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**Original article**

# Prepartum peripherally-induced hyposmia does not reduce postpartum anoestrus duration in nursing goats

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**Abstract** – Parturient goats rapidly develop exclusive nursing of their own litter that relies on olfactory recognition of the young. They also show a period of postpartum anoestrus whose duration depends on the presence of the kid. In cattle, maternal selectivity is one of the factors that delays the recovery of sexual activity. To investigate the possible influence of maternal selectivity on the duration of postpartum anoestrus in goats, we compared the recovery of estrus behavior by daily estrus detection with an active buck in intact and selective nursing goats ($n = 24$) with that of dams rendered non-selective by peripheral hyposmia with $ZnSO_4$ ($n = 18$). Postpartum anoestrus duration was shorter in intact (68 ± 7 days) than in hyposmic mothers (93 ± 7 days; $P < 0.05$). However, the cycles of normal duration were less frequent in intact goats ($P = 0.03$). We conclude that in nursing goats, preventing the establishment of selective nursing by prepartum peripheral hyposmia does not reduce postpartum anoestrus duration. Our results suggest that daily exposure to the buck may result in an earlier recovery of ovarian activity in intact mothers.

**postpartum anoestrus / anosmia / goat / maternal behavior / olfaction / selective bond**

## 1. INTRODUCTION

In several mammalian species, the presence of the young strongly inhibits the resumption of postpartum sexual activity [1], and the sensory stimulation caused by suckling is an important component in this process, although its mechanisms of action may vary between species. Thus in rats, the inhibition of reproductive activity during lactation involves many factors such as the presence of the pups, litter size, pup development and prolactin (PRL) secretion [2]. In primates, the only presence of the young or the suckling stimulus per se is able to inhibit ovulation independently of PRL release [3, 4]. In domestic ruminants, there is also some effect of the young on postpartum anoestrus duration (PPA), although the effect of nursing can only be evidenced during the reproductive season in species that show seasonal breeding [2, 5]. For example, in

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sheep there is a positive correlation between nursing activity and PPA duration [6], and ewes and goats separated early from their young and submitted to milking, return to heat sooner than mothers that keep nursing [5, 7], an effect partly due in sheep to an inhibitory action of PRL [8].

Another factor that can influence the duration of PPA in domestic ruminants is the presence of a selective maternal bond (i.e. exclusive acceptance of the own young at nursing). Sheep, goats and cattle show selective nursing of their own young, a process that depends mainly on maternal recognition of olfactory cues from the young [9–13]. Studies in cattle indicate that this maternal selectivity can be one of the factors that delays recovery of sexual activity in nursing cows [14]. These studies also indicate that the olfactory and visual cues involved in the identification of the calf by its mother play an important role in this respect, even though the respective roles of anosmia per se and lack of selectivity may need to be clarified, since they are confounded in anosmic mothers. Thus, cows impeded to bond to any calf, due to a blockade of the olfactory and visual cues, show an increase in LH secretion, indicating that the inhibitory effect of nursing on postpartum sexual activity is sustained only if the sensory cues necessary for calf identification are present. Since goats also develop an early selective nursing, in this species also, the presence of a selective bond may be an important factor inhibiting the recovery of postpartum sexual activity. Since in the goat this selective nursing depends solely on olfactory cues, contrary to what is found in cattle [11], this species is particularly well suited to study the importance of olfactory-dependent maternal selectivity in the regulation of postpartum anoestrus. To test this possibility, we investigated whether preventing the establishment of maternal selectivity by performing hyposmia during pregnancy, could facilitate the recovery of sexual activity measured daily through estrus detection by an active buck in goats nursing their kids.

## 2. MATERIAL AND METHODS

### 2.1. Animals and management conditions

The study was carried out at the experimental unit of the Instituto de Neurobiología, UNAM, located on the grounds of the farm belonging to the Veterinary School of the Universidad Autónoma de Querétaro, Mexico (latitude 20° N). The animals were kept under intensive management conditions at a density of about 5 $m^2$/animal, with permanent access to a shaded area. They were fed with lucerne hay, grain and minerals according to their physiological requirements [15], including litter size during lactation. Forty-two mixed-bred female goats (half of them nulliparous), bred in the spring using vaginal sponges (45 mg of fluorogestone acetate, Intervet) and PMSG (300 IU, Folligon Intervet), were used. One month before the expected date of kidding, 18 of these females, balanced for parity and body condition, were made hyposmic by two intranasal irrigations with a 2.5% $ZnSO_4$ + 2% Xylocaine solution under general anesthesia, according to a previously described method [13]. For ethical reasons, we followed the guidelines used for anosmia during other studies carried out in sheep in our laboratory, in accordance with authorization A37801 of the French Ministry of Agriculture [16]. Previous studies in sheep and goats have shown that this treatment has no effect on general behavior, body condition of the mothers or growth rate of the young [17, 18]. The olfactory deficit was confirmed at 24 h postpartum and at days 30 and 75 of lactation, using a behavioral food preference test [13]. The goats were also tested for their ability to accept an alien kid at the udder at 24 h postpartum.

During pregnancy, all females were kept in a single group and with a sexually active buck, for the last 30 days of pregnancy. Females gave birth in mid October (October 17 ± 4.5 days, mean ± sd). In the control group, 13 goats reared twins and 11 goats reared a single kid. In the hyposmic group,

the corresponding figures were 11 and 7, respectively. At parturition, the animals were penned individually for 12 h after which they were allocated to one of two adjacent shaded pens, according to their experimental group.

## 2.2. Behavioral observations

### *2.2.1. Olfactory discrimination test*

Females were given the choice between two feeding buckets for two minutes, one being associated with the odor of dog manure. Fifty grams of maize were placed in each bucket, without possible contact with the repellent in the contaminated trough. A female was considered to be behaviorally hyposmic if it consumed food from the contaminated bucket for more than 20 s.

### *2.2.2. Maternal selectivity test*

Each mother goat was tested for maternal acceptance of her own (or one of them, in case of twins) and an alien kid of similar age and appearance. Following a 10 min separation, the kids were presented separately for 5 min each, the alien being tested first. The frequencies of low (indicative of acceptance) and high-pitched bleats (indicative of rejection), acceptation and rejection at the udder, nursing duration and maternal aggression were focally recorded in the dam.

The mothers were classified in one of three categories, using definitions that gave a higher weight to aggressive and udder acceptance behaviors of the mother, and in which maternal vocal behavior was important only in the absence of these variables. These categories were as follows.

*2.2.2.1. Acceptance of the kid if*

– The mothers accepted the kid at the udder, and showed no aggressive behavior or if, in the absence of acceptance at the udder;

– They emitted more low-pitched bleats than high-pitched bleats and showed no rejection behaviors (rejection at the udder, higher frequency of high pitched bleats, or aggression).

*2.2.2.2. Rejection of the kid if*

– They did not accept the kid at the udder and showed either some aggressive behavior or rejection behavior at the udder or if, in the absence of these behaviors;

– They emitted more high-pitched bleats than low-pitched bleats and did not show any acceptance behavior.

*2.2.2.3. Ambiguous acceptance*

– All the other cases.

Finally, to obtain a global evaluation of maternal selectivity, an index of maternal selectivity was computed for each mother. The steps for computing this index were as follows. Firstly and for each variable, the values for the own and the alien kid were standardized together. Transformed values were then split to compute separately the indices of acceptance of the own and the alien kid, by summing up the values of all acceptance variables and subtracting rejection variables. The selective index was finally obtained by subtracting the acceptance index for the alien from that of the own young, resulting in the highest indices being indicative of higher maternal selectivity.

### *2.2.3. Postpartum sexual activity*

Starting 1 week after parturition, estrus behavior was monitored once a day up to 114 days postpartum, using an intact active buck fitted with an apron to avoid intromission. The male was introduced into each pen for 30 min. The spontaneous mounting behavior of the buck was observed during the corresponding time. A female was recorded as being in estrus when she accepted at least one mounting attempt by the male [19]. Postpartum anoestrus (PPA) duration was defined as the interval in days from parturition to the first recorded estrus behavior. An estrous cycle was defined as the interval between 2 non consecutive occurrences of sexual receptivity. Cycles were classified according to

their duration in short (< than 17 days), normal (between 17 and 25 days) and long cycles (> than 25 days) [20].

### 2.3. Statistical analyses

Due to the nature of the data (lack of normality or nominal data) the results were analyzed using non parametric statistics [21]. Bilateral probabilities were used.

## 3. RESULTS

### 3.1. Olfactory discrimination and maternal selectivity at 24 h

In all tests, including on day 56 of lactation, the 18 females from the $ZnSO_4$ treated group ate for more than 20 s in the trough contaminated with the repulsive odor, whereas none of the 24 control mothers ever did so. In fact, hyposmic mothers ate all the food from the contaminated trough in most instances while intact females never ate any food from that trough.

During the 5 min selectivity test at 24 h postpartum, in the intact group, all behaviors towards a mother's own kid differed significantly from those towards the alien kid (Wilcoxon test, $P < 0.05$ in all cases), while this was not the case in the hyposmic group. Also, when comparing the two groups, no differences were found for behaviors towards the mother's own young. On the contrary, behaviors towards the alien kid (frequency of acceptation at the udder, duration of nursing, frequency of aggressive behavior, proportion of maternal bleats/total bleats emitted) differed significantly between intact and hyposmic mothers (Mann-Whitney test, $P \leq 0.02$). In all cases, they indicated a better acceptance of the alien kid in hyposmic mothers (Tab. I).

When considering the various acceptance categories, 22 intact goats out of 23 accepted their own kid and one showed an ambivalent behavior, whereas only 4 accepted the alien; 17 rejected the kid and 2 showed ambiguous acceptance. In the group of hyposmic goats, 16/17 dams accepted their own young and one rejected their kid. Thirteen of the 17 mothers also accepted the alien kid, one showed ambiguous acceptance and 3 rejected the kid. The proportion of mothers accepting an alien kid was significantly higher in the hyposmic group (13/17 versus 4/23, Fisher exact $P < 0.001$).

The calculated selectivity score was significantly higher in intact goats (1.2 ± 1.9) than in hyposmic ones (–2.0 ± 2.9 ($P < 0.001$).

### 3.2. Postpartum sexual activity

In the control group, the mean date of first registered estrus was December 24 ± 7 days, while in the hyposmic group it was January 28 ± 7 days (mean ± sem), so that PPA was significantly shorter in intact mothers (68 days ± 7) than in hyposmic ones (93 days ± 7; Mann-Whitney $U = 131.5$, $P = 0.03$). The cumulated proportion of females showing estrus over the 16 weeks of the study differed significantly between the groups (Kolmogorov Smirnov test, MD = 0.463, $P = 0.02$; Fig. 1). In the intact group, 30 estrous cycles were registered, of which 2 were of normal duration (17 to 25 days), 24 were short cycles (< 17 days) and 4 were long cycles (> 25 days). In the hyposmic group, these figures were respectively 4/10, 5/10 and 1/10. The proportion of cycles of normal duration was significantly lower in the intact group than in the hyposmic group (2/30 vs. 4/10 respectively, Fisher exact $P = 0.026$).

Parity and litter size had no significant influence on PPA duration, although intact mothers nursing two kids showed a tendency to resume sexual activity earlier than the mothers of singles (60 days ± 10 vs. 78 days ± 10 respectively; Mann-Whitney U = 101.5, $P = 0.08$). Finally, in dams that showed estrus more than once, the cycle durations were not influenced by litter size or parity.

## 4. DISCUSSION

This is the first study concerning the effects of prepartum peripheral olfactory

**Table I.** Behavior (medians and inter-quartile ranges) of intact and hyposmic mixed-bred dairy goats towards the own and an alien kid during a test of maternal selectivity realized at 24 h postpartum. Hyposmia was peripherally induced one month before parturition by intranasal 2.5% $ZnSO_4$ irrigation. Intact and hyposmic goats showed different behavior toward the alien kid ($P \leq 0.02$ for all variables; Mann-Whitney test). Behaviors toward the goat's own kid were not different between the two groups ($P \geq 0.151$ for all variables; Mann-Whitney test).

| | Acceptation at the udder (frequency) | | Nursing time (s) | | Maternal bleats percentage | | Rejection at the udder (frequency) | | Aggressive behavior (frequency) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Own | Alien | Own | Alien | Own | Alien | Own | Alien | Own | Alien |
| Intact group ($n = 24$) | 2.0, [1.0, 2.5] | 0.0, [0.0, 0.0] | 16.0, [0.0,44.0] | 0, [0.0, 0.0] | 100, [80, 100] | 40, [10, 80] | 0.0, [0.0, 0.0] | 0.0, [0.0, 1.0] | 0.0, [0.0, 0.0] | 1.0, [0.0, 2.5] |
| Hyposmic group ($n = 18$) | 1.0, [0.0, 2.0] | 1.0, [0.0, 1.0] | 0.0, [0.0, 44.0] | 0.0, [0.0, 8.0] | 100, [100, 100] | 100, [100, 100] | 0.0, [0.0, 0.0] | 0.0, [0.0, 0.0] | 0.0, [0.0, 0.0] | 0.0, [0.0, 0.0] |

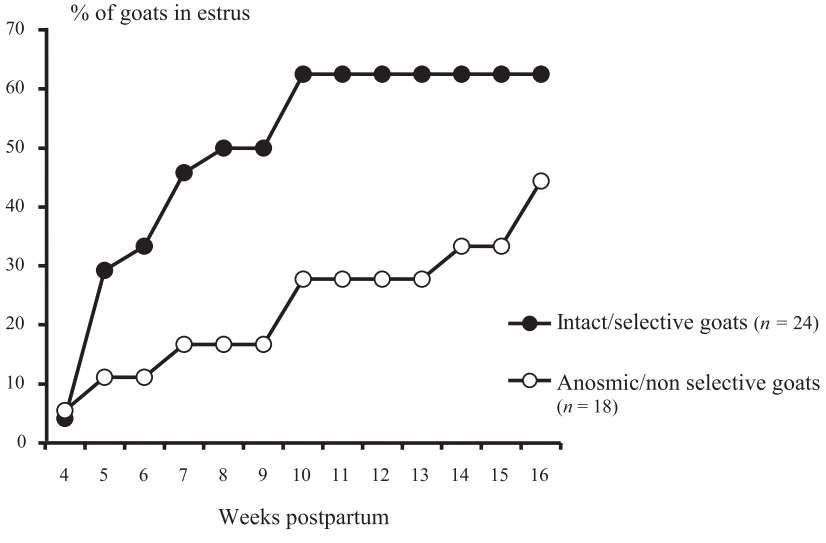


**Figure 1.** Cumulative percentage of females showing estrus behavior during the first sixteen postpartum weeks in intact and hyposmic nursing goats. (Significant difference between intact and hyposmic goats: = 0.02, Kolmogorov-Smirnov test.)

impairment on the recovery of postpartum sexual activity in nursing goats. The results did not support the hypothesis that preventing the establishment of the selective bond in nursing goats can result in a reduction of postpartum anoestrus duration, on the contrary to the findings reported in cattle, in which the nursing of an alien calf or impairing the perception of the calf's odor in intact mothers accelerates ovarian activity resumption or increases LH pulse frequency [10, 14]. There are several possible explanations for the apparent discrepancy in the regulation of postpartum anoestrus by olfaction

between goats and cattle. Firstly, this may reflect a difference between the two species, thus underlining the necessity of refraining from hasty extrapolations between species and the interest of comparative studies. Secondly, this discrepancy may also be due to several differences in the methodology used to evaluate the impact of selectivity impairment. In the present study, hyposmia took place before parturition and therefore inhibited the establishment of any selective bond. Nonetheless, it may have led to some compensatory process in the role played by the various sensory cues from the kid in the regulation of postpartum anoestrus. Even though visual and hearing recognition are not involved in selective nursing, they do exist very early after parturition [22, 23] and could play some role in the regulation of reproductive function recovery if olfactory cues are not available. By contrast, the studies performed in cattle concerned intact cows having already established a selective bond with their calves at least for the first 3 weeks postpartum. In these studies, depriving intact mothers of olfactory cues, either by tracheotomy or by presenting an alien calf may have resulted in a situation quite similar to a full mother-calf separation, thus mimicking more closely what would happen in the process of weaning and preventing any compensatory process. Also, in our study we measured the long term impact of the treatment on the global recovery of behavioral reproductive activity. On the contrary in the study of Griffith and Williams [10], only short term effects of calf olfactory cues deprivation on LH secretion were studied, while the effects on global estrous activity remain to be confirmed. Finally, it must be acknowledged that in our experimental situation, some additive confounding effect of hyposmia and lack of selective bond cannot be ruled out. Possible specific effects of anosmia are discussed in the next section. In any case, further studies comparing the various experimental conditions in the two species would be necessary to clarify whether the role of olfactory cues from the young in postpartum anoestrus differ between the two species.

In any case, the effects of hyposmia found in the present study are unlikely to be the result of disturbances in nursing behavior by hyposmia, since no differences were found between the two groups in spontaneous nursing frequencies and durations [18], as already reported in peripherally anosmic ewes [17].

Since the recovery of sexual activity was assessed through the detection of sexual receptivity by a buck, it could be argued that the duration of postpartum ovarian inactivity was over estimated, due to the possible existence of silent ovulations. While this cannot be totally ruled out, it must be stressed nevertheless that acceptation of the male reflects the ability of the female to resume reproduction which, at least in field conditions, necessitates acceptance of the male. Furthermore, it is well established in the goat that pre-exposure to a normal luteal phase is not an absolute requisite for estrus manifestation [24]. Indeed, during spontaneous seasonal variations of reproduction, there is a very close temporal association between ovulation and estrus behavior in this species [20] and this also applies for male effect-induced reproductive activity [25, 26] and postpartum sexual activity resumption [5]. Another factor which could theoretically account for the difference encountered between intact and hyposmic mothers, would be that the expression of sexual behavior had been impaired by hyposmia. In many mammals, some relation exists between olfaction, ovulation and sexual behavior [27, 28]. Various studies indicate that the percentage of anosmic sheep or goats ovulating and showing estrus behavior following a male effect is lower than that of intact females [25, 29]. At first sight, these results may appear to suggest that anosmia has some inhibitory effect on female sexual behavior expression. However, it must be pointed out that the lower percentage of estrus recorded in anosmic females is directly related to the importance

of olfaction for the induction of ovulation, and not with some inhibitory consequence of anosmia on the expression of sexual behavior. Indeed, in the study of Morgan et al. [29], while anosmic anoestrus females showed a lower ovulation and estrus response than intact ewes at the time of the male effect, the authors clearly state that all anosmic females cycled and became pregnant during the normal breeding season. Similarly, in the study of Chemineau et al. [25] while only 50% of anosmic goats responded to the buck effect, all those which responded showed sexual behavior with the same latency as the intact females.

Considering the various arguments mentioned above, we conclude that the difference of PPA duration found in the present study reflects a real effect of hyposmia on sexual activity resumption in nursing goats. Therefore, the presence of a selective bond is unlikely to be an important inhibiting factor of postpartum sexual activity in the goat. However at this stage, it is difficult to conclude that hyposmia actually lengthened PPA duration. Recently, it has been found that prepartum anosmia does affect PRL and oxytocin secretion during lactation in goats [18]. To which extent these changes affect the hypothalamo-pituitary-ovarian axis, and thus delay ovarian activity resumption, is not known. A possible alternative explanation would be that hyposmia prevented the perception of some olfactory cues from the buck that facilitated the resumption of sexual activity in intact dams. On the one hand, the progressive and widely spread resumption of estrous activity indicates that a true male effect (i.e. a rapid and synchronized triggering of ovarian activity in a majority of members in a group of anoestrus females) did not occur in our study. Moreover, the daily duration of contact with males was probably too short for such a teasing effect to occur [30]. On the other hand, chronic perception of olfactory cues from the males may have led to some advancing in the resumption of sexual activity in intact females, possibly through the diffusion from the males maintenance paddock situated at about 45 meters, or by buck scent-marking through urination and rubbing while inside the pens where the females were kept. For example, it is known that scent marks may remain active for as long as 7 days in some other ungulates such as the klipspringer antelope [31]. Such an interpretation would also be consistent with the fact that females maintained in permanent contact with bucks show shorter seasonal anoestrus than females totally isolated from males [32, 33], a phenomenon also found during postpartum anoestrus in cattle [34, 35]. It would also be congruent with the fact that cycles of abnormal duration were more frequent in intact females, which suggests that sexual activity had resumed before the reproductive system was fully ready to do so. Studying the recovery of ovarian activity through progesterone assay in intact and anosmic mother goats totally separated from any buck will be necessary to clarify this issue. In any case, these first results indicate that the use of bucks is a factor that should be taken into account when studying PPA duration, since it appears to have effects similar to those reported in seasonally anoestrous females. Also, given the little that is known about the relations between endocrinology of lactation, mother-young relationships and PPA in goats, further studies are also needed in these respects before comparisons with other species can be made.

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