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To cite this version:

Justine Alary, Bruno Patris, Catherine Husson, Benoist Schaal, Alexandra Destrez. Do lambs differentiate the odor of their mother from that of an alien ewe? Focus on inguinal wax. Applied Animal Behaviour Science, 2025, 283, pp.106496. 10.1016/j.applanim.2024.106496 . hal-04900951

HAL Id: hal-04900951
https://hal.inrae.fr/hal-04900951v1

Submitted on 20 Jan 2025

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Applied Animal Behaviour Science 283 (2025) 106496

Contents lists available at ScienceDirect

Applied Animal Behaviour Science

journal homepage: www.elsevier.com/locate/applanim

Do lambs differentiate the odor of their mother from that of an alien ewe? Focus on inguinal wax

Justine Alary [a], Bruno Patris [a], Catherine Husson [b], Benoist Schaal [a], Alexandra Destrez [a,b,*]

[a] Centre des Sciences du Goût et de l'Alimentation, CNRS, INRAE, Institut Agro, Université de Bourgogne, Development of Olfactory Communication and Cognition Lab, Dijon F-21000, France
[b] Institut Agro Dijon, Dijon 21000, France

ARTICLE INFO

Keywords:
Sheep (*Ovis aries*)
Inguinal glands
Olfaction
Preferences
Suckling
Mother-infant communication
Maternal behavior
Neonate

ABSTRACT

Ewes bear scent glands located near the udder which smelly secretion is interesting to newly-born lambs searching a teat to suck on. This glands' secretion – inguinal wax (IW) – was found to be reactogenic to lambs before they initiate suckling from the dam. The present study aimed to further assess whether IW odor affects two vital aspects of newborn lambs' behaviour: orienting and engaging suckling. First, forty-six lambs were tested in two-choice odor tests contrasting i) own mother's Inguinal Wax (IW-M) vs. unfamiliar mother's Inguinal Wax (IW-nM), ii) IW-M vs. control and iii) IW-nM vs. control, to measure their differential orientation to maternal IW odor presented against either IW from another dam or a control. Second, a bottle-feeding test (n = 41 lambs) assessed lambs' elicitation of suckling from IW-odorized vs. control bottles. Relative to the control, IW odor enhanced lambs' orientation during the two-choice odor test, but without differentiation of maternal IW from non-maternal IW. Otherwise, lambs did not engage more suckling on a bottle when odorized with maternal IW relative to a control bottle. Some aspects of lambs' responses were differentiated by sex, but this effect was independent on odor condition. In sum, this preliminary study indicates that ovine IW is somehow inherently attractive to lambs before they associate it with suckling.

1. Introduction

The survival of mammalian neonates depends on the expression of care by postparturient females, the ingestion of colostrum by offspring and the rapid establishment of a mutual bond among both partners (e.g., Nowak et al., 2000; Rosenblatt and Snowdon, 1996). In these primary interactions, mothers produce colostrum/milk and drive their offspring toward their nipples or teats (depending on species). These first vital interactions are affected by the neonates' motor abilities driven *a minima* by chemoreception and facilitated by the females' posture and multisensory stimulations, especially those mediated by touch and olfaction (e.g., Arteaga et al., 2013; Blass, 1990; Schaal, 2010; Schaal and Al Aïn, 2014). Odor-active compounds sourced in, on or around the mammary structures motivate and direct newborn offspring's movements (Rosenblatt, 2010; Schaal, 2014).

These functional principles stem mostly from studies on species with altricial neonates, which vision and audition are not functional at birth (e.g., rats, mice, cats, dogs; Arteaga et al., 2013; Blass, 1990; Schaal and Al Aïn, 2014; Rosenblatt, 1983). Less is known about semi-altricial (i.e., born with all senses functional to some extent, but no motor or thermogenic autonomy) newborns, who benefit of early visual and auditory inputs. However, research in human neonates (semi-altricial primates) indicates that functional audition and vision does not at all lessen the contribution of olfaction in the behavioral controls of neonates (e.g., Porter and Schaal, 1995; Schaal et al., 2020).

Even less is understood about how newborns of the precocial type - such as lambs, kids or calves, with motor/thermogenic autonomy and all senses functional among which vision and audition prevail - do explore the mother's body before finding a teat; what is then the part played by odors in successful suckling; how they rely on olfaction to elaborate their first perception on their mother. Focusing on *Ovis aries* as a biological model bearing precocial neonates, research was accordingly rather vision- and audition-centred in understanding the lambs' relationship with the dam (e.g., Ligout and Porter, 2004 a,b; Ligout, 2004; Sèbe et al., 2010, 2011; Nowak, 2006; Nowak and Poindron, 2006; Vince et al., 1985, 1986, 1987), with only a handful of studies showing interest in

* Corresponding author at: Centre des Sciences du Goût et de l'Alimentation, CNRS, INRAE, Institut Agro, Université de Bourgogne, Development of Olfactory Communication and Cognition Lab, Dijon F-21000, France.
E-mail address: alexandra.destrez@agrosupdijon.fr (A. Destrez).

https://doi.org/10.1016/j.applanim.2024.106496
Received 27 September 2024; Received in revised form 18 December 2024; Accepted 27 December 2024
Available online 28 December 2024
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olfaction (Vince and Ward, 1984; Vince et al., 1984, 1987; Schaal et al., 1995). But the domestic sheep is profusely odorous, emitting multiple excretions and secretions derived from species-specific metabolism and surface processes on skin and in wool (Lyne and Hollis, 1968). After parturition, ewes convey additionally amniotic fluid, blood, colostrum and milk, while on each side of the udder a pair of active glands, so-called inguinal glands, excrete a smelly wax. Due to their adjacency to the teats and the strong orientation effects of this inguinal wax (IW) on newly-born lambs (Vince and Ward, 1984), it was credited to play a special role in nursing (Vince, 1993; Alexandre-Pires et al., 2017; Schaal, 2010). Among these ovine body odors, lambs may indeed be eased in their way to a teat by the synergistic odor-heat combination at the ewes' inguinal and mammary areas. Lambs reacted positively to the IW odor right after birth, before its association with suckling (Vince and Ward, 1984). When lambs were anosmized, their time to seize a teat increased significantly (Vince et al., 1987), ascertaining that olfaction is involved in early suckling. Following Vince and Ward's (1984) seminal study, IW odor was acknowledged to play a role in suckling initiation and in postnatal thriving (e.g., Mora-Medina et al., 2016; Nowak, 2006; Schaal, 2010, 2014) although little additional empirical research was conducted based on this finding (i.e., Rietdorf, 2002, Alexandre-Pires et al., 2017; Alary et al., 2023).

The present study aims to further explore the role of IW odor on lambs' adaptive behavior in the species-atypical suckling context. The study focussed on hand-rearing systems developed to accommodate prolific breeds, which produce more lambs than an individual ewe can optimally sustain (Abraham and Thomas, 2012). Consequently, lambs, from litters exceeding two newborns, are separated at birth to be reared by hand. These mother-deprived lambs endure intense emotional, physiological and nutritional stress, and undergo therefore higher mortality than maternally reared lambs (David et al., 2014). We tested the ability of lambs to detect the odor of IW compared to control, discriminate own vs. unfamiliar IW and accept a feeding bottle filled with ovine colostrum. We hypothesized, following Vince and Ward (1984) in Clun Forest lambs, that IW odor will elicit interest and positive orientation in Romane lambs. Vince & Ward further found that IW conveyed two chemomessages, a species-specific one (response to any IW odor) and an individual-specific one (preferential response to own dam's IW). Therefore, we hypothesized that the IW odor might favor the lambs' engagement of suckling of an artificial teat and that the lambs will discriminate the maternal IW from the non-maternal IW. Sex-related behavioral or cognitive variations are often reported in sheep breeds, sometimes confounded with other sex-bound factors (such as birthweight, stress reactivity). For example, male Suffolk lambs are slower than females to stand, seek the udder and efficiently suck (Dwyer, 2003). More generally, female lambs are more active than males after birth and succeed earlier at suckling initiation (e.g., Abecia et al., 2022; Dwyer and Lawrence, 1999; Freitas-de-Melo et al., 2015), probably in relation with their higher survival rate (Burfening and Carpio, 1993; Gama et al., 1991; Huffman et al., 1985; Mandal et al., 2007; Nowak and Poindron, 2006). Hence, we hypothesized that female lambs will be more reactive to IW odor as a maternal odor factor potentially involved in survival.

2. Animals, material and methods

2.1. Ethical note

The present study took place in a commercial farm attached to the Charolles Agricultural high school (Burgundy, France). The study was run in the context of local management of breeding, lambing and artificial rearing of lambs. French sheep breeders being strictly enforced to follow French laws on animal welfare (Code rural et de la pêche maritime, chapter IV, Articles L214–1 to L214–23), the experimenters were constantly supervised by the farmer or her associates with respect to animal handling which anyway followed the principles stated in the 86/609/EEC European Community Directive regarding the treatment of animals used for experimental and other scientific purposes.

The experimental procedure which consists of exposing lambs to odorous stimulation was approved by the local ethics committees (Animal Experimentation Ethics Committee, University of Burgundy, Dijon, France, CEEA N°105).

2.2. Animal handling in the farm

The artificial rearing was part of the standard rearing methods in usage at the Charolles farm. Due to selection for multiple births in Romane and crossbred ewes, and risk of insufficient milk yield in dams bearing triplets or more, surnumerary neonate lambs were removed from them, kept in small groups in separate pens to be fed artificially (after first colostrum intake under the mother) and followed-up for health and growth. Hence, two lambs remained with any ewe and the other lambs (surnumerary lambs) were directed to artificial rearing based on the farmer's breeding method, following a criterion of body weight homogeneity of lambs remaining under the mother. In the present study, the initial colostrum feed was not drunk under the ewe but was hand milked from the dam and then given by bottle to the lambs as part of a test assessing the driving potency of maternal odor on first colostrum ingestion. In the present husbandry conditions, the farmer did not observe any difference in health issues between lambs separated from the dam and bottle-fed and lambs staying with their mother.

2.3. Animals and housing

The experiment was carried out in August and September 2020 in a flock of 199 ewes (Romane x Charolais crossbreed). Two hours before lambing (when the ewes separated from the flock), pregnant ewes were installed in individual lambing pens (1×1.5 m) where they remained with their two lambs for 2 days postpartum, before being re-grouped with other ewes and lambs. Lambing was constantly watched by experimenters so that new-born lambs could be submitted to the behavioural tests before any suckling experience, but after their mother licked them for 15–30 min after birth. Before these tests, the lambs were weighed on a scale (Terraillon, Croissy-sur-Seine, France; Precision: 1 g) by the same woman experimenter.

2.4. Inguinal wax collection

For both behavioural tests, inguinal wax (IW) was collected in the lambing pens just after the end of the licking by the ewe (15–30 minutes following lambing). For the two-choice odor test (described in Section 2.4), sampling was made manually using brown cotton pads (100 % cotton percale, Tex, 10 x 10 cm) previously spread with a dab (2 g) of petroleum jelly (Vaseline, Merck) to limit the effect of friction and facilitate IW adhesion. These pads were rubbed by gloved hand over the ewes' inguinal glands situated bilaterally of the udder. The IW secretion was then spread by folding the pad and pressing it inside the pad, before being put in air-tight plastic bags and kept in the refrigerator (4°C) for a maximum of 12 hours. Between each sampling, the experimenters' disinfected their hands (stericid gel, Steridis) and used new nitrile gloves (Eco nitrile PF 250, EcoShield).

For the bottle-feeding test (described in Section 2.5), the IW sampling procedure was the same, except that the pads (100 % cotton percale, Tex) were strips of cotton cloth of 30 x 3 cm to be fixed around bottles.

2.5. Two-choice odor test

2.5.1. Animals

Sixty just-born lambs (30 males, 30 females) were tested before they did engage suckling their dam. They were born between August, 17 and September, 06 (Saturdays and Sundays excepted) and between 07.00 a.

m. and 08.30 p.m. When the lambs were able to stand by themselves on four legs, they were carried to a test room adjacent to the sheepfold.

2.5.2. Test setting and device

The testing room was phonically isolated from bleating ewes. Individual lambs were placed into a 120-length x 100-width x 75 height-cm brown plastic box lined with straw to avoid slipping. Within this test box, the lambs were put in a Plexiglas device supporting paired odor stimuli conveyed by two pads disposed symmetrically at muzzle's height of a standing lamb (Fig. 1). These odor pads were heated to the body temperature of an ewe (i.e., 38–40°C) using a pocket heater (Rechargeable Hand Warmer, 1800 mAH, LBM) affixed bilaterally on the Plexiglas walls (Fig. 1). In an attempt to attenuate their separation stress during the tests (Schaal et al., 1995), the lambs faced a paper picture (40 x 58 cm) of an unfamiliar ewe while low-pitched bleats of a maternal ewe (not the mother) were played-back with a smartphone.

2.5.3. Stimuli and design

The lambs faced a pair of stimuli presented symmetrically at a distance of about 10 cm from muzzle at test onset. The odor stimuli consisted in IW sampled from their own postparturient mother (IW-M) or from an alien maternal ewe (IW-nM) at the same stage of periparturition. The control stimuli consisted in a brown pad spread with the same amount of petroleum jelly, but devoid of IW. Despite the sixty lambs were taken from their mother when they were able to stand, 14 of them had to be excluded because they later laid down or showed strong signs of stress (agitation, high-pitched bleating, turning opposite to the stimuli) during 1-min test.

Three pairs of stimuli pad were assayed in three tests considering different groups of lambs in order to assess i) their relative responsiveness between IW-M and IW-nM (16 lambs: 8 males, 8 females), and their absolute responsiveness ii) between IW-M and the control stimulus (15 lambs: 8 males, 7 females) and iii) between IW-nM and the control stimulus (15 lambs: 7 males, 8 females). The lambs were randomly allocated to the three groups following the lambing period i.e., the first-born lamb was allocated to the "IW-M/control" group then the second born lamb to the "IW-nM/control" group then the third lamb to the "IW-M/IW-nM" group, and so on.

2.5.4. Procedure

The two-choice odor test was adapted from similar assays perfected for ovine neonates (Vince and Ward, 1984; Schaal et al., 1995). Before the test, the odor pads were left on the heaters for 5 min to equalize their temperature. The lamb was gently positioned into the test device with the muzzle aligned on the midline between the two stimuli, at about 10 cm from them. The lateral position of the stimulus pad was inverted at each test, so that an equal number of lambs were exposed to a left vs. right lateral arrangement. The lamb was then left free to explore both odor pads for 1 minute which was video-recorded (Hero7 Black, GoPro) for subsequent off-line analyses. Between each two-choice odor test, the stimuli pads were changed, the Plexiglas device cleaned with alcohol and water, and the lining of the test-box renewed with clean straw.

2.5.5. Behavioural variables: definitions and coding

The video-records of the tests were analysed by the same trained coder who was unaware of the nature and presentation side of the paired stimuli. The behaviour items of interest (see below) were visualized from a computer screen and timed with the computer's internal clock (accuracy: 0.1 s) through the BORIS software (version 8.13, UniTo; 2016). The duration of head turning to either stimulus and the duration of muzzle direct contact with either stimulus was coded by tracking the tip of the lamb's muzzle in the accessible space over and between both stimuli. Any deviation of the cephalic midline was considered positive when the muzzle was positioned toward that stimulus pad, i.e., when both nares deviated from the experimental device's midline to the right or left sides. For each lamb, the orientation duration to a given stimulus was reported as the sum of all orientations toward that stimulus during a test. Following the above procedure, two variables were derived from the lambs' responses to the paired odor stimuli: i) the duration of head orientation to either stimulus and ii) the duration of muzzle in direct contact with either stimulus. These two variables were analysed during the 1-min test.

2.6. Bottle-feeding test

2.6.1. Animals

After the two-choice odor test, forty-one other neonate lambs (14 males, 27 females) were tested directly in their lambing case (with their mother and siblings) to avoid any emotional stress factors and to follow the farm practices (Fig. 2). As in the previous test, the lambs were tested when they were able to stand up on four legs and before they engaged their first suckling episode. The tested lambs were born between September, 06 and September, 17 (Saturdays and Sundays excepted) and between 07.00 a.m. and 08.30 p.m. The sex ratio of the group reflected the sex ratio of this lambing period.

2.6.2. Stimuli and design

This test took advantage of the first bottle-feeding of the lambs that were directed to artificial feeding on the farmer's decision. The bottles (lamb feeding bottle, anti-vac, Kerbl, Germany; with rubber nipple) were prepared with a content of 150 ml of colostrum milked from an unfamiliar ewe. The colostrum was warmed at 37°C in a water bath (Garcia Gonzalez and Goddard, 1998). Each bottle was fitted with a brown cotton pad (30 x 5 cm) tied around the bottle neck so that the considered odor stimulus was right under the lambs' nares. Two groups of lambs were considered: i) a group exposed to their postparturient

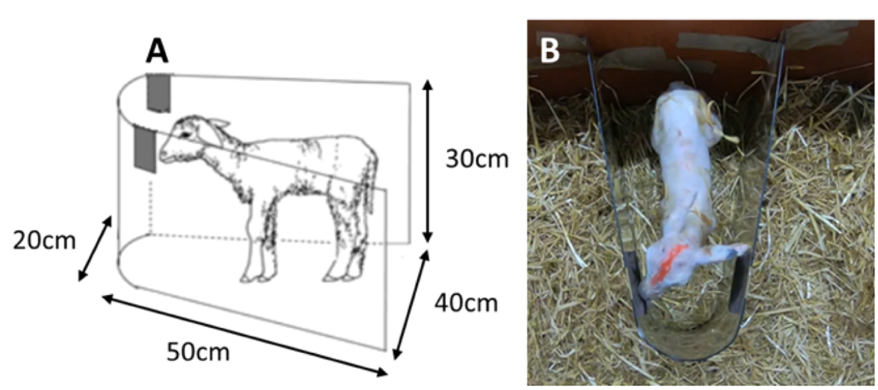

Fig. 1. Design of the two-choice odor test composed of a Plexiglas wall on which a pair of pads (brown cotton squares) was affixed: A. Dimensions and B. Screenshot from above showing a lamb with its muzzle in contact with a pad.

Fig. 2. Screenshot of the bottle-feeding test.

mother's IW mixed with petroleum jelly (IW-M group), composed of 19 lambs (5 males, 14 females) and ii) a control group composed of 22 lambs (9 males, 13 females) exposed to pure petroleum jelly. The lambs were randomly allocated to the two groups following the lambing period.

2.6.3. Procedure

The bottle-feeding test was adapted from a similar test situation (Alary et al., 2023). During the test, a woman experimenter (the same for all the tested lambs, wearing no perfume, Fig. 2) who was unaware of the stimulus presented (i.e., IW-M or control) held the lambs between her legs with one hand gently maintaining its mandible and the other hand presenting the bottle. Each assay comprised a minimum of 1 trial and a maximum of 10 trials, with a minimal inter-trial duration of 10 s. One trial consisted in introducing the bottle teat into the lamb's mouth for 3 s and assessing its spontaneous suckling response to the teat. A trial resulted in either a 'success' (when the lamb sucked actively before the end of the 3 s) or a 'failure' (when the lamb did not suck the teat before the end of 3 s). When a lamb experienced a failure, a new trial began 10 s after the end of the previous one. When a lamb experienced a success, the experimenter let the lamb ingested all the colostrum he or she wanted. Hence, the suckling duration (in s) was measured during the success trial and started when the lambs started to suck actively the bottle teat and stopped when they stopped to suck (during 3 s at least). The quantity of the colostrum ingested (in g) during the suckling period was measured during the success trial by weighing the colostrum in the bottle before and after the suckling (scale "pèse bébé évolutif", Terraillon, Croissy sur seine, France) and by calculating the weight difference. The test ended when the lamb achieved a success or 10 failures.

The test was video recorded (Hero7 Black, GoPro). The behaviour items of interest (see below) were visualized from a computer screen and timed with the computer's internal clock (accuracy: 0.1 s) through the BORIS software (version 8.13, UniTo; 2016).

2.6.4. Behavioural variables: coding and definitions

Four variables were recorded from this assay: the number of trials needed to achieve a 'success' in suckling (described in procedure, when the lamb sucked actively before the end of the 3 s), the quantity of colostrum ingested during the suckling period, the suckling duration and the suckling speed during this suckling period.

The suckling speed (in $g.s^{-1}$) was calculated by: $\frac{\text{the quantity of the colostrum ingested}}{\text{the suckling duration}}$.

2.7. Statistical analyses

As the within-subject data from the two-choice odor tests did not follow a normal distribution, the Wilcoxon test was used to compare the duration of lambs' head orientation toward, and duration of muzzle contact with, the odor stimuli. Then, separate analyses looked for sex effects. For the bottle-feeding test, between-subject data were analyzed using the Mann-Whitney test to assess the IW effect on our four variables (number of trials, quantity of colostrum ingested, suckling duration and suckling speed). We then tested for a sex effect on these variables. For both behavioral tests, the liveweights were analyzed using the Mann-Whitney test to assess the sex effect. All statistical analyses relied on the XLSTAT software (version 2015.3.01.19097; Addinsoft, Paris, France). The limit of significance was set at $p < 0.05$.

3. Results

3.1. Two-choice odor test

3.1.1. Odor effect

Neither the duration of lambs' head orientation, nor the duration of muzzle contact, differed significantly in the test contrasting the odors of IW-M and IW-nM (V=79, p = 0.6; Fig. 3a; V=81, p = 0.5; Fig. 3b, respectively). However, the lambs spent significantly more time oriented toward the IW-M pad when presented against the control pad (V=103, p = 0.01; Fig. 3a), without differential muzzle contact with either of these stimuli (V=49, p = 0.2; Fig. 3b). Likewise, they tended to spend more time oriented toward IW-nM pad than toward the control pad (V=92, p = 0.07; Fig. 3a), but without difference in muzzle contact toward these stimuli (V=65, p = 0.2; Fig. 3b).

In sum, while IW-M and IW-nM were more attractive than the controls for the whole group of lambs, they did not choose preferentially IW-M over IW-nM when both of these stimuli were presented simultaneously.

3.1.2. Sex effect

The durations of head orientation toward the stimuli (Fig. 4) and of muzzle contact on the stimuli (Fig. 5) were not significantly different between IW-M and IW-nM stimuli in female lambs (respectively V=21, p = 0.7 and V=18, p = 0.9) and in male lambs (respectively V=21, p = 0.7 and V=24, p = 0.5). Regarding lambs' responsiveness in the tests opposing IW-M or IW-nM with the control stimuli, the subgroup of females did not react differentially to IW or the control (IW-M vs. control test, orientation duration: V=24, p = 0.1; muzzle contact duration: V=19, p = 0.8; IW-nM vs. control test, orientation duration: V=23, p = 0.5; muzzle contact duration: V=12, p = 0.7; Fig. 4a et 5a). In contrast, male lambs' responses between IW-M and the control reached significance in duration of muzzle contact with IW-M (V=36, p = 0.008; Fig. 5b), but not in orientation duration (V=30, p = 0.1; Fig. 4b). Males also spent significantly more time orienting to IW-nM than the control (V=26, p = 0.047; Fig. 4b) and more time in muzzle contact with IW-M than the control (V=21, p = 0.028; Fig. 5b).

The liveweights were not significantly different between male and female lambs of each group (IW-M vs. IW-nM: females: 3.7 ± 0.5 kg and males: 4.0 ± 0.8 kg, U = 23, P = 0.61; IW-M vs. control: females: 4.0 ± 0.5 kg and males: 3.8 ± 0.9 kg, U = 26, P = 0.85; IW-nM vs. control:

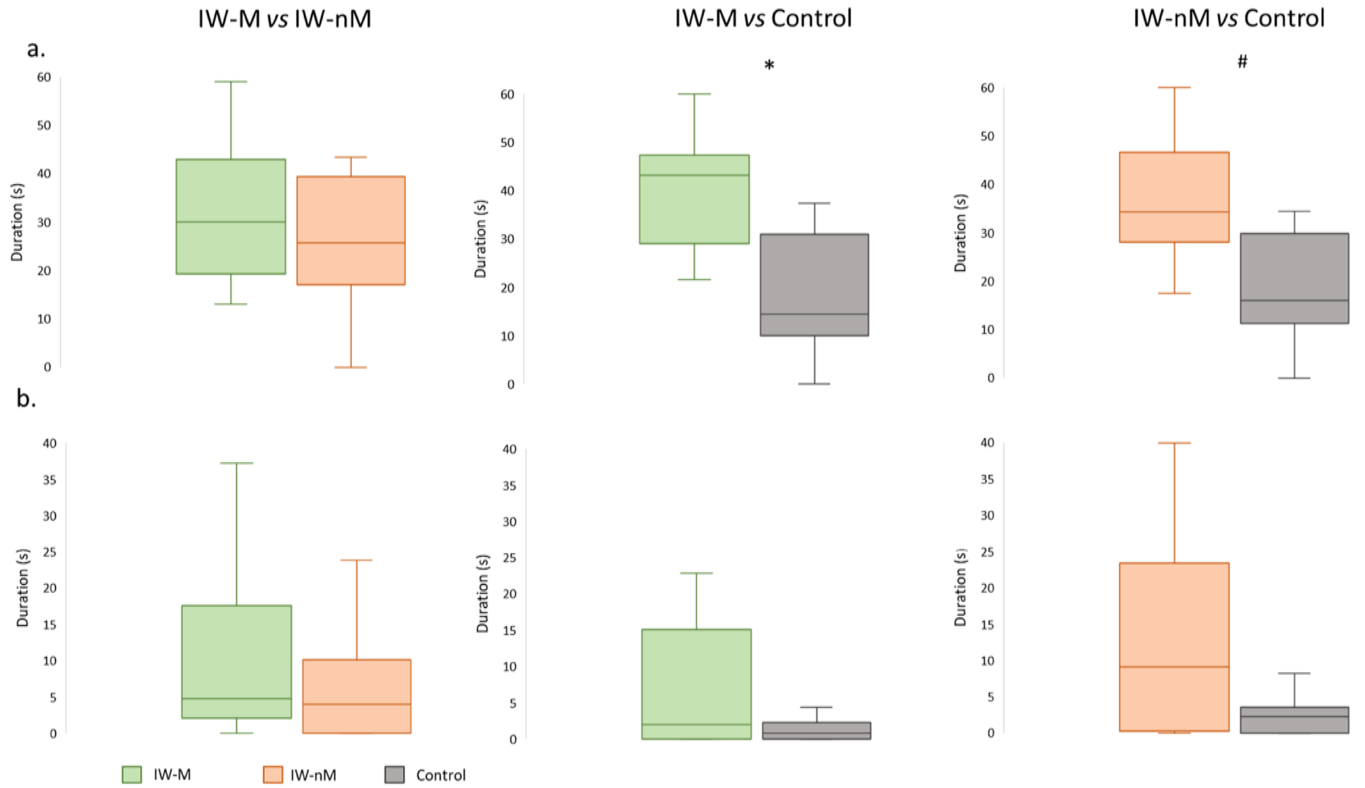

Fig. 3. Responses of lambs, during the two-choice odor test, to either stimulus presented in three pairs: Own Mother's Inguinal Wax (IW-M) vs. Unfamiliar Mother's Inguinal Wax (IW-nM), IW-M vs. control, and IW-nM vs. Control. **a.** Median duration (s) of head orientation to either stimulus; **b**. Median duration (s) of muzzle contact with either stimulus (Wilcoxon test, *: p < .05; #: 0.5 >p > .01).

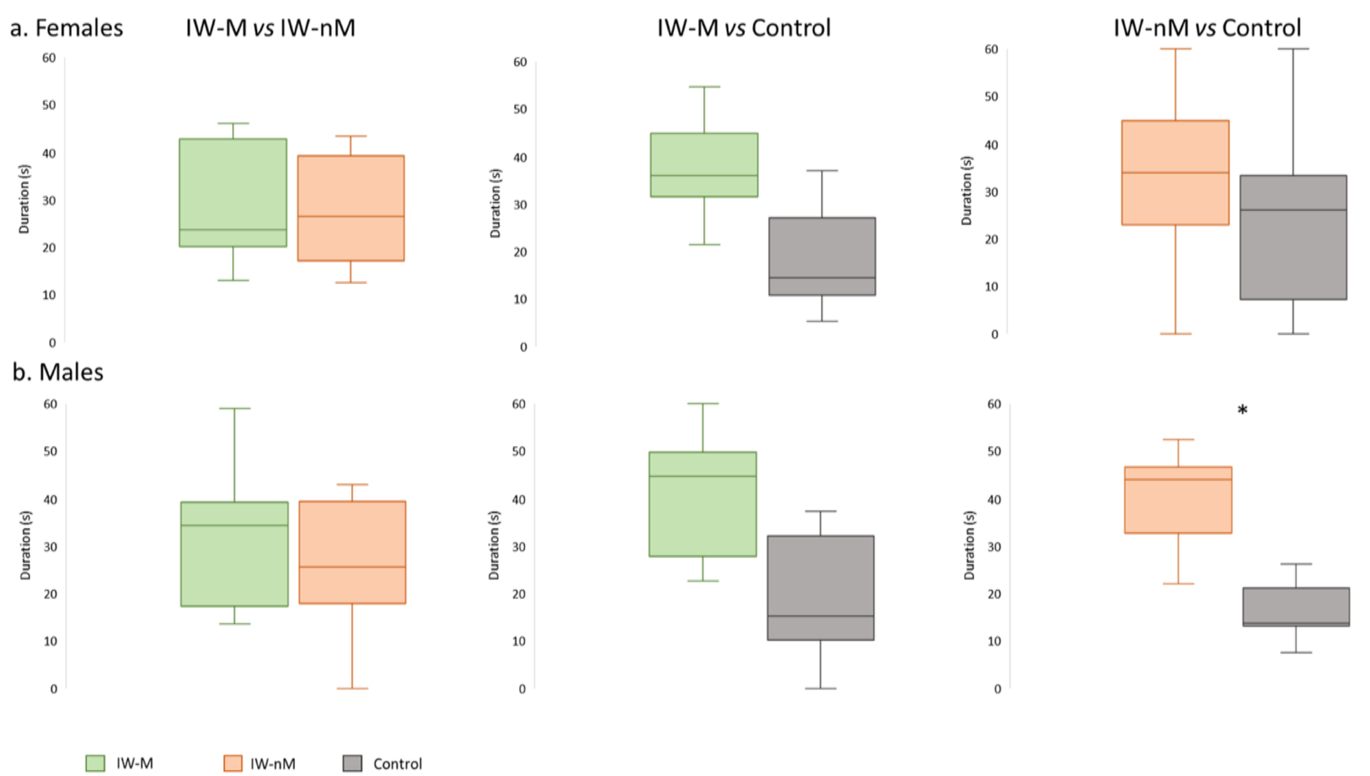

Fig. 4. Head orientation duration (s) toward the stimuli (Own Mother's Inguinal Wax: IW-M, Unfamiliar Mother's Inguinal Wax: IW-nM and Control) during the two-choice odor test for **a.** female lambs (group IW-M vs IW-nM: n = 8; group IW-M vs control: n = 7; group IW-nM vs control: n = 8) and **b.** male lambs (group IW-M vs IW-nM: n = 8; group IW-M vs control: n = 8; group IW-nM vs control: n = 7) (Wilcoxon test, *: p < .05; #: 0.5 >p > .01).

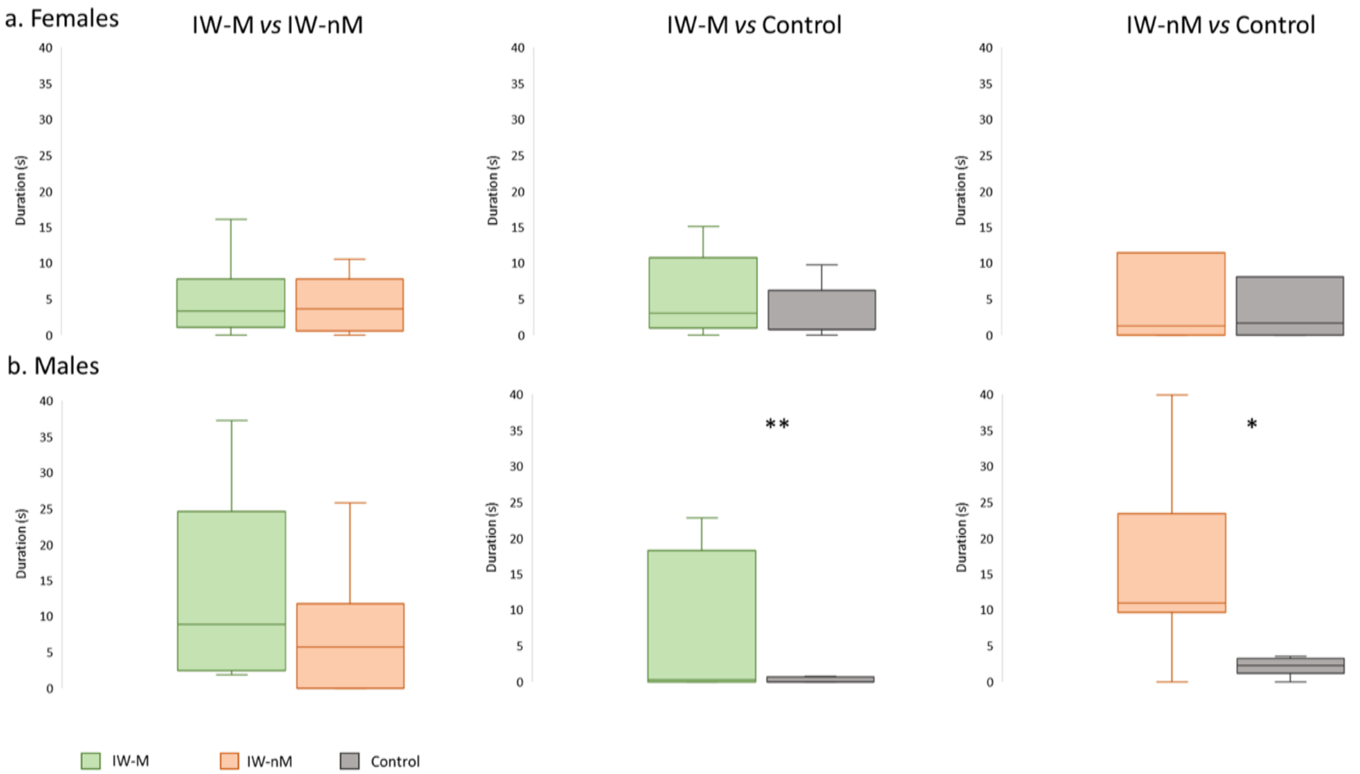

Fig. 5. Muzzle contact duration (s) on the stimuli (Own Mother's Inguinal Wax: IW-M, Unfamiliar Mother's Inguinal Wax: IW-nM and Control) during the two-choice odor test for **a.** female lambs (group IW-M vs IW-nM: n = 8; group IW-M vs control: n = 7; group IW-nM vs control: n = 8) and **b.** male lambs (group IW-M vs IW-nM: n = 8; group IW-M vs control: n = 8; group IW-nM vs control: n = 7) (Wilcoxon test, *: p < .05; #: 0.5 >p > .01).

females: 4.0 ± 0.7 kg and males: 4.0 ± 0.6 kg, U = 29, P = 0.96).

3.2. Bottle-feeding test

3.2.1. Odor effect

The odor on the bottle was without significant effect on the number of trials needed to engage successful suckling, on the quantity of colostrum ingested, on the duration of suckling and on the suckling speed (Table 1).

3.2.2. Sex effect

Regardless of the odor put on the bottle, female lambs needed significantly less trials than male lambs to engage suckling the artificial teat. They also tended to ingest more colostrum than the males (Table 2). However, the duration of suckling and suckling speed were not significant affected by sex (Table 2). Another analysis comparing the odor condition by sex subgroup [females/males on IW-M odorized bottle (n = 14 and 5, respectively), females/males on control bottles (n = 13 and 0)] did not lend significant differences between subgroups.

The liveweights were not significantly different between female and male lambs (respectively 3.6 ± 0.9 kg vs 3.2 ± 0.6 kg, U = 240, P = 0.17).

Table 1
Odor effect (IW-M odor vs. control stimulus) on the number of trials needed to display suckling on the artificial teat, suckling duration, quantity of colostrum ingested and suckling speed.

Variables	IW-M (n = 19) Mean ± SE	Control (n = 22) Mean ± SE	U	*p*-value
Number of trials	1.6 ± 0.2	1.8 ± 0.4	217	0.80
Suckling duration (s)	92.8 ± 12.0	90.4 ± 10.3	210	0.99
Quantity of colostrum (g)	85.8 ± 8.4	93.0 ± 8.7	185	0.54
Suckling speed (g.s^{-1})	1.1 ± 0.1	1.2 ± 0.2	187	0.60

Table 2
Effect of lamb's sex on the. number of trials needed to display suckling on the artificial teat, suckling duration, quantity of colostrum ingested, and suckling speed.

Variables	Females (n = 27) Mean ± SE	Males (n = 14) Mean ± SE	U	*p*-value
Number of trials	1.3 ± 0.1	2.6 ± 0.5	119	**0.02**
Suckling duration (s)	91.8 ± 8.2	91.0 ± 16.8	200,5	0.76
Quantity of colostrum (g)	97.4 ± 6.3	74.8 ± 12.3	250	*0.097*
Suckling speed (g.s^{-1})	1.3 ± 0.1	0.9 ± 0.1	246	0.12

4. Discussion

Our study aimed to investigate: **1/** the effect of the odor of IW emitted from postparturient (mother or alien) ewes on newly born lambs' teat-searching behavior and **2/** the effect of lambs' sex on these behavioural responses.

As to ***lambs' discriminative responsiveness toward maternal IW***, two alternative hypotheses were evaluated, either IW odor from own dam is more reactogenic than IW odor from any other postparturient ewe due to its greater familiarity, or lambs do not react to individual-specific cues conveyed in maternal IW because of co-occurring more salient odor factors. The results from the two-choice odor test inclines toward the second alternative, as lambs did not respond discriminatively in terms of head orientation toward, or muzzle contact with, the odors of their own mother's IW when paired with the IW from an alien dam. This result does not pertain to an incapacity of lambs to detect IW odor, as they were indeed mostly attracted to IW odors in the tests opposing them with the control stimulus. Suckling-inexperienced lambs may also differentiate the scent of their mother's IW from that of an alien dam but did not show it in our choice test. Our experimental setting was challenging for the new-born lambs that were put in an arena where they had to orient the head to the left or right. Consequently, the general attractivity of IW prevailed over its familiarity (own vs. alien). Vince and Ward (1984) found discriminative (behavioral and autonomic) responsiveness of suckling-naïve lambs to own mother's vs. alien mother's IW odors. Several differences among this study and the present study might explain such discrepancy, such as the IW sampling method (IW in petroleum jelly in the present study vs. "pure" IW in Vince & Ward), the experimental design (paired vs. serial presentation of stimuli), the nature of the response variables recorded (directional head movements vs. licking/heart-respiratory rates), the posture required from the tested lambs (standing vs. supported) and the breed of sheep investigated (Romane x Charolais crossbreed vs. Clun Forest; for breed effects on newly-born lamb behavior: Dwyer et al., 1996). Another study reports a capacity for odor-based individual recognition of agemates or twins in 4–5-day-old lambs (Ligout et al., 2004), suggesting that several days of interindividual experience may establish such social recognition performance more robustly. Likewise, lambs of the present ovine breed may need suckling experience and co-occurring exposition to maternal IW odor to be able to discriminate their dam's individual odor signature. Anyway, the present study needs replication in comparing a lambs' response to IW odor as a function of amount of exposure during suckling.

Moreover, the present results suggest that odor cues in the IW from categories of social meanings might supersede those from individual identity or familiarity meanings. Such categories of social meaning would apply to factors that are common to all sheep, such as, e.g., genetics underlying odor emission/reception in individuals of the breed or flock (inbreeding), an evolved chemosignal active on *Ovis aries* neonates, or prenatal priming by the same nutritional ecology. For example, odorous components of IW do also occur in ovine amniotic fluid which collects nutrient metabolites to which lambs have been already exposed prenatally from the fodder eaten by pregnant ewes (Rietdorf, 2002; Schaal et al., 1995). Also, as in other species of mammals (Schaal, 2010; Schaal and Al Aïn, 2014), one cannot exclude that ovine IW might convey a species-specific chemosignal that overpowers any idiosyncratic experience in the earlier or current odor environment. In sum, much like other mammalian excretions, ovine IW may encode multiple informational regularities that neonates can possibly process in parallel to organize developing social cognition. These various, probably redundant, IW-mediated mechanisms of chemo-communication now await detailed investigation.

As to ***IW-related facilitation to engage suckling an artificial teat***, it was predicted that lambs should more rapidly/efficiently suck any object impregnated with an attractive odor. In the case of IW odor, this prediction was not verified in the present conditions: the lambs did not suckle more readily or more insistently, or with more efficiency in terms of volume of fluid extracted, during our test with an IW- scented bottle than with a control bottle.

One possible explanation of this outcome resides in the fact that both bottles contained ovine colostrum, which odor may have leaked and competed with IW odor on the bottles. Species-specific colostrum odor is indeed attractive to matching neonates (e.g., Al Aïn et al., 2015; Klaey-Tassone et al., 2021) due, as well as for IW, to prenatally-experienced odor cues or to an experience-independent predisposed chemosignal. This applies also to lambs who prefer ovine milk to any other fluid (e.g., Belanche et al., 2019; Sevi et al., 1998). In the present experimental conditions, the test-bottles may thus have conveyed odor agents and probably also taste agents from ovine colostrum which may have eclipsed the orthonasal salience of IW odor on the bottles. In previous studies investigating orthonasal odor effects on neonates' nutritive suckling patterns from a bottle, its content was indeed chosen to be species-atypical (artificial formula; synthetic odorant) or more or less neutral (water) (e.g., Mizuno & Ueda, 2004; Delaunay-El Allam et al., 2010; Malidaki and Laska, 2018). This experiment should thus be replicated with bottles containing non-specific or scentless fluids.

Finally, the task expected in our tests to be performed by the lamb was a substantively different one from a situation where the lamb has to identify a teat and engage with it without direction. Thus, further tests on suckling engagement in lambs is clearly needed to replicate this preliminary study. On a more general level, the present bottle-test remains interesting to compare with the results of a similar earlier study, but in which the lambs were suckled colostrum *before* the test (Alary et al., 2023). Regardless of IW-odorization of the bottles, 42.8 % of these suckled lambs (n = 28) did not even initiate suckling during the test, while in the present study 100 % of the unsuckled lambs (n = 41) initiated suckling and ingestion from an artificial teat. This supports Vince and Ward's (1984) finding that the suckling-experience status is decisive in the lambs' proneness to seize and suck an artificial teat, and is part of shepherds' practical knowledge that the earlier the separation from the dam after birth, the more rapidly lambs learn to suck an artificial teat (Ørskov, 1983).

As to ***sex-related modulation of lambs' responsiveness to IW odor***, no compelling evidence for a sex effect arouse. Female lambs were not differentially reactive to IW-M in any of the three double (when presented against either IW-nM or control stimuli). Male lambs were consistent with females in not differentiating simultaneously presented IW-M and IW-nM, although, in the IW odor vs. control tests, they oriented or contacted either IW stimuli rather than the control, further indicating their attraction to ovine IW odor irrespective of the emitting ewes' identity. Some sex-differentiated trends appeared in the bottle-feeding test, but without regard of the odor applied on the bottle: female lambs required fewer trials to initiate suckling of the artificial teat and tended to drink more colostrum than males. But such sex differences are relatable to more general breed- and/or sex-related differences in neonatal lambs' dispositions, such as e.g. arousal, susceptibility to stress, vitality, or mobility and exploratory drive toward the mother ewe/environment (e.g., Abecia et al., 2022; Degenhard, 2004). For example, male Suffolk lambs were reported to be slower than females to stand, seek the udder, and efficiently suck, although such sex-dependent contrast was not seen in Blackface lambs (Dwyer, 2003; Dwyer and Lawrence, 1999). Whether the Romane-Charolais crossbred lambs of the present study follow the Suffolk or the Blackface pattern of initial behavior toward their dam remains to be substantiated.

We acknowledge multiple **limitations** in this study. Although 101 neonate lambs were tested overall, the sample size of subgroups, especially when the sex factor was entered, was low (sometimes too low as in the bottle-feeding test); accordingly, the sex-related outcomes of the present study are to be considered as provisional. A major complication of the study was to submit newly-born lambs, able to stand on four legs before they had any suckling experience, to the tests. Stable standing being difficult to reach, about 16 % of lambs had to be excluded (from the two-choice odor test). Future behavioral tests on early reactivity to IW odor might thus consider breeds giving birth to lambs having greater vitality (e.g., Dwyer and Lawrence, 1999) or consider behavioral/cognitive variables that do not depend on lambs' standing ability (e.g., heart rate, respiratory rate and amplitude, licking, suckling). Finally, the use of petroleum jelly on the cotton pads to sample IW (to prevent friction effects) then applied in the two-choice tests was not the best option: grease might indeed function as a "fixator" of volatile compounds of IW, and as such may have interfered with the odor intensity of native IW, in the sense of reducing volatiles' evaporation rate (Finnerty et al., 2017) and attenuating lambs' responses to them; but they nevertheless appeared able to differentiate pure petroleum jelly from petroleum jelly loaded with IW in the present and earlier studies (Alary et al., submitted). If possible, future similar studies should thus use pure biologic odor matrices.

5. Conclusions

Inguinal wax (IW) odor is attractive to suckling-naïve lambs, as measured by head-turning and muzzle contact, but without differentiation of the maternal IW and IW from an alien postparturient ewe, in interim disagreement with Vince and Ward's (1984) results. There was also no difference in lambs' suckling behaviour between IW-scented and control bottles, but all of the lambs did successfully suckle. The effects of IW odor do not appear to be affected by the lambs' sex, although females and males showed some general behavioral differences. To better understand the behavioral effects of ovine IW odor on newly-born lambs, future studies should leverage more ethologically valid methods in the species-specific context of nursing, e.g. in using live ewes as the immediate test background and measuring neonatal lambs' responsiveness without direct human intervention (for example, in using remote-sensing technologies; e.g., Abecia et al., 2022).

CRediT authorship contribution statement

Alexandra Destrez: Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Catherine Husson:** Writing – review & editing, Resources, Data curation. **Benoist Schaal:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Justine Alary:** Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Bruno Patris:** Writing – review & editing, Methodology.

Declaration of Competing Interest

The authors have no competing interests to declare.

Acknowledgments

The authors would like to thank the farmers Julien Meloux and Agathe Chevalier in Charolles, Burgundy, for their exceptionally kind welcome and generous logistic and technical assistance. Acknowledgements are also expressed to the French Ministry of Agriculture who funded a doctoral grant to J Alary and to Institut Agro Dijon who funded the study.

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