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Economical and technical consequences on French breeding schemes of a possible ban of hormones for sheep and goat reproduction

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INSEMINATION AND HORMONAL TREATMENT WERE SET UP TOGETHER IN SMALL RUMINANTS

The research on animal insemination (AI) started during the 1940s in France on bovine, ovine and porcine species and on caprine species about 10 years later (Bougler 1984). The use of animal insemination on farm followed rapidly for cattle, but it took much more time for small ruminants, due to technical difficulties and cost, in relation with the lower value of goat and sheep products.

For small ruminants, the difficulties to develop insemination came mainly from semen preservation and from the detection of natural oestrus by a "teasing" male to determine the right moment to inseminate, what was found too much a constraint. The first interesting results on semen preservation were obtained in the 1960s, but only few hundreds of females were inseminated on natural oestrus in this period.

The development of AI on a large scale started in the 1970s with the adjustment of an hormonal treatment to induce and synchronise the oestrus and ovulation of females. Thus the detection of oestrus by a male wasn't required any more and it became possible to inseminate a group of females at the same time, and therefore to reduce the cost of insemination.

An other problem is that most French sheep and goat breeds have a seasonal reproductive period, during the second semester of the year when day time is decreasing. For this reason, hormonal treatments were particularly appreciated to control the reproduction of females out of the mating season, in order to adapt milk and meat production to the demand of consumers. In these years, AI was performed with fresh semen within an interval less than 24 h after collection time, what allowed to fertilize a number of animals.

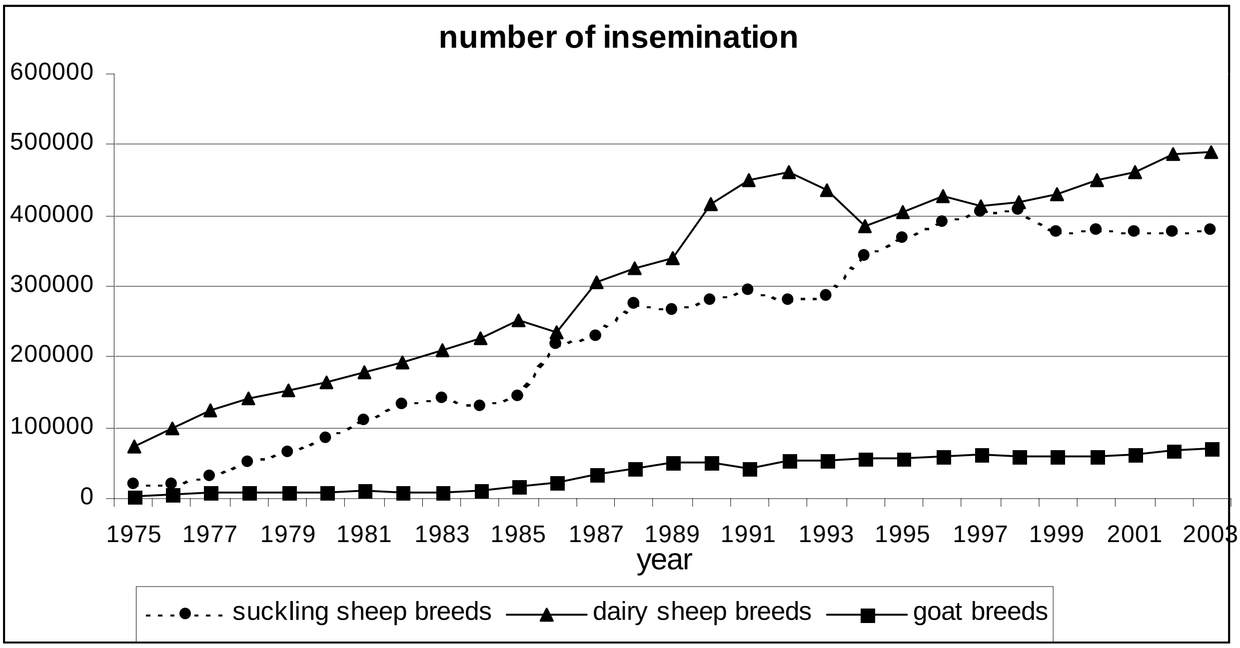
Besides its ability to induce a reproductive cycle, it appeared that the hormonal treatment induced an increase by 20 to 40 % of prolificacy of ewes and around by 10 to 15% of goats (Barillet et al., 1984). This effect has few interest for goat breeding because the kid has a poor value. On the contrary, it is particularly interesting for sheep breeders in relation with the lamb market and even for milk producers, because the lambs still have a great part in their income.

Insemination associated with hormonal treatment allows the emergence of selection programs

Genetic improvement is based on the possibility for the best reproducers to have more offspring than the others, but a special technique is needed to multiply the reproductive ability of the selected animals. Insemination is the major tool for realizing this objective. In cattle it is possible to get several tens of thousands doses of frozen semen for a bull. However for rams and bucks, the results are much lower. In sheep, the technique is based on fresh semen, which has a conservation ability for some hours only. So the semen production of a ram is about 400 -

800 doses per year, according to the length of the collection period, which is limited to the few months when females need to be inseminated in the farming system. For bucks, the fertility results with frozen semen are quite good (around 60 %) so the collection period may be longer than for rams. Their semen production reached 800-1000 doses when they produced during the whole mating season only ; it has increased to 2000 doses per year as soon as a photoperiodic treatment allowed semen collection all the year long.

Thus, during the 1970s the development of insemination, associated with hormonal treatments for out of season and synchronised reproduction allowed to start selection programmes for several ovine and caprine breeds. The development of insemination in each of the 3 production contexts of small ruminants is shown in figure below. It must be compared to the number of animals in the whole country : about 850 000 goats, 7 million, decreasing to 5 million suckling ewes during the period, 1 million, increasing to 1.4 million dairy ewes during the period.



In 1970, the breeders of the milked Lacaune sheep organised the first breeding scheme to become effective with more than 50 000 inseminations. Insemination has developed rapidly because the hormonal treatment has allowed them to organise the lambing period according to the collect of milk by the factories, what had an obvious economic interest.

As regards suckling breeds, some of them have developed AI for crossing, mainly with Charollais or Texel rams to produce lambs out of sexual season, whereas the others set up genetic improvement schemes using AI (Suckling Lacaune, Ile de France, Berrichon du Cher breeds), sometimes in association with a zootechnical purpose. In 1980, 10 breeds used AI (more than 1 000 IA/breed and per year). To date, 16 breeds are concerned. In an almost constant way, for the 1975 - 2002 period, 50 % of the inseminations were carried out to produce meat lambs out of season.

As insemination was late to develop in goats, the selection programme started with progeny-testing in a single experimental farm where the daughters of about 15 bucks were bred together in order to evaluate the genetic merit of the sires. The semen of the selected sires was then collected, but few herds used insemination at that time. The selection scheme for Alpine and Saanen became more effective during the 1980s, when the number of inseminations on farm passed beyond 20 000.

DURING THE YEARS 1980 –1995 INSEMINATION ASSOCIATED WITH HORMONAL TREATMENT BECAME A USUAL TECHNIQUE

Hormonal treatments along with natural mating

For long, milk or meat products have got a better price when they were marketed out of season, so that breeders decided to use hormonal treatment to make their animals reproduce by natural mating out of season, without any purpose of genetic improvement. Information about this technique is not easily available, but we assess it could still concern in France at least 500 000 ewes and 50 000 goats, perhaps more (Baril et Saumande, 2000). But as the hormonal treatment concerns exclusively the female, it could be a problem for the males to serve the females, what was an incitement to use insemination.

Controlling oestrus and ovulation to inseminate

To obtain a high level of fertility, the insemination have to be performed in the second part of oestrus, few hours before the time of ovulation. That situation leads to synchronizing the time of oestrus and ovulation of cyclical females in sexual season or not cyclical females out of breeding season, in order to inseminate at the right moment.

The hormonal treatment generally used to induce oestrus and ovulation in sheep and goats is based on a combination of a progestagen treatment followed by an injection of PMSG, and may include a treatment with commercial prostaglandin analogues (goats only). Treated goats are inseminated once with 100 millions frozen_thawed spermatozoa 43 h after sponge removal. In sheep the progesterone is administrated during 10 to 14 days, then PMSG is administrated at the same time as sponge is removed and AI is performed 55 h later.

Insemination practice is regulated

Insemination has developed within the frame of the 1966 French Breeding Law.

Specialized breeding centres for males have been built for semen production. They are submitted to special regulations concerning genetic and sanitary aspects. Males have a sanitary check up during quarantine when they enter the centre. If they pass the sanitary tests they go on with the genetic programme set up for the breed, with an annual sanitary control. The sanitary security of semen is therefore an argument in favour of insemination practice.

For technical and economical reasons, it’s not possible to inseminate more than once females that haven’t been fertilized at the induced cycle. So it is still necessary to keep males in flocks and goat herds. That’s why the sanitary argument in favour of insemination has not been as strong for small ruminants as it used to be for cattle or pigs (Barillet et al, 1984). Nevertheless insemination makes it possible to bring in new genetic origins in the flocks without entering animals from other farms and with a smaller number of males to breed. From a sanitary point of view it’s an advantage which has some economical value.

Performing insemination is also a regulated activity : inseminators must have a State diploma and their activity is under the responsibility of a granted insemination centre. In France, sheep and goat breeders do not inseminate with frozen semen their own animals even if it is perfectly legal.

Importance of insemination for selection programmes

As regards genetic improvement, insemination has two advantages : a significant increase in the offspring bred per year and male, and a space dissociation between production and utilisation of the semen ; if frozen semen can be used, a time dissociation also may occur (Mocquot et al, 1984; Barillet et al, 1989; Bodin et al, 1997).

In the case of pure breed selection, the more animals you get in the breeding scheme, the more efficient it is. For ruminants, several tens to several hundreds breeders are involved in the project, according to the breed. Thus insemination allows to set up efficient breeding schemes in spite of difficulties due to the dispersion of animals among herds, sometimes located within a wide territory.

1) Estimation of breeding value

Breeding evaluation is based on the on-farm performance recording of the animals themselves or their relatives. To avoid systematic bias it is necessary to dissociate the effect of environmental factors like year, season and herd, etc… from the genetic effects. This objective is achieved if there are genetic connections between years and flocks due to the pedigree links between animals. As insemination allows to procreate half-sibs from a sire in a great number of flocks during one or few consecutive years, it is the best mean to create the connections needed by the breeding evaluation and thus to improve the computation of breeding values of reproducers, males as well as females.

Besides, the possibility for some males to have a great progeny, well distributed across flocks, allows to evaluate their breeding value precisely, according to the size of their progeny. If these males are still alive after progeny-testing, or if their semen has been kept in stock, it’s possible to disseminate widely the genes of the better ones. In France, several ovine breeding schemes (Lacaune, Ile-de-France, Blanc du Massif Central …) and the two caprine schemes (Alpine and Saanen) include progeny-testing in their programme.

2) Utilisation of males

The ability to diffuse males is essential concerning the efficiency of a selection scheme, for the creation as well as for the diffusion of the genetic improvement.

As regards the creation stage, each new generation of elite males must be procreated by the elite reproducers of the previous generation. It means that the best females (whose proportion is determined by their reproductive abilities) must be inseminated with the semen of the best males at disposal. Insemination allows to reduce the number of males which are necessary and thus the selection intensity at this stage is higher.

As regards the diffusion stage, insemination makes it possible to diffuse the selected males themselves (after an ancestry selection, joined or not with a progeny testing) because the number of necessary males is lowered and because their physical presence in the flocks is not obligatory. This remark is true for the diffusion in the breeders flocks as well as the commercial flocks.

However, the use of insemination is sometimes limited by technical problems or by its cost. So a stage of multiplication exists in herds which procreate and sell sons of elite sires. This practice exists at a more or less large scale in all ovine and caprine breeds. The diffusion of genetic improvement by the sons is slower than it could be with the proven sires themselves because it adds one generation of animals between the elite males and the animal which gives a production (the dairy or suckling female). However if the flow of reproducers become stable within the various stages of the breeding scheme (breeders and commercial flocks), it has been demonstrated, then shown in some situations, that the speed of improvement is the same for all the flocks, with a remaining difference of genetic level according to the position of the flocks in the scheme.

These general principles have been set in practice within all the selection programmes of ovine and caprine breeds in France, according to the various conditions, i.e. economical and technical opportunities or constraints, to which they had to fit. We will now give a few examples of selection schemes chosen according to the more or less importance they give to insemination.

Use of insemination in dairy sheep populations

As said above, the Lacaune dairy sheep organisation of the Roquefort area (in south of France) was the first in small ruminants productions to take advantage of the new insemination technique associated with hormonal treatment in the selection programme of the Lacaune dairy breed (Cottier et Briois, 1984). Previously their programme laid on mass selection of females and ancestry selection for young rams. Thanks to insemination, they could set up a breeding scheme much more effective, based on progeny testing of rams and planned mating between proven sires and elite females.

Using fresh and low-diluted semen produced within a short period (2 months) leads to a limited diffusion of rams. Consequently the cost of insemination is rather high, at least 3 or 4 times more expensive than for cattle. Nevertheless insemination did not stop developing since the 1970s. There are two reasons for this favourable evolution : first, insemination brings an evident genetic improvement in the flock, and second, it is a simple mean, with the hormonal treatment, to meet the needs of the dairy industry for a controlled timing of reproduction.

The organisation of the Lacaune breeding scheme has not changed since the end of the 1970s (Astruc et al, 1995). Insemination (which concerned in 2003 90 % of the ewes in the nucleus flocks) plays a major role in the efficiency of the scheme, with 400 rams progeny tested, planned mating and diffusion of the proven rams. Out of the nucleus, the diffusion of the rams is realised by the mean of insemination, or the sale of reproducers sons of the elite sires. Therefore, this technical organisation has reached an economic equilibrium, for the breeders as well as the technical organisations responsible for the selection programme, what is of great importance to make the breeding scheme last for long.

In the Roquefort area where dairy industries stop their activity between July and November, sheep breeders try to group the lambing and the beginning of the milking of their ewes, and thus to make them fertilized at the same moment, just before the natural reproductive period (June July). With insemination and hormonal treatment this will is easily satisfied, and simultaneously the number of males necessary to serve the females is limited. Ewe lambs, as well as old ewes, do not participate to the procreation of the replacement reproducers ; thus they are inseminated with semen from meat breeds to produce lambs for slaughter. This purpose represents about 20 % of AI in dairy flocks. So, insemination is a good way to optimise the income of breeders allowed by meat and dairy production, and to avoid keeping in the flock the great number of males that would be necessary to serve all the females in so short a period.

Other breeding schemes exist for dairy sheep breeds located in the Pyrenees Mountains and in Corsica. They tend more or less towards a similar organisation as the Lacaune scheme, but as they have been set up many years later and because their economic environment is quite different, they have not reached yet the same efficiency. For instance, at the time, the insemination rate in Pyrenees breeders flocks is 60 % (within nucleus). It has been shown that the efficiency of breeding schemes directly depends on the rate of insemination used for progeny testing (Astruc et al., 2002). Furthermore the important use of AI has been a precious help in the fight against scrapie disease to select for resistant rams (genotyped ARR/ARR). The programmes of eradication of the unfavourable alleles and the selection for the resistant ones have been efficient according to the percentage of inseminations with rams carrying the good alleles (Palhière et al, 2003).

Settlement of genetic schemes in suckling sheep

As meat lamb production developed in out of season period with hormonal treatments and AI, the genetic schemes aimed to improve meat abilities (growth rate, conformation and fat thickness). About 80 rams of different breeds were tested on their progeny in Berry-Test station. As regards the improvement of meat production abilities, it is to be noticed that the performances can be measured on the animal itself and on its offspring of both sex. Consequently, the number of inseminations needed to have a sufficient progeny to evaluate the animal is lower than in dairy breeds.

Simultaneously it was necessary to improve the maternal abilities of females (prolificacy, dairy value) to increase the productivity of the livestock. In 1985, 21 AI centres produced fresh ram semen and some of them had been engaged for several years in operations of planned mating, progeny testing or large spreading of ram improvers. In 2002, there were 31 400 AI for planned mating, 30 900 AI for progeny testing and 87 300 AI in order to produce females and mainly males of renewal. So, one can estimate at 24 000 the number of meat breeds rams necessary each year at the national level. At present, 1/3 of these needs are covered directly by the organizations of selection and on these 8 000 rams, 1 500 arise from meat ram improvers, the others being grandsons of these males.

The development of AI and genetic schemes simultaneously was also a good opportunity to face the crisis induced by scrapie in the last 90'. The generalization of PrP genotyping of rams showed the importance of the efforts to carry out to increase the level of genetic resistance of the livestock, mainly the rams, with strong breed disparities. Thanks to AI, planned mating were carried out to pass quickly at a first resistant heterozygous stage then at a homozygous stage ARR / ARR. A good example is provided with the Causse du Lot breed (Perret and al, 2003) where starting with an initial frequency of 15 % of ARR allele in 1999, we were able to reach the rate of 97 % ARR / ARR for the rams born in 2002/2003 and used for natural mating in the breeding flocks (16 000 females). To achieve this goal, 11 600 AI were carried out between 1999 and 2002.

Use of insemination for the two main dairy goat breeds

The development of insemination in dairy goat breeding systems has been much slower than in Lacaune dairy ewes. It began to increase in the 1980s from 20 000 in 1983 to 50 000 with an annual 10 % growth rate. Then it slowed down to an average 2 % rate and reached the number of 71 000 goats inseminated in 2003.

With the development of insemination it became possible to set up a classical dairy breeding scheme with planned mating, progeny testing and proven sires AI diffusion in the nucleus herds. The genetic improvement obtained by this organisation has been evaluated to 12.5 kg and 13.6 kg of milk yield for Saanen and Alpine breeds respectively (Clément et al, 2002). In return this efficient selection programme incited breeders to invest in insemination as a good method to improve their milk production and income. However insemination associated with hormonal treatments to synchronize oestrus and induce out of season reproduction didn't diffuse much out of the breeding scheme nucleus, for economical reasons and because of uncertainty in fertility results (Leboeuf et al, 1998).

Till the years 90' the hormonal treatment associated with AI was the only method allowing an out of season reproduction (from March to July), what breeders tried to obtain for part of their herd. But they were not completely satisfied with the technique because the goats which remained unpregnant (around 40 %) had their next heat not until several month, at the beginning of the sexual season. Thus, the dates of kidding in the herd were disorganised, leading to negative economical consequences. These difficulties were overcome thanks to the photoperiodic treatment which has been popularised with success among goat breeders in the 1990s. Both techniques used together make the result more secure, in terms of global fertility, because it restores cyclicity for 2 or 3 cycles after the oestrus induced by the hormonal treatment and the bucks can serve the remaining goats.

THE HORMONAL TREATMENT IN QUESTION

Drawbacks of hormonal treatments

1) Costs

The main impediment to a larger use of hormonal treatment associated to AI in small ruminant farming is its cost comparing to the gross margin per production (lactation or lambs sold). As reported by Barillet *et al.* (1989), AI is three times more expensive for dairy sheep than for dairy cattle; in relation to gross margin per lambing, AI is twice more expensive for suckling sheep than for dairy sheep (18% vs 9%); and finally, comparing fertilisation with sire of similar genetic value, the cost of cervical insemination in sheep is twice the natural mating one. There are no evidences that this situation will get better in a near future.

2) Result variability

To ensure good fertility results after insemination, it is important to respect a large number of prerequisites from the female selection to their management before and after AI, a strict respect of delay between sperm collection and the time of AI, and also between sponge withdrawal and AI, and so on. Even when AI is performed in the best conditions, fertility results are highly variable and depend on many environmental factors which are not easy to control. Sometimes, occurrence of too low fertility discourages breeders to keep going on with AI.

3) immunity response to PMSG injection

Studies on lack of fertility after insemination have shown off that part of the animals, ewes as well as goats, product antibodies against the PMSG that they are injected according to the hormonal treatment protocol (Bodin et al, 1995 ; Baril et al, 1996). PMSG is made from pregnant mare serum and it has an antigenic potential that induces a reaction of variable intensity. Taking this fact into account, the hormonal treatment has been adapted : it recommends 1) to make no more than one treatment each year, 2) to observe (with a teasing male) the oestrus of treated females in order to keep off those whose oestrus is delayed, 3) to select females and not present for insemination those which remained barren after previous insemination and those which have had already more than 5 treatments. These recommendations can only lower the problem but not solve it.

4) maximum residue limit

At present, for the synthetic progestagen (fluogestone acetate) used in the vaginal sponges, a Maximum Residue Limit (MRL) is fixed by a European regulation (EEC 665/2003 and EEC 739/2003). This provisional regulation will expire on January 2008 and the MRL will be reviewed. For PMSG there is no need for MRL at the present time according to the EEC 2377/90.

5) risk of disease transmission with natural hormones

The need to avoid disease transmission leads to limit injection or consumption of natural products from animal origin like PMSG. To get rid of this risk can be considered as a precautionary measure that gives a chance to sheep and goat production to keep going on in the future.

6) animal well-fare – market requirements – organic farming

These elements, although more subjective than the previous ones, must be considered, as they have taken a great importance in consumer's mind. Breeders cannot withdraw themselves from this context ; it leads them to prefer natural methods, which even become the rule in the case of organic farming and for some Protected Designations of Origin. For instance there are yet about 20 000 goats in 350 herds bred according to organic methods.

Anyway, as far as meat production is concerned, it should be kept in mind that in this case, the animal which is slaughtered is not the one which has been treated.

Alternatives to hormonal treatments

1) Alternative to impede seasonality of sheep and goat reproduction

a. Photoperiodic treatment

The annual cycle of photoperiod is the most important synchronizer of reproduction rhythm. At a certain time of the year, the neuroendocrine system responds to the appropriate short day length with a physiological cascade that includes an increase in gonadotropin secretion leading to a set of reproductive processes, including reproductive behaviour. This ability to respond to short day lengths is known as the state of photosensitivity. However, after about 70 days ewes become insensitive to the previously stimulatory photoperiods and stop their reproductive activity. Sheep also become refractory after prolonged exposure (about 150 days) to long photoperiods. Thus two physiologic states are important to the regulation of seasonal function - photosensitivity and photo refractoriness and this can account for transitions into and out of the reproductive season under natural conditions.

Artificial photoperiods that are used in all photoperiodic treatments are composed of two phases: first a long day period is provided to inhibit the current sexual activity and to prepare females to respond to the subsequent stimulatory effect of short days (Chemineau et al., 1988). Long days can easily be done in late fall or winter, providing extra-illumination to the animals. Furthermore, it is known that this long day period can be efficiently obtained by 1 hour light pulse given each day 16-17 hours after a fixed dawn.

According to latitude and season, short days can be more difficult to provide. In north regions, if the starting short day period starts before mid February, the natural day length is still short enough to induce the stimulatory effect, but if this period begins later, natural day length is already large, what may require dark proof sheds. An alternative is to provide melatonin treatment in order to mimic this short day period (see below).

The photoperiodic treatment in itself does not clutch the oestrus synchronisation. Therefore, under a classical mating organization, it does not impose a large number of males. However, since females are in a physiological status similar to that found at the beginning of the breeding season, they can respond to a male effect which can interestingly be associated to the photoperiodic treatment.
Association of these two methods is very efficient, and easy to apply for an early out of season breeding. However it presents some inconvenient which stops its large use. - On a farm, the number of sheds will determine the number of groups which can be managed. For instance if there is only one shed, all animals will receive the same photoperiodic treatment and will have to be bred altogether. That imposes a unique mating period which can be a problem for young replacement females. - Grouping animals for photoperiodic treatment have to be done a very long time before mating, and that cannot be changed during this period. – Although this treatment is efficient, there are few females which remain barren after the mating period. By a residual effect, these females will recover their "natural" sexual activity later in the following autumn. – Photoperiod can modify more functions than reproduction. For instance, food intake or milk production may also depend on photoperiod and could interact with the reproduction treatment.

b. Male effect

The duration of the anoestrus season in sheep and goats is also affected by social interactions between males and females. Hence, females permanently maintained in contact with rams have a longer sexual season than those kept separated from males (O'Callaghan et al., 1994). On the other hand, a sudden introduction of a male (ram or buck) in a group of slight anoestrous females, previously separated from males for at least one month, induces a sexual activity in a variable proportion. This phenomenon is known as the "male effect" or "teasing" (Signoret, 1990) and has long been used by breeders. A first silent (without oestrus) ovulation occurs within 2-3 days of male introduction; it is referred to as a "silent heat', meaning the females are not receptive to the males. This event, however, starts her natural biological clock and a fertile heat will follow in approximately 17 days (21 days for goats) in a variable proportion of the ewes (goats). The remainder of the flock will short cycle, having another silent heat at 6 or 7 days. These females will have a fertile heat at approximately 24 days (28 days for goats) after the introduction of the males. However, the proportion of females responding to the male effect as well as the proportion of females presenting a short cycle depends on several factors among which are the species, the season, the breed, the age and the nutritional status of females (Thimonier et al., 2000). It is generally accepted that the efficiency of the male effect is inversely related to the proportion of cyclic females (Martin et al., 1986) and to the libido of the male (Flores et al., 2000). There are, however, numerous contradictory results (Tournadre et al., 2002) on the efficiency of this effect, as it depends on a large number of factors cited above. The synchrony of oestrus obtained by such methods has not been fully studied in the aim of inseminating females because this body of work was initially done to be used with natural mating.

Major interest of this male effect is its low cost in contrast with the benefit of high parturition grouping. However, it presents some inconvenient which limits its use. - It works only when females are in slight anoestrus, at the beginning or at the end of the breeding period, and does not allow the onset of oestrus and ovary activity in full out of season breeding. - Active males secrete pheromones (a hormonal substance that females can smell) which have this dramatic and immediate effect upon females which have been kept apart from them for several weeks. But to be efficient, the separation prior to the sudden joining has to be total (view, smell, voice) and consequently supposes a specific shed for males. When combined with a photoperiodic treatment, which leads females to the precise physiological status to respond at a male effect, males should be also submitted to a similar photoperiodic treatment in order to stimulate their libido and their semen production, but that has to be done in another shed. – As synchronisation of oestrus is relatively good (70% of mating within 10 days), the number of males has to be sufficient to ensure fecundations (1 males/15 females).

c. Melatonin

Pineal secretion of melatonin is crucial to photoperiodic signal transduction. Secretion of this small molecule is regulated by circadian rhythm and serum concentrations at night exceed the daytime values, in sheep and goat by about 50-fold. The duration of the circadian phase of elevated pineal melatonin serves as an endocrine 'code' for day length. In short-day breeders (e.g., sheep), short-duration melatonin peaks (associated with long days) inhibit reproduction, whereas long-duration peaks (associated with short days) are stimulatory. Melatonin treatments tend to mimic short or long days by controlling artificially the concentration in the blood. Subcutaneous implant or oral administration in food may be read as short days. Even, the typical nocturnal phase of pineal melatonin secretion is not essential for evoking photoperiodic responses.

As for photoperiodic treatment of short days, a melatonin treatment should last at least 50 days to be efficient. It also has to follow a natural or artificial long day period of about 2 to 3 months.

Thus, photoperiodic and melatonin treatment can be interestingly associated, since photoperiodic treatments permit to mimic short days easier than long days while melatonin treatments simulate natural long days.

For our concern, the major inconvenient of this treatment is that melatonin is a hormone, although it is a small synthetic, non antigenic and totally safe molecule which is naturally very abundant. But as a hormone, melatonin does not represent a real hormone-free alternative to hormonal treatments.

2) Alternative treatments to use with artificial insemination

a. Problems to be solved.

Female ovulation appears at the end of the final follicular maturation process. To minimize the gamete waiting time and maximize the mating success, fertilization may occur at a given time of this physiological process. In natural mating, males are warned of the beginning of this process by the oestrus onset of each female. It is possible to perform artificial inseminations on natural oestrus. It only needs to inseminate individual female at a given time after the onset of its oestrus which should be carefully controlled. The use of hormonal treatment permits to get in this physiological process and to dispose of an event which strongly and accurately provokes its start and fix the ovulation time. PMSG injection associated to sponge withdrawal realizes synchronization of individual female process, that also triggers within 36 hours the preovulatory LH surge and consequently ovulation.

Alternatives to hormonal treatment used for artificial insemination should 1) restore sexual activity for female in anoestrus, 2) synchronize cycles of a group a females, 3) trigger the final follicular maturation until ovulation and 4) give to the breeder an accurate reference time for insemination.

b. Natural alternatives.

At the present time, there are no good alternatives which can substitute the current hormonal treatment. Restoration of sexual activity for females in anoestrus is possible at any time of the year with photoperiodic treatments which can be interestingly associated with melatonin treatment (see above). But that does not induce synchronization, and overall does not deliver an accurate starting point for the final follicular maturation until ovulation. Nowadays, in sheep and goat, the efficiency of the male effect (i.e. the ability to induce cyclicity in a large proportion of non-cyclic ewes and to synchronize oestrus occurrence among females) performed alone when females are not in deep anoestrus, or in association with treatments for deep anoestrus cited above, is not sufficient for a systematic animal insemination (i.e. at a given time after male introduction).

c. AI practices according to the synchronisation level

It is necessary to adapt the practice of AI to the lack for a good method of synchronization and a fine control of ovulation time which are free of hormones. However animal insemination in natural oestrus was quite used some years ago in large sheep flocks from South America and Eastern Europe and is still used in Nordic and Middle and Eastern European countries (Kukovics, 1997; Perret et al., 1997). The lower degree of synchronization due to the suppression of hormonal treatments necessitates to detect females in oestrus and to perform the AI at the most appropriate time, using semen with an enlarged fertilizing ability. Both techniques are currently used.

Determination of individual ovulation time can be done through the detection of oestrus onset at a regular time in a group of females previously synchronized by a natural method (male effect at the end of a photoperiodic treatment). Insemination of females after this oestrus detection (by a teaser male) twice or once a day is possible and provides good results. However, it is time consuming and imposes several visits to perform the inseminations. Detection of LH surge in blood or in milk is also possible and has been used in experimental conditions.

On the other hand, with poor synchronization of females, the time of insemination is less predictable, what induces a more variable delay between sperm collection and AI (in case of insemination with fresh semen) and also between ovulation and insemination. To conserve a high fertility rate, spermatozoa have to maintain their ability over a long time both in vitro after collection and later in the female tract.

At the opposite to the other cell types and due to the inactive genome, the male germ cells have lost their ability to repair and renew their intra-cellular components. The spermatozoa is not a cell programmed to survive a long time, but it can stay alive and fertile at least two weeks in the male genital tract and several days at 4°C in vitro in the epididymal fluid (Guérin et al., 2003). The survival of fertile sperm in vitro is possible according to these observations, but, to take advantage of this ability and to use it in vivo, new researches have to be developed either in extender technologies or in sperm preparation.

Possible consequences on breeding schemes

As described above, alternatives to hormonal treatments yet exist but they are still less convenient : more work time needed, less secure results in terms of fertility rate. Thus, if breeders were obliged to use these methods in the present state of things, we guess that negative consequential effects would happen.

In dairy goat production :

1. out of season reproduction in order to spread the milk production

Since 1989, the photoperiodic treatment has been popularised with goat breeders (Chemineau, 1989). For out of season reproduction (March to May), the protocol works perfectly well, it is not expensive, and the fittings of the shed are simple. As the protocol applies to females and males as well, natural mating makes no problem. An inquiry hold in 2002 (Brice et al, 2002) has brought to light that more than 25 % of breeders with milk recording already use a photoperiodic treatment for at least a part of their herd.

2. reproduction with insemination for genetic improvement

As insemination is no more necessary for out of season reproduction, the only reason to use it is genetic improvement. Thus it is to be used at the moment chosen by the goat breeder, in season, just before season or out of season as well.

At present, insemination of a group of females synchronised with a male effect needs that an oestrus detection occurs with a teasing male, and it takes quite a lot of time to the breeder (about 1 hour for 50 to 70 females according to the organisation of work and to the male sexual behaviour). In the present context, with goat herds of quite a great size (150 animals in average for milk recorded herds in 2003), every time-consuming work represents a major constraint, all the more when other duties need also manpower like cultural works in spring.

Thus, at the present state of technique, we conclude that the economical profit of insemination without hormonal treatment is lower than with it, and so demand for insemination in these conditions would decrease comparatively as what it is yet.

Consequently, in the breeding scheme, we ought to reduce the number of inseminations to what is strictly necessary. But till now, insemination is hardly used out of the nucleus of the breeding scheme, 20 to 25 000 inseminations at the most. Therefore we think that insemination would be used only in the restricted nucleus of breeders, those who have a sufficient genetic level to sell young bucks to the AI centres, that mean about 500 breeders who keep about 100 000 animals for the 2 main breeds.

According to the organisation of the caprine breeding scheme, the necessary inseminations include, for both breeds, the planed mating (about 1000), and the progeny testing (about 10 000) and about 30 000 AI to insure a good connection of herds (essential for breeding evaluation) and dissemination of genetic progress .Females born from the proven males would be used for replacement and the males could be sold to commercial flocks in order to diffuse genetic improvement. However it must be kept in mind that in the present sanitary context, breeders are not quite willing to exchange animals as sellers or buyers (remind the scrapie regulation that makes suspicious the herd where the sick animal is born).

In fact, the situation described above is not very different from the present one, but with less inseminations realised, perhaps half of the number we get now, for a breeding scheme of the same size, about 70 bucks progeny tested for both breeds. Obviously, the profitability of the AI centre would decrease, unless the price of the semen could be multiplied by 2. Breeders couldn’t afford the new price of insemination unless they could save costs by realising themselves the insemination. This possibility hasn’t been tried already with frozen semen, but it seems also difficult, as there is few work-time available in farms and as the fertility result could be at risk.

Could semen exports develop enough to balance the decrease of sales in France ? French exportations of bucks semen are about 9000 doses in 2003. 37 % are sold in Europe (Italy, Switzerland, Cyprus …). In these countries, the European regulations would apply as in France, so we can predict a decrease in sales in case of a ban of reproductive hormones, for the same reasons as in France. As regards exports in extra-European countries, it remains a difficult activity, for economical, political and sanitary reasons. Moreover a competition exists with other countries (USA, New-Zealand …) so it's hazardous to expect that exports could increase rapidly in order to compensate the inner decrease.

Therefore, the lack of profitability of the production of buck semen would jeopardize the existence of the breeding scheme, through the involved technical organisations : AI centres, breeders association, milk recording structures, whose activity is for a part linked to the genetic improvement process. At least, this loss of profitability would delay or cut out the development projects of the activity, like taking into account new goals as udder morphology or improvement of cells number in milk. The ban of hormones for reproduction in small ruminants would then damage the French caprine breeding scheme.

In suckling sheep production

To get sexual activity of females in out of season period, photoperiodic treatment is applied by few breeders (same protocol than for goats), but this technique competes with the large use of hormonal treatment and the breeding technical advisers don't encourage it. Some other breeders search for breeds with a short out of season period such as INRA 401 or Ile de France breeds. In spring, light treatment can be applied on rams used for crossing (light pulses to simulate the long day period or darkening the sheep housing to simulate the short day period). This last technique is widely used in the AI centres.

Nowadays, none of both considered techniques - ram effect and photoperiodic treatment of ewes – allows a sufficient synchronisation of ovulation to inseminate only once with success. For a sheep breeder it is unthinkable to make daily detection of ewes oestrus because it needs too much workforce. In addition, to inseminate a group of females, the inseminator ought to come on farm many times, so the mean cost of AI per ewe would increase a lot. Therefore, the ban of the artificial synchronization of oestrus would mean the stop of the existing genetic schemes becoming less effective: loss of connections between flocks, abandonment of planned mating and disappearance of ram progeny testing. More generally the strain father-son or father-daughter would be lost. The improvement of the maternal characters would then be strongly slowed down such as prolificacy with a weak heritability. In the case of meat characters, the loss of AI would be less harmful. All the effort would then be related to the individual control of the young rams in station: more controlled rams and stronger intensity of selection. The qualitative characters measured on carcass would be abandoned. In such a prospect, one could place a lot of hope in the genomic for a direct selection of the gene of interest. But to reach this goal it is necessary to have beforehand real measures on the individuals and their relatives born from planned mating what still requires AI to be efficient.

For dairy sheep production :

The consequences described above for suckling sheep would apply also for dairy sheep. However they would be more acute, according to the greatest importance of insemination in the dairy context. Besides, as dairy characters are recorded only on females (milk production, fat and protein content, somatic cell count, udder traits), progeny testing, which needs the use of insemination, is the only way to get an efficient breeding scheme. In the Lacaune farming system with an average size of flocks of 430 animals, it is frequent to inseminate 200 ewes or more the same day ; so it seems impossible to organise the detection of natural oestrus at such a large scale. In this context, the ban of hormones would lead to a very damaging decrease of insemination. The efficiency of the breeding scheme would be questioned, and thus its economic equilibrium. Anyway the new selection goals would be abandoned, despite their interest for animal welfare (disease resistance, functional longevity) or for consumers benefit (chemical and sanitary qualities).

PROSPECTS AND CONCLUSION

Owing to the low productivity of sheep and goat females, a selection programme for these species needs the co-operation of a large network of breeders. In these conditions, insemination remains an essential tool to get genes circulate among flocks. Till now, the use of insemination has been inseparable from the use of hormonal treatments, needed to synchronize oestrus in order to inseminate a group of females together.

According to the present state of art, it seems difficult to find a new valuable synchronization method totally hormone free. Finding natural vegetable molecules with progestative and estrogenic effects will surely improve synchronization of females. But it is uncertain that it could both, efficiently substitute to the current hormonal treatment and be accepted by consumers. Works are nevertheless done in this way. Other existing natural techniques of synchronisation have to be improved to ensure better results. For instance it appears reasonable to obtain a significant increment of efficiency of the male effect by a transient nutritional improvement (flushing) of female, and a better preparation of males.

Adapting the animal insemination technique to females less synchronized is also critical. In that purpose, it is essential to easily detect females in oestrus. Efficiency of this detection can be improved in two ways. The first one is to clearly identify behavioural signs which could be taken as criteria for oestrus. The second is to try to set up a method in which the spontaneous behaviour of the oestrus female (interest for the male) could be used to let the females self-select themselves when in oestrus and then limit man-manipulation for oestrus detection. It is also essential to improve the semen preservation technology in order to better preserve the fresh semen quality during its storage, and to enlarge the possible time for its use. The objective would be to maintain the fecundity of spermatozoa during at least 48 hours for sheep and 72 hours for goat and also to increase its resistance within the female tract.

In this purpose, 14 small and medium enterprises (SME) representing sheep and goat breeders & AI centres of 6 countries (France, Italy, Nether Lands, Poland, Portugal, Spain) and 9 Research and Technology Development performers recognized for their experience in small ruminants reproduction have proposed to join their efforts to develop alternative techniques to hormonal treatments in small ruminants reproduction and to adapt animal insemination to these techniques and finally prepare the future of goat and sheep reproduction in Europe.

In the short term, as long as other techniques are not as performing as the hormonal treatment, we think that reproductive hormones should stay under control and regulations. It would be damaging to deteriorate sheep and goat genetic improvement activity, which has been of great importance for the whole economic sector for the last 40 years, for improving the food qualitative and quantitative aspects. Besides, the scrapie crisis has shown how it is important to be able to diffuse quickly a favourable gene, for disease resistance or whatever other interesting character, in the breeder's or in the consumer's point of view.

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