# Oestrus induction and synchronisation during anoestrus in cashmere goats using hormonal treatment in association with "male effect"

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## SUMMARY

*During the anoestrous period, induction and synchronisation of oestrous was obtained in 30 cashmere goats at 54±5 days after kidding. A treatment for 11 days with vaginal sponges containing FGA (Fluorogestone-acetate) 45 mg and an injection of 400 I.U. of PMSG and 125 mg of $PGF_{2a}$ i.m. two days before sponge removal, was used. Goats were equally divided into two groups: A and B. In order to verify the effect of male introduction on the resumption of sexual activity and on oestrous fertility, males were introduced three days before sponge insertion into group A and 24 hours after sponge removal into group B. Males were equipped with a ram-harness carring a coloured crayons. Before their utilization, each buck had been housed for one week in a small pen with an estradiol injected female. After male introduction, oestrous was checked daily looking to the colored signs on the female back. Blood samples from each goat starting from delivery until the 35th day after male introduction were collected and plasma progesterone profiles by RIA method were determined. Pregnancy diagnosis by ultrasonography was performed at on the 35th day after male introduction. The precocious introduction of males exerted no influence on the resumption of female sexual activity. Plasma progesterone before sponge removal was always less than 1.5 ng/ml indicating the absence of luteal activity in all goats After sponge removal, progesterone profiles indicated luteal activity in 82.8% of the goats in both groups. Though 68.9% of the goats were mated, fertility was very low: only one goat of group A and one of group B became pregnant. Oestrous induction schedule was effective in inducing a normal luteal cycle in 45% of goats but males were not active and their sperm probably not sufficiently fertile.*

## INTRODUCTION

Cashmere goats, as well as most other goats, present sexual activity during the fall, while kidding occurs between the end of winter and the early spring. Reproductive performance may be improved by induction and synchronisation of oestrous during the anoestrous period, but fertility of induced oestrous is generally low. This is due to sexual inactivity of both males and females during spring, but also to lactating post-partum anoestrous after delivery (for a review see: Malpaux et al., 1994, Thimonier 1981). Several authors have shown that it is possible to induce the resumption of the sexual activity in anovulatory lactating goats out-of season using the "male effect" in association with different hormonal treatments or artificial light regimen (Ott et al., 1980; Pearce, 1985; Claus, 1990; Chemineaux, 1985, 1986b, 1987).

In this work, we verified the possibility of inducing oestrus in out-of-season lactating cashmere goats by the association of hormonal treatment with male effect, which is considered a natural method for oestrous synchronisation (Chemineau, 1987; Walkden-Brown et al., 1993 a, b, c).

## MATERIALS AND METHODS

Pregnancy diagnosis by ultrasonography, performed on February 1st, 1995 showed that more than 90% of the goats were pregnant. Deliveries were distributed as follows: 60% (25/42) during the second part of March, 16% (7/42) during April and 23% (10/42) during May. For the present experiment we chose 30 goats which delivered before April 20. One of them died during the experiment. Non-pregnant goats were not choosen because they were too young in relation to the adult goats.

At the time of oestrous synchronisation, goats the average time since last kidding was 54±5 (mean ± standard deviation) days , although some individuals were no more than 40 days. Experimental goats were separated from the rest of the herd but, since they were lactating, kids were not removed. Adult males had been kept isolated for almost four months. Goats were housed and fed with hay and alfalfa concentrate. A week before the beginning of the experiment, females were divided into two groups, A and B, balanced according to date of delivery. Hormonal treatments for oestrous induction were the same for both groups, while time of exposure to males was different. The experimental schedule is summarized in Table 1.

TABLE 1

Experimental schedule

| Date | Day | Group A | Group B |
|---|---|---|---|
| 08-05-95 | -3 | Male introduction | / |
| 11-05-95 | 0 | Sponge insertion | Sponge insertion |
| 20-05-95 | 9 | PMSG and PGF2a i.m. | PMSG and PGF2a i.m. |
| 22-05-95 | 11 | Sponge removal | Sponge removal |
| 23-05-95 | 12 | / | Male introduction |
| 26-05-95 | 46 | Male removal and pregnancy diagnosis | Male removal and pregnancy diagnosis |

*Oestrous induction and synchronisation*: Vaginal sponges containing 45mg of FGA (Fluorogestone acetate, Crono-gest, Intervet, Italy) were inserted for 11 days by vaginal way. Two days before sponge removal, each goat was injected with 400 I.U. of PMSG (Pregnant Mare Serum Gonadotropin, Crono-gest, Intervet Italy) and mg125 of synthetic $PGF_{2a}$ (Cloprostenol, Estrumate, Pitman-More) i.m.

*Male preparation*: In order to stimulate sexual activity, each male was housed with a female in oestrus the week before its introduction into the experimental group. Oestrus was induced in the stimulus females by administration of 10 mg i.m of estradiol benzoate (Progynon Depot, Schering).

*Exposure to the male*: Groups A and B were divided into two subgroups ($A_1$, $A_2$, $B_1$ and $B_{2)}$ each with 7-8 females. In each subgroup, $A_1$ and $A_{2,}$ one male was introduced to the females three days before the insertion of vaginal sponges. In subgroups $B_1$ and $B_{2,}$ one male was introduced to the females 24 hour after sponge removal - i.e., 15 days later than in subgroups $A_1$ and $A_2$. All males carried a “Ram-harness” with a coloured crayon. Sexual activity was registered by daily observations looking for the coloured marks on the female's backs. Males were left into the mating groups for 35 days after sponge removal.

*Blood sample collection*: Blood samples were collected weekly from kidding to the beginning of hormonal treatment, and twice weekly until the 35th day after sponge removal. Plasma progesterone was assayed by RIA method.

*Pregnancy diagnosis*: Fertility was evaluated by plasma progesterone assays and by ultrasonography performed on the 35th day from male introduction using a Toshiba Sonolayer B with 5MHtz probe.

## RESULTS

During the preparation phase, males did not mate the estradiol-injected females and did not show the characteristic ircin odour. Similarly, the precocious introduction of males into the group A, 3 days before sponge insertion did not lead to any mating, neither before vaginal sponge insertion nor during hormonal treatment.

Plasma progesterone concentrations between delivery and the fourth day after sponge removal were always lower than 1.5 ng/ml in all goats.

One day after vaginal sponge removal, 68.97% of females (10/14=71% in group A and 10/15=66.7% in group B) showed oestrus. These animals were mated within three days after sponge removal. All males but one (the male of group $A_2$) showed normal sexual behaviour. 24 hours after his introduction, the inactive male was removed and the females were introduced with the active male of group $A_1$.

Plasma progesterone concentrations between the 2nd and 9th day after sponge removal showed that 89.6% of goats (12/14=86.7% in group A and 14/15=93.3% in group B) presented luteal activity, with a mean progesterone level of 3.3±1.3 ng/ml at the 8th day (see Table 2).

TABLE 2
Oestrous behaviour and luteal activity after sponge removal

| | Group A | Group B | Total |
|---|---|---|---|
| oestrous behaviour | | | |
| | 10/14 | 10/15 | 20/29 |
| % | 71 | 66.7 | 68.97 |
| luteal activity between 2nd and 9th day after sponge removal | | | |
| | 12/14 | 14/15 | 26/29 |
| % | 86.7 | 93.3 | 89.6 |

According to the progesterone profiles of the 20 mated goats, 3 were pregnant, 7 showed a normal luteal cycle at the sponge removal but did not become pregnant, 8 showed a short luteal cycle (plasma progesterone fell to levels less than 1.5 ng/ml before the 21th day after oestrous) and 2 never showed progesterone level higher than 1.5 ng/ml, although they were regularly mated.

According to the progesterone profiles of the 9 non-mated goats, 6 showed a normal luteal cycle, 2 showed a short luteal cycle and 1 never achieved a plasma progesterone level higher than 1.5 ng/ml, and did not show oestrous behaviour. Data are summarized in Table 3. Progesterone profiles are shown in Fig. 1.

TABLE 3.

Results after hormonal treatment

| | Mated goats | Non mated goats | Total | % |
|---|---|---|---|---|
| Pregnant | 3 | 0 | 3 | 10% |
| Normal luteal cycle | 7 | 6 | 13 | 45% |
| Short luteal cycle | 8 | 2 | 10 | 34% |
| Progesterone less than 1,5 ng/ml | 2 | 1 | 3 | 10% |
| Total | 20 | 9 | 29 | 100% |
| % | 68.97 | 31.03 | 100 | 0 |

Pregnancy diagnosis performed by ultrasonography on the 35th day after male introduction showed that only one goat of group A and one of group B were pregnant, while the third goat which had shown high progesterone concentrations on the 27th day after sponge removal, did not become pregnant.

## DISCUSSION

This experiment confirms that cashmere goats, as most other goats (Malpaux B. et al. 1994), are in deep seasonal anoestrous during springtime. Plasma progesterone profiles showed that before, and until four days after sponge removal, 100% of the goats were in deep seasonal and lactational post-partum anoestrous since none of them presented progesterone levels higher than 1.5 ng/ml.

Males too showed deep anoestrous since they did not present the typical ircin odour of the active male (Claus *et al*., 1986; Over *et al*., 1990) and did not mate the estradiol-injected females during the preparation phase. The failure of this treatment may be due to the stimulus females being too young, and thus failing to respond to estradiol, consequently not adequately stimulating the males.

Contrary to reports by several other authors (Chemineaux 1986a, 1986b,1987, Walkden-Brown *et al*., 1993a, 1993b, 1993c), the introduction of males did not induce ovarian activity: Group A females showed the same low levels of plasma progesterone as those in group B and no goat was mated until sponge removal.

Ten goats (34.4% of total, 8 of them mated) had low progesterone concentrations on the 8th day after sponge removal (see fig. 1). Such abnormal duration of luteal activity is generally due to a short life of the *corpora lutea* and is usually called "Short Luteal Phase" (SLP). SLP has been reported to occur on the introduction of males into female flocks in both goats and ewes as the very first ovarian activity (Chemineaux 1983, 1985, 1986a, 1986b, 1987, 1989; Ott et al. 1980 in goats; Pearce et al. 1985 in ewes). However, in the present experiment the male introduction before hormonal treatments failed to induce either short or normal duration ovarian activity. We observed ovarian activity in both experimental groups only after hormonal treatment, and the observed 34.4% of SLP of the present experiment is much higher than the 5% reported by Chemineaux (1985) after both long or short duration FGA-treatment. Our results demonstrated that the hormonal treatment (either in association with exposure to the males or not) failed to induce a complete luteal activity. Moreover, none of the SLP goats presented a second oestrous, as normally happens after the male effect (Chemineaux, 1985, 1987, 1989, Carnevali et al., 1994), demonstrating that male introduction and hormonal treatment were not able to completely overcome the out-of season and lactational post-partum anoestrous and to restore cyclicity of sexual activity (see Pearce G.P. and Oldham C.M., 1988 and Malpaux et al., 1994 in the ewe). In fact, even in those goats in which the hormonal treatment induced a normal luteal activity, cyclicity was not induced and progesterone concentrations returned to typical out-of-season values (less than 1.5 ng/ml). Furthermore, about half of these latter goats were not mated and probably had silent ovulations without sexual behaviour (Thimonier, 1981, Chemineaux, 1985, 1987, 1989, Carnevali et al., 1994), further supporting the hypothesis that the hormonal treatment was not able to completely overcome the post-partum anoestrous.

The low overall fertility we observed was mostly due to those females that either did not mate or showed a short luteal phase, or had progesterone concentrations always lower than 1.5 ng/ml (19/29). Of the remaining ten females, in which hormonal treatment was effective and that were regularly mated, only three become pregnant. In our opinion, this low fertility might be due to the anoestrous of the male. In fact, male cashmere goats show a circannual cycle of LH and testosterone production associated with the seasonal sexual activity (Walkden et al., 1993a, 1983b, 1983c, 1994). Ovine and caprine males showing a normal sexual behaviour during the anoestrous season, present low sperm fertility because of a poor quality and quantity of sperm production (Dacheux et al., 1981; Grasselli et al., 1992, Susha et al., 1992), therefore the observed low fertility in this experiment may also be attributed to poor sperm quality.

In conclusion, the schedule of oestrous induction and synchronization was only moderately effective in inducing oestrous in female cashmere goats and was not able to restore normal cyclicity. Males also presented a deep seasonal anoestrous and their fertility was probably low.

Future studies will evaluate whether artificial insemination with frozen semen could partially overcome these problems and to improve fertility of cashmere goats during the lactational post-partum anoestrous.

## REFERENCES

Chemineaux P. (1983). Effect on oestrous and ovulation of exposing Creole goats to the male at three times of the year. *Journal of Reproduction and Fertility*, 67: 65-72.

Chemineaux P. (1985). Effects of a progestagen on buck-induced short ovarian cycles in the creole meat goat. *Animal Reproduction Science*, 9: 87-94.

Chemineaux P., Levy F. & Thimonier J. (1986a). Effect of anosmia on LH secretion, ovulation and oestrous behaviour induced by males in the anovular creole goat. *Animal Reproduction Science*, 10: 125-132.

Chemineaux P., Levy F. & Thimonier J. (1986b). Induction and persistence of pituitary and ovarian activity in the out-of-season lactating dairy goat after a treatment combining a skeleton photoperiod, melatonin and the male effect. *Journal of Reproduction and Fertility*, 78: 497-504.

Chemineau P. (1987). Possibilities for using bucks to stimulate ovarian and oestrous cycles in anovulatory goats - a review. *Livestock Production Science*, 17: 135-147.

Chemineaux P. (1989). L'effet bouc: mode d'action et efficacité pour stimuler la reproduction des chèves en anoestrous. *INRA, Production Animal*, 2 (2): 97-104.

Carnevali F., Castrignanò F., Capozzoni A. & Misiti S. (1994). Male effect in Angora goats. *Atti XI Congresso Nazionale SIPAOC,* pp 351-354.

Claus R., Over R. & Dehnhard M. (1990). Effect of male odour on LH secretion and the induction of ovulation in seasonally anoestrous goats. *Animal Reproduction Science,* 22: 27-38.

Dacheux J.L., Pisselet C., Blanc M.R., Hocherau-de-Reviers M.T. & Courot M. (1981). Seasonal variation in rete-testis fluid secretion and sperm production in different breeds of ram. Journal of Reproduction and Fertility, 61: 363.

Grasselli F., Gaiani R. & Tamanini C. (1992). Seasonal variation in the reproductive hormones of male goats. Acta Endocrinologica, 126.

Malpaux B., Viguié C. Ravault J.P., Thiéry J.C. & Chemineaux P. (1994). Photoperiodic and neuroendocrine control of seasonal reproductive functions in the ovine and caprine species.*European Fine Fibre Network. Occasional Publication No. 2*, Macaulay Land Use Research Institute, Aberdeen, UK. pp 3-21;

Pearce D.T., Martin G.B. & Oldham C.M. (1985). Corpora lutea with a short life-span induced by rams in seasonally anovulatory ewes are prevented by progesterone delayng the preovulatory surge of LH. *Journal of Reproduction and Fertility,* 75: 79-84.

Pearce G.P. & Oldham C.M. (1988). Importance of non-olfactory ram stimuli in mediating ram-induced ovulation in the ewe. *Journal of Reproduction and Fertility,* 84: 333-339.

Ott R.S., Nelson D.R. & Hixon J.E. (1980). Effect of the presence of the male on initiation of estrous cycle activity of goats. *Theriogenology* 13,(2): 183-190.

Over R., Cohen-Tannoudji J., Dehnhard M., Claus R. & Signoret J.P. (1990). Effect of pheromones from male goats on LH-secretion in anoestrous ewes. *Physiology and Behavior,* 487: 665-668.

Susha N., Metha V.M., Deshpande S.B. & Shah R.G. (1992). Recent advances in Goat production. "*Proceedings of the Vth International Conference on Goats*". New-Delhi. pp1192-1194.

Thimonier J. (1981). Control of seasonal reproduction in sheep and goats by light and hormones. *Journal of Reproduction and Fertility,* Suppl. 30: 33-45.

Walkden-Brown S.W., Restall B.J. & Henniawati (1993). The male effect in the Australian Cashmere goat. 1. Ovarian and behavioural response of seasonally anovulatory does following the introduction of bucks. *Animal Reproduction Science* 32: 41-53.

Walkden-Brown S.W., Restall B.J. & Henniawati (1993). The male effect in the Australian Cashmere goat. 2. Role of olfactory cues from the male. *Animal Reproduction Science,* 32: 55-67.

Walkden-Brown S.W., Restall B.J. & Henniawati (1993). The male effect in the Australian Cashmere goat. 3. Enhancement with buck nutrition and use of oestrous females. *Animal Reproduction Science,* 32: 69-84.

Walkden-Brown S.W., Restall B.J., Norton B.W. & Scaramuzzi R.J. (1994). The “female effect” in Australian casmere goats: effect of season and quality of diet on the LH and testosterone response of bucks to oestrous does. *Journal of Reproduction and Fertility*, 100: 521-531.