




Human neonates prefer colostrum to mature milk: Evidence for an olfactory bias toward the “initial milk”?

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Funding information

Agence Nationale de la Recherche, Grant/Award Numbers: ANR-12-BSH1-0008-01, ANR-15-CE21-0009-01, ANR-15-IDEX-0003

Abstract

Objectives: Colostrum is the initial milk secretion which ingestion by neonates warrants their adaptive start in life. Colostrum is accordingly expected to be attractive to newborns. The present study aims to assess whether colostrum is olfactorily attractive for 2-day-old newborns when presented against mature milk or a control.

Methods: The head-orientation of waking newborns was videotaped in three experiments pairing the odors of: (a) colostrum (sampled on postpartum day 2, not from own mother) and mature milk (sampled on average on postpartum day 32, not from own mother) (n tested newborns = 15); (b) Colostrum and control (water; n = 9); and (c) Mature milk and control (n = 13).

Results: When facing the odors of colostrum and mature milk, the infants turned their nose significantly longer toward former (32.8 vs 17.7% of a 120-s test). When exposed to colostrum against the control, they responded in favor of colostrum (32.9 vs 16.6%). Finally, when the odor of mature milk was presented against the control, their response appeared undifferentiated (26.7 vs 28.6%).

Conclusions: These results indicate that human newborns can olfactorily differentiate conspecific lacteal fluids sampled at different lactation stages. They prefer the odor of the mammary secretion - colostrum - collected at the lactation stage that best matches the postpartum age of their own mother. These results are discussed in the context of the earliest mother-infant chemo-communication. Coinciding maternal emission and offspring reception of chemosignals conveyed in colostrum may be part of the sensory precursors of attunement between mothers and infants.

1 | INTRODUCTION

From birth onwards, milk conveys protection, nutrition, hydration, and neurobehavioral regulations to neonates (eg, Hale & Hartmann, 2007; Zibadi, Ross Watson, & Preedy, 2013). More specifically, the initial mammary

secretion - colostrum - transfers components that ensure, among other functions, passive immunization, oro-gastro-intestinal conditioning, reduced oxidative stress, competitive colonization by nonpathogenic microbiota, and the regulation of biorhythmic and neurocognitive processes (eg, Bode, Raman, Murch, Rollins, &



Gordon, 2020; Clancy, Hinde, & Rutherford, 2012; Toscano, De Grandi, Grossi, & Drago, 2017). However, the newborns' receipt of these nutritional and protective assets depends on a surprisingly neglected forerunning function of the lactating breast: **communication** through chemosensory cues or signals.

This chemocommunicative function of the lactating breast instigates neonates' attraction and orientation, latching on a nipple, and efficient sucking leading to ingest colostrum. Mothers and infants cooperate to attain this goal, the former easing neonates' efforts to get through the multiple constraints of beginning breastfeeding (Dewey, Nommsen-Rivers, Heinig, & Cohen, 2003), while the latter stimulate sensitive mothering and lactational psychophysiology (eg, Lau & Henning, 1990). Several sensory features of the lactating mother's breast evolved to attract the offspring's attention (Schaal & Al Ain, 2014). Among these, odors play a central role (Schaal, 1988; Winberg, 2005). Specifically, the postparturient mother's breast emits an odor factor that elicits positive responses in infants from the first postnatal minutes up to several weeks postpartum (eg, Macfarlane, 1975; Russell, 1976; Schaal et al., 1980; Porter, Makin, Davis, & Christensen, 1992; reviewed in Porter & Winberg, 1999; Schaal et al., 2019; Schaal, Saxton, Loos, Soussignan, & Durand, 2020). This mammary odor factor drives neonates to the nipple, sustains sucking, and may boost the learning of multisensory cues of the nursing context and of mother's identity (eg, Durand, Baudouin, Lewkowicz, Goubet, & Schaal, 2013; Durand, Schaal, Goubet, Lewkowicz, & Baudouin, 2020).

The odor of the lactating breast results from the mixing of several substrates. Colostrum and milk are the most obvious and profuse ones, but parsimonious secretions from areolar Montgomery's glands are also involved. Human milk and Montgomerian secretions have indeed been found to be attractive and appetitive to newborns (Doucet, Soussignan, Sagot, & Schaal, 2007, 2009; Marlier & Schaal, 2005; Marlier, Schaal, & Soussignan, 1998; Schaal et al., 2019), regardless of their prior experience with breastfeeding (Doucet et al., 2009; Marlier & Schaal, 2005), suggesting that the mammary odor bears some species-specific properties. However, newborns differentiate the milk of their own mother from the milk of an unfamiliar mother matched in lactational age (Marlier & Schaal, 1997), indicating that they can also detect individual-specific odor cues. Therefore, mammary secretions convey multiple odor-based meanings to newborns, combining species-specific signals and individual-specific signatures from the mother and her chemoecology (ie, diet, atmosphere, cosmetics, drugs, stress, etc.; eg, Mennella & Beauchamp, 1991).

Beyond its physicochemical, biochemical and microbiological complexity, milk is also highly complex from a temporal or developmental perspective. Some aspects of its composition follow circadian rhythmicity (eg, Gunther & Stanier, 1949; Hahn-Holbrook, Saxbe, Bixby, Steele, & Glynn, 2019; Jenness, 1979), while other aspects change within and between feeds (eg, Daly, Di Rosso, Owens, & Hartmann, 1993; Hall, 1975; Saarela, Kokkonen, & Koivisto, 2005). However, the most marked changes occur progressively along lactation: mammary secretory activity starts at the end of pregnancy when the glands undergo morphological and intracellular changes, and already produce colostrum in the last weeks of pregnancy (Kulski & Hartmann, 1981; McManaman & Neville, 2003; Neville, Morton, & Umemura, 2001; Pang & Hartmann, 2007). Immediately before and after birth, mammary glands increase in activity, first producing colostrum (on days 0–5), then “transitional” milk (on days 5–10), and thereafter ever increasing amounts of “mature” milk (Kulski & Hartmann, 1981). Colostrum contains higher concentrations of proteins, in the form of bioactive compounds such as immunoglobulins, hormones, enzymes, growth factors, and antioxidants (lactoferrin, vitamins), whereas mature milk becomes additionally richer in lactose and lipids (eg, Boersma, Offringa, Muskiet, Chase, & Simmons, 1991; Buescher & McIlheran, 1988; Chapman & Pérez-Escamilla, 2000; McManaman & Neville, 2003; Xu, 1996). These biochemical changes of milk composition with advancing lactation probably also result in the alteration of their chemosensory properties, although not much data is available on this issue (reviewed in Loos, Reger, & Schaal, 2019). To adult noses, the odor of colostrum and transitional milk is extremely weak in intensity and close to hedonic neutrality (Marlier et al., 1998; Soussignan, Schaal, Marlier, & Jiang, 1997), while the odor of mature milk is described as “hay-like, metallic, sweet, fatty or cooked milk-like”, but overall also of very low intensity (Spitzer & Buettner, 2010). However, the adult nose may not be the best judge of the sensory qualities of a biological fluid whose properties may be evolutionarily optimized for detection by the neonate's nose.

There are at least three reasons to hypothesize that, in addition to its chemical and biochemical uniqueness, colostrum is *chemosensorily* special for newly born infants. **First**, as the fluid that inaugurates postnatal ingestion, colostrum is a key transitional medium from the fetus to the neonate. Despite compositional differences with amniotic fluid, colostrum is chemically overlapping with it (Hartmann et al., 2012; Schaal, 2016), prompting odor resemblance between both substrates and equivalent attraction in newborns (Marlier, Schaal, & Soussignan, 1997; Schaal, Marlier, & Soussignan, 1998).

Secondly, and related to the previous point, the chemosensory familiarity of colostrum is probably higher in the days right after birth, during the phase of colostrum stability (ie, postnatal days 0–2; Patton, Huston, Montgomery, & Josephson, 1986; Montgomery, Patton, Huston, & Josephson, 1987). At this time, the chemical similarity between colostrum and amniotic fluid is maximal because both fluids are under the same sources of variation (Schaal, 2005, 2016). Accordingly, during this phase, colostrum should overrule mature milk in terms of olfactory familiarity and attractiveness for newly born infants. **Thirdly**, due to its importance for neonatal viability, one cannot exclude that colostrum conveys one (or several) chemosignal(s) to which neonates' perceptual abilities are inherently attuned.

To the best of our knowledge, the relative potency of colostrum and mature milk odors to affect behavior remains unknown in human newborns. A straightforward appraisal of the relative preference of neonates for early-lactation (colostrum) vs later-lactation milk consists in pairing them in a choice test. The choice paradigm has provided ample evidence for neonatal abilities to discern odor qualities (eg, Balogh & Porter, 1986; Cernoch & Porter, 1985; Delaunay-El Allam, Marlier, & Schaal, 2006; Delaunay-El Allam, Soussignan, Patris, Marlier, & Schaal, 2010; Macfarlane, 1975; Marlier et al., 1997, 1998; Marlier & Schaal, 1997, 2005; Schaal et al., 1980; Schaal et al., 1998). Among other results, these studies found that neonates aged 1–5 days show preference for the odor of colostrum against those of formula (crafted from bovine milk) or a control (water).

The present study aims to complement current knowledge about the responsiveness of human newborns to the odor of conspecific lacteal secretions in examining whether they display more orienting behavior toward age-matched milk (ie, colostrum) than to developmentally mismatched milk (ie, collected from a woman which lactation stage is much later than the target infant's mother is). Based on aforementioned arguments, and in line with data from other mammalian newborns, human neonates are expected to manifest a preference for the odor of age-matched milk.

This study is therefore composed of three experiments, each pairing two stimuli to assess the time they spend orienting to each odor. In Experiment 1, the newborns were exposed to age-matched colostrum vs age-mismatched mature milk. Experiments 2 and 3 aimed to assess the absolute attractiveness of either colostrum or mature milk against the control stimulus. Head-turning was the main dependent variable as this behavior initiates the ordered display of actions at a typical breastfeed (Koepke & Bigelow, 1997; Prechtel, 1958; Widström, Brimdyr, Svensson, Cadwell, & Nissen, 2019). Also,

developmental research on perception largely used this variable as an index of neonates' general attraction, sensation seeking and recognition memory (eg, Papousek, 1961; Kuhl, 1985), and it was already fruitfully applied to investigate neonatal olfaction (Balogh & Porter, 1986; Cernoch & Porter, 1985; Delaunay-El Allam et al., 2006, 2010; Macfarlane, 1975; Marlier et al., 1997, 1998; Marlier & Schaal, 1997, 2005; Schaal et al., 1980; Schaal et al., 1998).

2 | PARTICIPANTS, MATERIALS AND METHODS

2.1 | Participants

The study was conducted according to the principles of the Declaration of Helsinki on Biomedical Studies, and ethical approval was obtained from the Ethics Committee of the National Center of Scientific Research (CNRS), Agence Nationale de la Santé et du Médicament, and the regional Commission for the Protection of Persons (CPP-EST 1, authorization # 2016-A00542-49). Parents provided written informed consent to allow infant testing or to donate some colostrum or milk.

A total of 58 newborns were recruited on postnatal days 0–3 in the maternity ward of Dijon University Hospital (Bourgogne, France). The following inclusion criteria were applied: (a) mothers should be healthy before and during a pregnancy leading to (b) a full term healthy singleton newborn (gestational age > 36 weeks; Apgar score of 10 at 10 min), with (c) a birth weight > 2500 g. In addition, to be eligible for the final data analyses, the newborns had to (d) be in a stable arousal state compatible with active choice behavior (State 3–4 of Prechtel's (1974) scale), (e) display spontaneously a minimum of bilateral orientations to ensure sampling of both stimuli (see details in section 2.5), and (f) display head orientation responses devoid of any tactile elicitation (see section 2.5). Thus, out of the 58 newborns tested, 21 were dropped from final analyses (13 were not in stable arousal state, three did not orient bilaterally, and five oriented after unwanted tactile elicitation).

The 37 newborns finally included (Table 1) were full term (gestational age: $M = 39.9$ weeks, $SD = 1.2$ week; range: 36.4–42.3 weeks), with an average birthweight of 3189 g ($SD = 357$ g; range: 2620–3970 g). They all had an Apgar score of 10 at 10 min after delivery and none of them had any pathology before the moment of testing. Among the 37 mothers (age: $M = 32.5$ years, $SD = 4.4$ years; range: 23–45 years; 19 primiparae; cf. Table 1), 34 gave birth through the vaginal route and three through Cesarean section. At the time of testing,

Experiment	1	2	3
Infants (<i>n</i>)	15	9	13
Females/Males (<i>n</i>)	6/9	7/2	7/6
Gestational age at birth (weeks) ^a	40.1 ± 1	40.2 ± 1.7	39.5 ± 1.1
Range (weeks)	38.4–42.3	36.4–41.4	37–41.7
Birthweight (g) ^a	3214 ± 338	3231 ± 391	3131 ± 376
Range (g)	2800–3830	2620–3760	2755–3970
Age at testing (h) ^a	55.3 ± 16.3	58.9 ± 35.7	46.7 ± 12.9
Range (h)	22.5–85.3	32–150.4	18–65.1
Time from last feed (h) ^a	2.9 ± 1.9	2.2 ± 0.7	2.3 ± 1.2
Range (h)	0.6–7.6	1–3.2	0.8–4
Feeding method (<i>n</i>) Excl. breast/Excl. formula/Mixed ^b	7/5/3	6/1/2	9/1/3
Mothers			
Age (y) ^a	32.1 ± 3.3	30.3 ± 2.9	34.5 ± 5.8
Range (y)	27–38	26–36	23–45
Mode of delivery (<i>n</i>) vaginal/cesarean	12/3	9/0	13/0
Parity (<i>n</i>) primiparae/multiparae	10/5	4/5	5/8

^aMean ± *SD*.

^bExcl.: exclusive breast/formula feeding; Mixed: breast + formula feeding.

28 mothers were breastfeeding their infants (six with occasional complementary formula feeds), and nine were feeding them formula (two having given colostrum in the very first feeds). Finally, 30 mothers were born in Europe, 6 in Africa and 1 in Asia. They were all of middle-to-high socioeconomic level.

In addition, 34 other healthy mothers donated a sample of colostrum or mature milk. Their pregnancy, delivery, and lactation onset and course were uneventful. Their mean age was 29.2 ± 6.0 years (range: 18–43 years), and 18 of them were primiparae. They were from Caucasian or African origin (*n* = 27 and 7, respectively), and of middle-to-high socioeconomic level. Among these women, 18 donated colostrum during their hospital stay, and 16 were home-visited for the collection of mature milk by a trained research assistant.

Three independent groups of infants were tested in distinct experiments (see Table 1). The infant groups

TABLE 1 Descriptive characteristics of infants and mothers in the three experimental groups

differed neither in terms of gestational age, birth weight, and postnatal age at testing ($F[2, 34] = 1.370, 0.262, \text{ and } 0.912; p = 0.268, 0.771, \text{ and } 0.412$, respectively), nor in terms of composition by sex and feeding mode ($\chi^2[2, n = 37] = 3.233 \text{ and } 1.526, p = 0.199 \text{ and } 0.466$, respectively). The present study assessing whether species-level chemocommunicative processes are involved, infant sex and mode of feeding were not considered in the following analyses.

2.2 | Stimuli

Two different odor substrates were used, colostrum and mature breast milk. Colostrum was sampled on postpartum days 1–3 ($M = 58.6 \text{ h}, SD = 10.9 \text{ h}$; range: 44.7–75.4 h) and mature milk between 2 to 7 weeks postpartum ($M = 32 \text{ days}, SD = 10 \text{ days}$; range: 15–50 days).

Both substrates were collected using an electric breast pump (brand “Calypso”, Ardo, Switzerland), which elements were carefully cleaned between uses (glass bottles and breast shields washed with water and odorless detergent [L'Arbre Vert, Saint-Benoît, France], shields being then disinfected with 90% ethanol). Both lacteal substrates were sampled 1–2 h after a mothers' meal. It took approximately 5–15 min to trigger ejection and to collect the secretion from both breasts, which was then pooled and refrigerated immediately (see below). In order not to interfere with the infants' exposure to nutritive and immunity benefits of colostrum, each mother was sampled only once for limited amounts of colostrum and of mature milk (maximum 10 and 40 mL, respectively). Assuming that a chemocommunicative cue, if any, should be more concentrated in foremilk as an incentive to locate the breast and sustain sucking, we opted to collect foremilk and not to empty the breasts.

The colostrum and milk samples were conveyed to the laboratory on ice (at most within 30 min following sampling). Aliquots of 1 mL were stored in 1.5-ml glass vials (VWR International, Leuven, Belgium) prefilled with argon to minimize oxidative processes, and immediately frozen at -20°C . They were used the same or next day, within a 24-hour time-window. For Exp. 1 contrasting two lacteal stimuli, both substrates were sampled on the same day and stored in the exact same conditions. Before a test was started, the samples were thawed for 5 min at room temperature. The 1-ml samples of colostrum/milk were pipetted in a standardized way on a 7.5 x 7.5-cm pure cotton gauze pad (Urgo, Chenôve, France). The control stimulus consisted of 1 mL of mineral water similarly applied on gauze pad.

Breastmilk storage conditions (time elapsed since the beginning of sampling, temperature) in the present study were based on previous work using freezing to stabilize milk (eg, Lawrence, 1999; Vangnai, Phamornsuwana, Puhin, Sribunsua, & Rathanachai, 2017), and on a pilot study on 4–5 day-old infants' response to the odor of human milk (Couegnas, 2003). It came out from the latter that: (a) Milk stored 30 min at ambient temperature remained as attractive to infants as fresh milk; (b) milk stored 3 h at ambient temperature was less attractive than fresh milk, although without inducing repulsion; and, finally, (c) milk frozen immediately after ejection and stored for about 3 h at -20°C remained as attractive as fresh milk. Therefore, to ensure preservation of their odor activity on infants, colostrum/milk samples were either kept on ice between ejection and the test (for the tests made with fresh secretion), or immediately frozen (-20°C) when the tests had to be postponed (within a 24-h period following sampling).

The infants' response to qualitative contrasts between colostrum and mature milk being the target point of the study, it is important to exclude the possibility that they primarily react to simple intensity differences between them. To compare the odor of colostrum, mature milk, and water in terms of subjective intensity, 15 adults (mean age \pm SD: 29.3 ± 7.4 years, 9 women) were asked to rate the intensity of each stimulus on a Likert scale ranging from 0 (“no odor”) to 9 (“extremely intense odor”). The stimuli consisted in 1 mL of each fluid applied on a pure cotton gauze pad that was placed into a dark glass flask and presented randomly under the nose of participants. All stimuli were judged of very weak intensity, not significantly different from that of water (colostrum: $M = 3.7$, $SD = 2.5$; mature milk: $M = 2.9$, $SD = 1.5$; water: $M = 2.2$, $SD = 1.3$), $F(2, 42) = 2.37$, $p = 0.11$). All stimuli conveyed thus a very weak odor, including the control stimulus which odor derives from wetted pure cotton (as also noted in previous animal research; e.g. Gregg & Thiessen, 1981).

Finally, it is important to note that the study aimed to evaluate whether human newborns differentiate the odor properties of human colostrum/milk beyond their individual specificity. Therefore, the newborns were exposed only to the odors of colostrum/milk from unfamiliar, genetically unrelated women (ie, women who were not the own mother or mother's kin of the tested newborn).

2.3 | Experimental device and setting

All tests were conducted between 10:00 AM and 4:00 PM in our neonatal “Babylab”, a dedicated room in the maternity ward equipped for infant psychobiology research. The ambient noise, light and temperature of the room were held constant during the study period ($22\text{--}24^{\circ}\text{C}$).

Infant testing was conducted within a “chamber” (1 x 0.8 x 0.5 m) made of dark fabric to minimize light intensity and equalize frontally and laterally the visual scene to which infants were exposed. The newborns were seated in a special chair with semi-reclining back set at a 25° -angle with the vertical. To limit a potential head orientation bias, their posture was adjusted so that their body axis was aligned on the sagittal plane. A small sheet was tightened on each side of the seat to cover the body of the infants up to the neck in order to secure them in the seat and minimize their arm movements toward the device.

The odor-presentation device was composed of two arms positioned on each side of the infant's head. Both gauze pads impregnated with an odor stimulus were attached to each arm of the device (Figure 1). During the

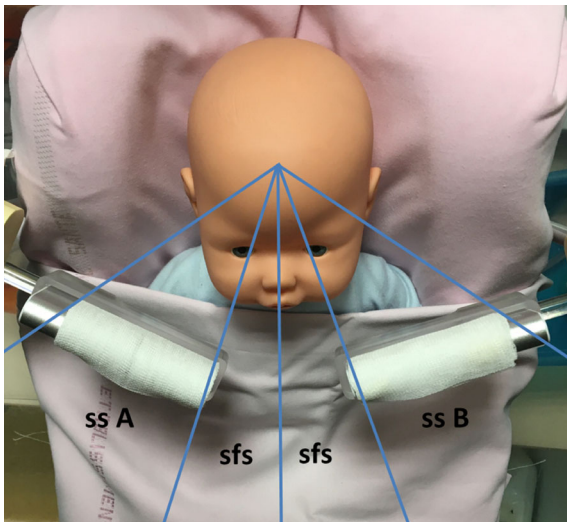


FIGURE 1 The odor-presentation device. Two odor stimuli (A and B) are presented on gauze pads, which are fixed on the device symmetrically on each side of the infant's face. On each side of the midline, stimulus-free sectors (sfs) range up to an eccentricity of 20°, followed by two stimulus sectors (ss) corresponding to odor A (ssA) and to odor B (ssB). Note that the size of the infant model is 25% smaller than a real newborn of average birth weight

tests, the experimenter stayed behind the infant without movement and without any interaction with the newborn. In order not to interfere with target odor stimuli, the experimenters handling the infants abstained from wearing or consuming any scented products.

A remote-controlled video camera (DCR-SR190E, Sony Corporation, Tokyo, Japan) was placed in the sagittal plane of the infants, 40 cm above their head, to record their cephalic movements.

2.4 | Testing procedure

All tests were performed upon spontaneous waking of the newborns and before a feed (time elapsed since the last feed: $M = 2.5$ h, $SD = 1.3$ h; range: 0.6–4.7 h; see Table 1). Before testing started, the newborns were required to be in an alert, calm-to-active state (states 3–4 of Prechtl's scale). Occasionally, sleepy infants were awakened by gentle strokes on both palms and simultaneous vocal stimulation by an experimenter; if too aroused, infants were rocked or given a pacifier until the optimal state was attained. When in the right state, the infant was installed in the seat and brought in front of the odor-presentation device.

Both arms of the device were adjusted for each infant so that the stimuli were presented at the level of their nose, a distance of 6 cm being set between both gauze pads. Both arms of the device were positioned

symmetrically so that both stimuli were equidistant from the infant's nose at the test onset. In addition, a distance of 2–4 cm was kept between the odor-presentation device and the nose of the infant to avoid any somesthetic elicitation of head orientation (5 infants were dropped from the analyses because of tactile elicitation of head turning by clothes touching their lower jaws or chin). Following Macfarlane's (1975) procedure, infants were first familiarized with both stimuli by manually turning their head so that their nose faced each stimulus for 5 s. Both side of presentation and nature of the first stimulus presented were counterbalanced across infants. The head of the infant was then brought into the sagittal plane, and the first 1-min trial started when the head was released. During an inter-trial pause of about 1 min, the lateral position of stimuli was alternated to control for any lateral bias of head motricity. The head of the infant was then again manually centered in the sagittal position and released for the second 1-min trial. The behavior of the infant was video-recorded all along. When the test was over, the odor stimuli were removed, and the device cleaned with 90° ethanol and water.

The experimenters who administered the tests to the neonates were not systematically blind to the nature of the stimuli presented on the white cotton gauze. Colostrum and milk are indeed more or less pigmented by carotenoids (their color ranging from bright yellow to translucent; Patton, Canfield, Huston, Ferris, & Jensen, 1990) and therefore can sometimes be visually discriminable from the competing stimuli (water).

2.5 | Behavior coding and data analysis

Blindness was however ensured at the coding stage as the color of the stimuli was not visible from the side of the camera. The video-recorded tests were analyzed frame-by-frame by a coder who was unaware of the nature and lateral position of the stimuli. The spatial distribution and duration of the behavioral events were recorded with an accuracy of 0.1 s by the computer using the Observer software (Noldus, Wageningen, NL). The behavioral coding aimed to first characterize each infant's general pattern of response to the stimuli in terms of bilateral head-turning and side bias. Therefore, the number of nose (nasal columella) crossings of the midline of the odor-presentation device, of entering into the stimulus sectors (see below) and of turning first to the right/left side or to stimulus A/B, were systematically coded. A minimal criterion of bilateral exploratory response consisting in two crossings of the device's midline per test was required for an infant to be included in the final analyses. This criterion was set to ensure that both stimuli in a pair were

sampled *spontaneously* and that head-turning was not unilateral due to an inborn orientation bias. Then, in order to assess the differential response of the newborns toward each odor stimulus, we coded the latency and duration of each head (nose) orientation toward each stimulus in a pair.

The duration of head orientation was coded by tracking the infant's columella over its 180° possible trajectory around the odor-presentation device. The whole trajectory was divided into four sectors on each side of a line representing the mid-sagittal plan: two "stimulus sectors" corresponding to both gauze pads, and a central "stimulus-free sector" devoid of stimulus pads; this latter sector was subdivided in two <20°-angle sectors on each side of the infant's sagittal plane. The amplitude of a positive head-turning toward a stimulus was defined as a deviation greater than 20° from the midline, when an infant's columella enters a stimulus sector, and less than 60° from the midline (beyond this 60° threshold, the nose was considered out of the stimulus sector). Infants' nose orientation to each zone (stimulus sectors or stimulus-free sectors) was timed for each trial, and then summated for both trials composing each test. The orientation duration was reported as the mean proportion (%) of the time the infant spent oriented toward the stimulus sector to the total time of observation (120 s).

Inter-observer reliability was obtained by having a second coder analyzing 29% ($n = 11$) of the tests. Both coders were blind to the lateral position of the stimuli, and one of them was blind to the nature of the stimuli. Spearman rank-order correlation coefficients were computed to verify their agreement regarding head orientation. The correlations between both coders regarding head orientation toward stimulus A or B were $r = 0.95$ and 0.97 , respectively.

2.6 | Statistical analyses

The data were analyzed using the Statistica software (version 13, Statsoft, Paris, France). The assumption of normality of the data being found unsatisfactory, nonparametric statistics were performed. The infants' general behavior pattern in the paired-stimuli device was first analyzed with a Kruskal-Wallis test to compare the number of side alternations between the three experiments; Wilcoxon tests were used to compare, within each experimental group, the number of entries into the different sectors of the device. Numbers of first head-turns to the right/left or to stimulus A/B were analyzed with χ^2 tests where the occurrences of deviations were combined between the two trials and compared with a chance distribution (ie, 50%). Finally, infants' differential response to the odor stimuli (ie, relative duration of head orientation, and

latencies to reach either stimulus sector) were compared using Wilcoxon tests. For all analyses, the tests were two-tailed and statistical significance was set at $p < 0.05$.

3 | RESULTS

3.1 | General behavior

Several aspects of the infants' head orientation served to assess their alertness and general pattern of responsiveness in front of the experimental setting, as well as to evaluate potential interferences with lateral biases.

Crossings of the midline of the odor-presentation device. We computed the number of times the infants' nose crossed the midline of the device (Table 2). No side alternation differences were found between the three groups of infants ($\chi^2[2, n = 37] = 4.046, p = 0.132$), indicating that they had similar rates of head movements across the midline in the three experiments.

Entries into the stimulus sectors. Infants' choice behavior was defined as the number of times the infants' nose crossed the limits of both stimulus sectors (Table 2). The mean number of entries into either stimulus sector was similar in Exp. 1, opposing colostrum and mature milk ($z = 1.475; p = 0.140$), and in Exp. 3, opposing mature milk and the control ($z = 1.478; p = 0.139$). In Exp. 2, pairing colostrum with the control, the newborns entered more often into the colostrum sector than the control sector ($z = 2.014; p = 0.044$).

Lateral Bias. To assess whether there was any tendency for the infants to first orient to the right or to the left after their head was released from the central position (regardless of the presented stimuli), the number of infants deviating to each side was computed (Table 2). First orientations to the right or to the left did not differ in the three experiments (Exp. 1: $\chi^2(1, n = 15) = 0.067, p = 0.796$; Exp. 2: $\chi^2(1, n = 9) = 1.526, p = 0.217$; Exp. 3: $\chi^2(1, n = 13) = 0.185, p = 0.667$). Thus, no lateral bias influenced initial head orientation.

Odor-induced initial Directionality. The number of infants orienting first to Stimulus A was compared with the number of infants orienting first to Stimulus B (Table 2). The newborns first orientation toward either stimulus was randomly distributed in all experiments, indicating that the pretest exposure to both stimuli was not influential on their first orientation response (Exp. 1: $\chi^2(1, n = 15) = 0.867, p = 0.352$; Exp. 2: $\chi^2(1, n = 9) = 1.526, p = 0.217$; Exp. 3: $\chi^2(1, n = 13) = 0.481, p = 0.488$).

In all three experiments, the newborns actively explored the scene to which they were confronted by alternating their head-turns between both sides of the odor-presentation device and more specifically over both

TABLE 2 General pattern of the neonates' head-orientation responses when they faced the odor-presentation device

Experiment	Midline crossing ^a	Entries into stimulus sectors ^a		Side of first orientation ^b		First stimulus sector reached ^b	
	Trials 1 + 2	Trials 1 + 2		Trial 1	Trial 2	Trial 1	Trial 2
				L/R	L/R		
1	12.13 ± 8.43	Col: 6.87 ± 3.38	MM: 5.40 ± 3.98	9/6	5/9	Col: 9 MM: 6	Col: 8 MM: 6
2	18.78 ± 10.28	Col: 8.33 ± 3.91	Wa: 5.44 ± 3.40	4/4	2/7	Col: 4 Wa: 4	Col: 7 Wa: 2
3	13.08 ± 10.14	MM: 6.77 ± 4.88	Wa: 4.77 ± 4.27	8/4	4/9	MM: 6 Wa: 6	MM: 8 Wa: 5

Note: The behaviors coded were (i) the alternations of head movements across the midline, (ii) the entries of the nose into either stimulus sector, (iii) the side (left/right) of first head deviation, and (iv) the nature of first stimulus sector reached by the nose. Variables (i) and (ii) combine the data of both trials.

Abbreviations: Col, colostrum; MM, mature milk; Wa, water; L, left side; R, right side.

^aMean number of occurrences ± SD.

^bNumber of infants.

stimulus sectors. Their initial head-turns appeared not to be affected by a lateral bias inherent to the infants or the test setting, or to the preexposure maneuver.

3.2 | Experiment 1: Colostrum vs Mature Milk

The head orientation latencies and durations of 15 infants were measured when they were simultaneously exposed to the odor of colostrum and mature milk.

Latencies to reach either stimulus. When comparing the time that newborns needed to reach each stimulus, no significant difference emerged ($z = 1.537$, $p = 0.124$) although they tended to orient more quickly to the colostrum odor than to the milk odor (15.8 ± 19.1 s vs 24.6 ± 25.7 s, respectively).

Relative duration of orientation. As shown in Figure 2, the newborns oriented for a significantly longer duration toward the odor of colostrum ($M = 32.8\%$, $SD = 17.1\%$) than toward the odor of mature milk ($M = 17.7\%$, $SD = 15.8\%$), $z = 2.30$, $p = 0.021$.

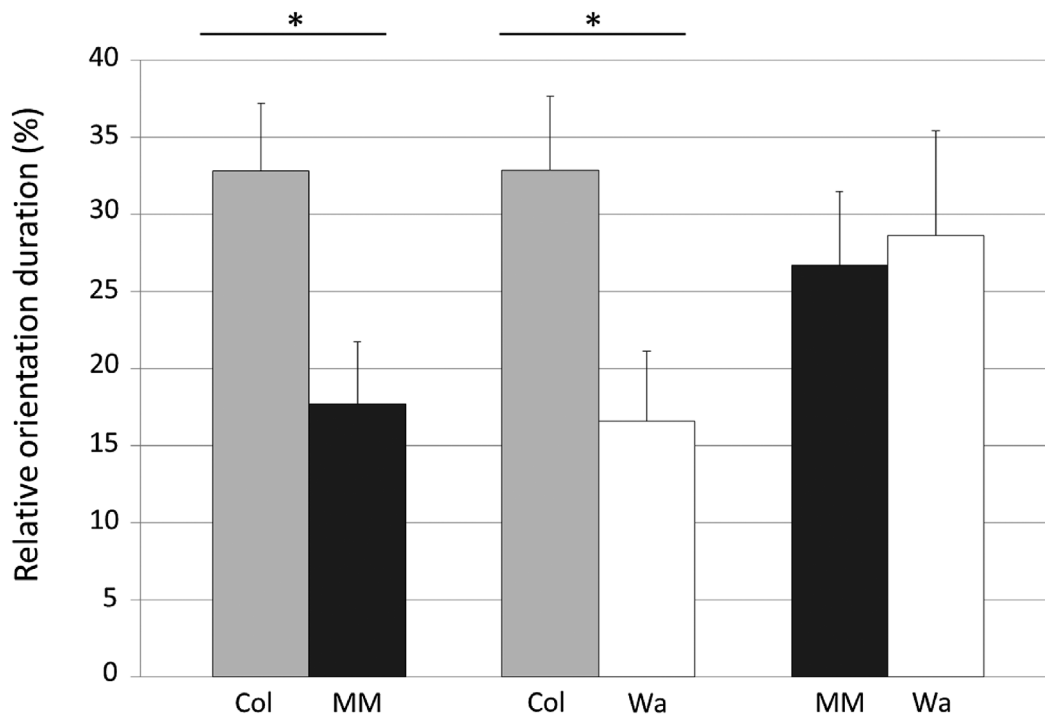


FIGURE 2 Mean percentage of relative duration (\pm SEM) of head orientation of three independent groups of 2 day-old newborns exposed to paired-choice tests opposing the odor of colostrum (Col) vs mature milk (MM) (Exp. 1); the odor of Col vs control (water, Wa) (Exp. 2); and 3/ the odor of MM vs control (Wa) (Exp. 3). Wilcoxon's tests: * $p < .05$

Overall, when simultaneously facing colostrum and mature milk, newborns displayed longer orientation toward the colostrum than toward the mature milk, indicating discrimination between them and preference for colostrum.

3.3 | Experiment 2: Colostrum vs Control

The outcome of Exp. 1 might be interpreted in terms either of a true preference for the odor of colostrum or of a false preference resulting from avoidance for the odor of mature milk. Here, we aimed to appraise the first possibility, testing newborns' attraction to colostrum odor against the control stimulus. This experiment replicating earlier studies (Marlier et al., 1998; Marlier & Schaal, 1997), only a small sample of infants ($n = 9$) was investigated.

Latencies to reach either stimulus. The latency to first orientation toward the colostrum odor (16.1 ± 21.0 s) did not differ from the latency toward the control stimulus (23.7 ± 27.0 s), $z = 0.852$, $P = 0.394$.

Relative duration of orientation. Results showed that infants spent significantly more time orienting to the colostrum sector than to the control sector ($M = 32.9\%$, $SD = 14.3\%$; control: $M = 16.6\%$, $SD = 13.5\%$, $z = 2.251$, $p = 0.024$; see Figure 2).

In sum, the newborns expressed longer head orientation toward the odor of colostrum when presented against the control.

3.4 | Experiment 3: Mature Milk vs control

This experiment further ensures that in Exp. 1 the positive response toward colostrum odor does not stem from turning away from the competing mature milk odor. It tests whether the odor of mature milk was not somehow repulsive to 2 day-old newborns. To do so, 13 newborns were simultaneously exposed to the odor of mature milk and to the control stimulus.

Latencies to reach either stimulus. Times to reach the stimulus sectors of mature milk (21.2 ± 24.6 s) and of the control stimulus (23.2 ± 26.8 s) were not significantly different, $z = 0.174$, $p = 0.861$.

Relative duration of orientation. The neonates spent about as much time orienting to the odor of mature milk ($M = 26.7\%$, $SD = 17.3\%$) than to the control odor ($M = 28.6\%$, $SD = 24.5\%$), $z = 0.245$, $p = 0.807$.

These results indicate that newborns did not react differently to the odor of mature milk when paired with the control stimulus (water). Thus, the results of Exp. 1 likely

reflect a true preference for the odor of colostrum over mature milk.

4 | DISCUSSION AND CONCLUSION

4.1 | Neonates prefer the odor of colostrum

The present study examined whether 2 day-old human newborns display a preference between the odors of colostrum collected on postpartum days 2–3 and mature milk collected on average on day 32. It comes out that they exhibit a preference, as they turned their head (nose) longer to colostrum than to the simultaneously presented mature milk. Although both secretions conveyed low odor intensities (to the adult nose), infants were able to smell them apart, and responded in favor of colostrum.

Two ancillary experiments further ascertained that this pattern of response actually derives from a true preference for colostrum and is not secondary to an avoidance from mature milk. First, when facing the odor of colostrum along with the control (Exp. 2), newborns turned their nose longer toward colostrum. They are thus able to detect the odor of colostrum against the control odor of wet cotton and prefer the former, corroborating earlier studies (Marlier et al., 1998; Marlier & Schaal, 1997). Secondly, and rather surprisingly, the newborns did not show any preference when exposed to the odor of mature milk against the control (Exp. 3). The present testing conditions evidenced neither positive nor negative head orientation to the odor of mature milk relative to the control. This raises the issue of whether 2 day-old newborns failed to *detect* the odor of mature milk or whether they did detect it, but were not *attracted* to it because it had not yet acquired any motivational meaning for them. The present test paradigm cannot decide between these alternatives and other approaches are needed (eg, habituation-dishabituation paradigm or psychophysiological testing of reactivity). Nevertheless, we favor the second alternative: the odor of mature milk, even though of human origin, may bear relatively low ecological validity for newborns because of a potential mismatch with their prior chemosensory experience with it (see below). Two-day-olds behave as if they were not “interested” in the odor of mature milk, and do not show more attraction to it than to the control. Previous studies relying on the same variable (duration of head-turning) and paradigm (two-choice test) came to similar interpretations after exposing newborns to paired odor stimuli (cf., e.g., Exp. 3–5 in Cernoch & Porter, 1985; Balogh &



Porter, 1986; Exp. 2 in Porter, Makin, Davis, & Christensen, 1991; Exp. 2 in Porter et al., 1992; Exp. 2–3 in Delaunay-El Allam et al., 2006), or, closer to our argument, to an unfamiliar odor against a scentless control pad (eg, Exp. 1 and 4 in Makin & Porter, 1989; Schaal, Marlier, & Soussignan, 2000). Converging interpretations were also raised for nondiscriminative responses of neonates to auditory or visual stimuli (eg, Farroni, Menon, Rigato, & Johnson, 2007; Trehub, 1973). Therefore, Exp. 3 indicates that the odor of mature milk is neither attractive nor aversive to newborns relative to the control, leading us to suggest that the newborns' head movements in Exp. 1 indicate attraction toward colostrum odor.

4.2 | How and why is colostrum attractive to newborns?

Obviously, the attractive potency of colostrum odor derives from stimulus-bound factors interacting with neonatal factors. Regarding the *stimulus side*, colostrum conveys odor properties that appear more attractive for neonates than those of later-lactation milk. The colostrum odor factor might depend on quantifiable properties of mammary secretions collected at various stages of lactation. One strong possibility is that the colostrum attractant potency relies on the prevalence of key-compound(s) or of a complex signature of compounds in colostrum, which progressively fade(s) after the stage II of lactogenesis sets on. Alternatively, these odor-active compounds might be emitted equally in colostrum and later-lactation milk, but their release from the milk matrix may change as a function of lactation-related variations in proteins or lipids that bind them (such binding processes being otherwise known to influence volatiles release from milk; e.g., Druaux & Voilley, 1997; Hansen & Heinis, 1991; reviewed in Hansen, 1997).

Surprisingly few chemo-analytic attempts have scrutinized the volatilome of human lacteal secretions, and these have been mainly directed at mature milk that is easy to collect (eg, Bingham, Lavin, & Acree, 2003a; Buettner, 2007; Hausner, Philipsen, Skov, Petersen, & Bredie, 2009; Le Roy, Villière, Fillonneau, & Prost, 2018; Macy, 1949; Shimoda, Yoshimura, Ishikawa, Hayakawa, & Osajima, 2000). From these analyses, varieties of compounds that are odorous to the adult were identified, but so far, none of them were tested with infants. Colostrum, which is much more difficult to sample in quantity for both ethical and practical reasons, underwent much rarer chemical dissections of its volatiles (Hartmann et al., 2012; Loos et al., 2019; Macy, 1949). These last studies found odorous

compounds that are inclusive to colostrum and later-lactation milk, although in graded quantities. For example, Hartmann et al. (2012) detected higher concentrations in amino acid conjugates of pungent odorants in colostrum than in milk samples. More recently, applying gas chromatography coupled with olfactometry to solvent extracts of colostrum and transitional milk, Loos et al. (2019) identified shared odorants in both fluids. Some of them, such as the blood-like odorant trideca-2,-4,7-trienal, the violet odorant β -ionone, and the fatty smelling non-2-enal, evinced higher contributions to colostrum odor than to the odor of the same women's transitional milk. These first instrumental analyses support the infant-based diagnostic of a qualitatively and/or quantitatively differentiable volatilome of colostrum and of later-lactation milk. Future research awaits to characterize the odorants that are proper to, or more typical of, human colostrum, evaluate their attractiveness to neonates, and track their occurrence in lacteal secretions throughout the lactation-span in relation with other nutritive and bioactive compounds (eg, Newburg, 2001).

From the *infants' side*, how are the aforementioned compositional changes of milk detected and, perhaps, potentiated by the chemoreceptive abilities of neonates? Early perceptual abilities may indeed be either tuned to detect a configuration of multiple compounds conveying a given aroma gestalt or odor signature to milk, or they may be specialized to detect one compound or a small subset of compounds in milk. The *first option* relies on the attractive potency of colostrum as a by-product of the fetuses' experience of amniotic fluid odor, the environment that shapes the operational properties of early olfaction (eg, Miranda-Morales, D'Aloisio, Anunziata, Abate, & Molina, 2020; Schaal et al., 1998, 2000; Smotherman & Robinson, 1985; Todrank, Heth, & Restrepo, 2011). Neonates' attraction to colostrum relative to later-lactation milk could therefore originate from the greater resemblance of colostrum's odor profile with the one of amniotic odor (Schaal, 2016). Similarly, newly born mice are more attracted to the odor of early-lactation, age-matched milk than to the odor later-lactation milk (Al Aïn, Belin, Patris, & Schaal, 2012). But the chemical correlates of milk odor qualities that unfold with developing lactation remain elusive in mice as well as in humans. In both species, the memory trace of the amniotic odor signature may decline as a function of postnatal experience with changing milk composition, rendering any contemporaneous milk odor always more familiar and, hence, more attractive to the growing offspring (eg, Al Aïn, Goudet, Schaal, & Patris, 2015; Al Aïn, Mingioni, Patris, & Schaal, 2014; Logan et al., 2012; Marlier et al., 1997).

Following the **second option**, neonatal olfaction may detect in colostrum inherently attractive odorants, which sensory salience fade as the proportion of colostrum drops when milk comes in. So far, data for such coevolved coupling between maternal release of a given odor factor and neonatal olfaction are rare. One interesting mammalian case is 2-methylbut-2-enal, a chemosignal released by rabbit females to attract their pups that is more concentrated in early-lactation milk than in late-lactation milk (Schaal et al., 2003). The early expression of selective sensitivities or related molecular mechanisms (eg, Fleischer, Schwarzenbacher, & Breer, 2007; Mamasuew, Hofmann, Breer, & Fleischer, 2011; Zhang et al., 2004) may underlie such neonates' olfactory sensing coinciding with maternal emission. One human study suggests that neonatal olfaction may "track" volatiles from changing milk, as attested by their hemodynamic brain response (Bartocci et al., 2000): upon inhaling their own mother's colostrum odor, the neonates' brain activation correlates negatively ($r = -0.64$) with postnatal age within the 6-to-192 h window. This result suggests either changing neonatal receptivity to a constant stimulus or constant receptivity to a changing stimulus.

A **final option** is that both above-sketched generalist and specialized processes relying on prenatal learning or on inborn perception operate in concert, mediated by different neural subsets of peripheral receptors and pathways in the olfactory system. The plausibility of such a functional duality of neonatal olfaction in humans is backed by comparative evidence for similar effects in other mammals (eg, Schaal, 2014; Schaal et al., 2009).

Future research may progress along the above lines in systematically testing: (a) infants of variable postnatal age with the odor of milk from fixed lactation age, and (b) neonates of fixed age with lacteal secretions from variable postpartum age. This approach would improve our understanding of the overlooked domain of milk-mediated odor communication through the perspective of its evolved target, the neonate infant.

4.3 | Limitations

Because recruitment has been stopped earlier than planned due to COVID-19, this study is limited by small sample sizes. However, the size of groups is aligned with those of previous comparable studies laboring with the challenge of testing newborns in the very unsteady alert state, especially before a feed when they are prone to begin crying. This sample size precluded analyses of the effects of infant sex and mode of feeding. Otherwise, on the stimulus side, the postpartum age when mature milk

was collected is wide ranging (15–50 days), raising the possibility of heterogeneous composition of what is considered "mature milk". Although textbooks and breastfeeding professionals vary in their definition of when human milk is termed "mature" [e.g., by postpartum days 10 (Boersma et al., 1991), 15 (Pham, Patel, Baban, Yu, & Bhatia, 2020) or 30 (Castellote et al., 2011; Zanardo et al., 2001; Zarban, Taheri, Chahkandi, Sharifzadeh, & Khorashadzadeh, 2009)], this point should be better controlled in future studies. Finally, the chemosensory difference of early- vs late-lactation milk may be confounded by the fact that these milks were not sampled from the same donors, introducing an additional source of potential chemosensory variation. However, as both donors were unrelated and unfamiliar to all tested newborns, we ensured that both kinds of milks did not convey unbalanced cues of genetic relatedness or familiarity. Would we have used colostrum and mature milk from the same donor (especially in Exp. 1), both samples would obviously not have had the same level of freshness, thus introducing another confound. Therefore, we opted to prioritize the freshness of both samples in pairing them in odor tests within maximum 24 h after sampling and immediate freezing, ensuring their pristine odor qualities. Despite these limitations, we hope this study serves to invite others to consider refining the methods to investigate the newborn infants' responsiveness to elements of their natural chemosensory ecology in the critical transition from fetal to postnatal life.

4.4 | Conclusion and implications

Relative to a developmentally mismatched milk, 2-day-olds appear to prefer the odor of a milk collected at the lactation stage that matches their age (here, colostrum), or rather their own mother's postpartal age. This phenomenon has both theoretical and clinical implications. It suggests that neonatal olfaction, is somehow synchronized with the mother's lactational physiology, in line with homologous evidence of early odor communication in other species (mouse: Al Ain et al., 2012; rabbit: Coureaud, Langlois, Perrier, & Schaal, 2006). Further, these results make a case for a pan-mammalian coevolutionary mechanism linking the mothers' production of vital secretions with an offspring's ability to sense them. Postparturient females thus yield an initial mammary secretion—colostrum—that appears to be most attractive to neonates when these face maximal challenges to their viability (eg, Edmond et al., 2006; Schaal & Al Ain, 2014). Consequently, in addition to its many other benefits, colostrum also conveys an odor-based behavioral regulator facilitating the onset of breastfeeding interactions and

stimulating the lactation process itself (eg, Lau & Henning, 1990; Peaker, 1998).

Infant attraction toward colostrum odor also has implications for the bioinspired management of neonates, especially those born preterm and/or with (very) low-birthweight. Whether such infants do *prefer* the odor of colostrum remains untested, but repeated observations indicate that exposure to human milk odor influences their readiness to feed orally (eg, Bingham, Abassi, & Sivieri, 2003b; Davidson, Ruthazer, & Maron, 2019; Lee, 2019; Yildiz, Arikan, Gözüim, Taştekin, & Budancamanak, 2011) or their self-regulatory responses when facing a pain challenge (eg, Alemdar & Özdemir, 2017; Badiee, Asghari, & Mohammadzadeh, 2013; Baudesson de Chanville et al., 2017; Goubet, Rattaz, Pierrat, Bullinger, & Lequien, 2003; Maayan-Metzger et al., 2018). Additionally, there is evidence for better developmental outcomes of premature infants when fed with their own mothers' milk compared to the mismatched milk of an anonymous donor (eg, De Halleux et al., 2019; Heiman & Schanler, 2006; Montjoux-Régis et al., 2011). The oral administration of the mother's colostrum to preterm neonates additionally canalizes their oral microbiota and immunity (Glass, Greecher, & Doheny, 2017; Maffei, Brewer, Codipilly, Weinberger, & Schanler, 2020; Snyder et al., 2017), but it may also subject them to chemosensory incentives. Finally, suffusing own mother's milk odor into the incubator quasi-continuously for 3 days promotes the psychophysiological stabilization of preterm infants (Park & Im, 2020), further suggesting sustained adaptive effects of the odor of age-matched milk.

By its transitional situation between the amniotic niche and the milk niche, colostrum plays an essential behavioral role in the coordination between the neonate and the mother, usually only acknowledged from the immune-protective and nutritional-energetic points of view. The present study adds the possibility that mothers emit colostrum odor cues or signals that are somehow tailored to the olfactory sensitivity or expectations of the neonate. Their coinciding emission and reception may be precursors to the synchrony between the mother and the infant (eg, Feldman, 2006).

ACKNOWLEDGMENTS

The authors are grateful the mothers who donated milk and who let their infant take part in the study, to Michel Tavan for his technical skills in realizing the odor-presentation device, Véronique Boulanger for her efficient help in collecting mature milk samples, and the constant support of the staff from the Maternity of Dijon University Hospital. We also thank Dr Carolyn Granier-

Deferre for discussion and multiple inputs on a previous draft of the manuscript, Dr Melvin W Hanna for linguistic help, and the AJHB editor and an anonymous referee for useful comments. This work was supported by the Agence Nationale de la Recherche (Paris) under grants ANR-12-BSH1-OO08-01 (to Joël Candau), ANR-15-CE21-0009-01 (to BS), and ANR-15-IDEX-0003 (International Junior Fellowship from ISITE-BFC to FD).

AUTHOR CONTRIBUTIONS

Magali Klæy-Tassone: Data curation; formal analysis; writing-original draft; writing-review and editing. **Karine Durand:** Data curation; methodology; formal analysis; writing-review and editing. **Fabrice Damon:** Data curation; funding acquisition; writing-review and editing. **Katrin Heyers:** Data curation. **Nawel Mezrai:** Data curation. **Bruno Patris:** Writing-review and editing. **Paul Sagot:** Project administration; resources; writing-review and editing. **Robert Soussignan:** Formal analysis; methodology; writing-review and editing. **Benoist Schaal:** Project administration; funding acquisition; methodology; writing-original draft; writing-review and editing.

CONFLICT OF INTEREST

The authors declare no competing interest.

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How to cite this article: Klaey-Tassone M, Durand K, Damon F, et al. Human neonates prefer colostrum to mature milk: Evidence for an olfactory bias toward the “initial milk”? *Am J Hum Biol*. 2020;e23521. <https://doi.org/10.1002/ajhb.23521>