presented here were supported by grants from Région Centre and Région Poitou-Charentes and by grants of the French Ministry of Agriculture and of SANOFI Santé Nutrition Animale. Authors want to thank P. Lonergan for help in the english version of this manuscript.

REFERENCES

AKINLOSOTU, B.A. and WILDER, C.D. (1993). *Theriogenology* **40**: 895-904.

BARIL, G. (1995). Proceedings of XI Congreso Brasileiro de Reproduçao Animal, Belo Horizonte, pp. 110-20.

BARIL, G., LEBOEUF, B. and SAUMANDE, J. (1993). *Theriogenology* **40**: 621-8.

BARIL, G., POUGNARD, J.L., FREITAS, V.J.F., LEBOEUF, B. and SAUMANDE, J. (1995a). *Theriogenology* (accepted for publication).

BARIL, G., REMY, B., LEBOEUF, B., BECKERS J.F. and SAUMANDE, J. (1995b). *Theriogenology* (accepted for publication).

BARIL, G., REMY, B., VALLET, J.C. and BECKERS, J.F. (1992). *Reprod. Dom. Anim.* **27**: 161-8.

BECKERS, J.F., REMY, B., BARIL, G., FIGUEREIDO, J.R., BUREAU, F., SULON, J. and SAUMANDE, J. (1995). *Theriogenology* **43**: 165 (Abstract).

BERGER, T., DROBNIS, E.Z., FOLEY, L., METZLER, J.K. and HORTON, M. (1994). *Theriogenology* **41**: 711-7.

BORQUE, C., PINTADO, B., PEREZ, B., GUTIERREZ, A., MONOZ, I. and MATEOS, E. (1993). *Theriogenology* **39**: 192 (Abstract).

BREBION, P., BARIL, G., COGNIE, Y. and VALLET, J.C. (1992). *Ann. Zootech.* **41**: 331-9.

BRETZLAFF, K.N., NUTI, L., ELMORE, R.G., MEYERS, S;A., RUGILA, J.N., BRINSKO, S.P., BLANCHARD, T.L. and WESTON, P. G. (1992). *Am. J. Vet. Res.* **53**(6): 930-4.

CHEMINEAU, P., (1992). Proceedings of the 5th Int. Conf. on goats, New Delhi, Vol. II, Part II, 355-368.

CHEMINEAU, P., MALPAUX, B., DELGADILLO, J.A., GUERIN, Y., RAVAULT, J.-P., THIMONIER, J. and PELLETIER J., (1992). *Anim. Reprod. Sci.* **30**: 157-184.

COGNIE, Y., GUERIN, Y., POULIN, N., CROZET, N. (1992). Proceedings of the 5th Int. Conf. on goats, New Delhi, Vol. III, 1248-1252.

CORTEEL, J.M., LEBOEUF, B., BROQUA, B. (1993). *Elevage et Insemination* **255**: 1-8.

COX, J.F., AVILA, J., SARAVIA, F., SANTA MARIA, A. (1994). *Theriogenology* **41**: 1621-1629.

CROZET, N., AHMED-ALI, M. and DUBOS, M.P. (1995). *J. Reprod. Fertil.* **103**: 293-8.

CROZET, N., DE SMEDT, V., AHMED-ALI, M. and SEVELLEC, C. (1993). *Theriogenology* **39**: 206 (Abstract).

DE SMEDT, V., CROZET, N., AHMED-ALI, M., MARTINO, A., COGNIE, Y. (1992). *Theriogenology* **37**: 1049-1060.

DELGADILLO, J.A., HOCHEREAU-DE-REVIERS, M.T., DAVEAU, A. and CHEMINEAU, P. (1995). *Reprod. Nutr. Dev* **35**(1): (in press).

DELGADILLO, J.A., LEBOEUF, B. and CHEMINEAU, P. (1991). *Theriogenology* **36**: 755-70.

DELGADILLO, J.A., LEBOEUF, B. and CHEMINEAU, P. (1992). *Small Rumin. Res.* **9**: **47-59**.

DEVESON, S., FORSYTH, I. A. and ARENDT, J. (1992a). *Anim. Reprod. Sci.* **29**: 1-5.

DEVESON, S., FORSYTH, I.A. and ARENDT, J. (1992b). *J. Reprod. Fertil.* **95**: 629-37.

FIENI, F., BECKERS, J.F., BUGGIN, M., BRUYAS, J.F., PERRIN, J., DAUBIE, M. and TAINTURIER, D. (1995). *Reprod. Nutr. Dev.* **35**: 367-73.

FLORES-FOXWORTH, G., McBRIDE, B.M., KRAEMER, D.C. and NUTI, L.C. (1992). *Theriogenology* **37(1)**: 213 (Abstract).

GEBBIE, F. (1993). Ph.D. Thesis, University of Surrey, UK, pp. 205.

GRAFF, K.J., MEINTJES, M., PAUL, J.B., DYER, V.W., DENNISTON, R.S., ZIOMEK, C. and GODKE, R.A. (1995). *Theriogenology* **43**: 223 (Abstract).

HESSELINK, J.W.(1993). *Veterinary Record* **30**: 110-112.

KESKINTEPE, L., DARWISH, GM., KENIMER, A.T. and BRACKETT, BG. (1994) *Theriogenology* **42**: 527-35.

KRISHER, R.L., GWAZDAUSKAS, F.C., PAGE, R.L., RUSSEL, C.G., CANESECO, R.S., SPARKS, A.E.T., VELANDER, W.H., JOHNSON, J.L. and PEARSON, R.E. (1994). *Theriogenology* **41**: 491-8.

LE GAL, F., BARIL, G., VALLET, J.C. and LEBOEUF, B. (1993). *Theriogenology* **40**: 771-7.

LEBOEUF, B. (1993). *La Chèvre* **195**: 13-16.

LEBOEUF, B., BERNELAS, D., POUGNARD, J.L., BARIL, G., MAUREL, M.C., BOUE, P. and TERQUI, M. (1993). Proceedings 9th A.E.T.E. Meeting, Lyon, p. 226 (Abstract).

LEBOEUF, B., BARIL, G., MAUREL, M.C., BERNELAS, D., MARCHETEAU, J., BERSON, Y., BROQUA, B. and TERQUI, M. (1996) Proceedings 6th Int. Conf. on Goats, 5-11 May Bejing, China (this volume).

LEBOEUF, B., RENAUD, G., DE FONTAUBERT, Y., BROQUA, B., CHEMINEAU, P. (1994). Proceedings 7th Intern. Meeting on Animal Reprod. MURCIA, 6-9 July . pp 251-255.

MALPAUX, B., CHEMINEAU, P. and PELLETIER J. (1993). In "Melatonin, biosynthesis, physiological effects and clinical applications" (Eds H.S. Yu and R.J. Reiter) pp. 253-287 (CRC Press: Boca Raton).

MANI, A.U., WATSON, E.D. and McKELVEY, W.A.C. (1994). *Theriogenology* **41**: 1673-8.

MARTINO, A., MOGAS, T., PALOMO, M.J. and PARAMIO, M.T. (1995). *Theriogenology* **43**: 473-85.

MARTINO, A., PALOMO, M.J., MOGAS, T. and PARAMIO, M.T. (1994). *Theriogenology* **42**: 859-73.

MAUREL, M.C., LEBOEUF, B., BARIL, G. and BERNELAS, D. (1992). Proceedings 8th A.E.T.E. Meeting, Lyon, p. 186 (Abstract).

MIALOT, J.P., SABOUREAU, L., GUERAUD, J.M., PRENGERE, E., PARIZOT, D., PIROT, G., DUQUESNEL, R.,PETAT, M., CHEMINEAU, P. (1991). *Rec. Med. Vet., Spec. Reprod. Rum.* **1** : 383-390.

NOWSHARI, M.A., BECKERS, J.F. and HOLTZ, W. (1995). *Theriogenology* **43**: 797-802.

PAWSHE, C.H., TOTEY, S.M. and JAIN, S.K. (1994). *Theriogenology* **42**: 117-25.

POULIN, N., GULER A., PIGNON, P. and COGNIE, Y. (1996). Proceedings 6th Int. Conf. on Goats, Bejing 5-11 May, International Academic Publishers, Beijing, 838-340.

PRICHARD, J.F., THIBODEAUX, J.K., POOL, S.H., BLACKEWOOD, E.G., MENEZO, Y. and GODKE, R.A. (1992). *Hum. Reprod.* 7(4): 553-7

RITAR, A.J. and BALL, P.D. (1993). *Anim. Reprod. Sci.* **31**: 249-62.

RITAR, A.J., ROBERTSON, J.A., EVANS, G. (1994). *Reprod. Fert. Dev.* **6**: 737-747.

ROY, F., MAUREL, M.C., BRIOIS, J.P., COMBARNOUS, Y. and DELETANG, F. (1996). Renc. Rech. Ruminants **2**, INRA-IE Paris, 395-398.

SOONEN, A.H., LEWALSKI, S., MEINECKE-TILMAN, S. and MEINECKE, B. (1991). Proceedings 7th Sci. Meet. Europ. Emb. Trans. Assoc. Cambridge, **1**: 208 (Abstract).

SOULIERE, I., (1991). Mémoire de fin d'Etudes. Ecole Nationale Supérieure Féminine d'Agronomie. Rennes, 28pp.

TODINI, L., COGNIE, Y., POULIN, N., GUERIN, Y. (1994). Proceedings Congresso SIPAOC 331-334.

VALLET, J.C., CASAMITJANA, P., BREBION, P. and PERRIN, J. (1991). *Rec. Med. Vet., Spec. Reprod. Rum.* **167**: 293-301.

VON BRACKEL-BODENHAUSEN, A., WUTTKE, W. and HOLTZ, W. (1994). *J. Anim. Sci.* **72**: 955-62.

WRATHALL, A.E. (1995). *Theriogenology* **43**: 81-8.

YUSIWIATI, E. and HOLTZ, W. (1994). *Theriogenology* **34**: 629 (Abstract).