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The effect of amniotic fluid olfactory stimulation on salivary oxytocin in anestrus female dogs

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Abstract: Amniotic fluid is known to attract mammals around parturition, but its hormonal impact through sensory stimulation remains unclear. This study investigated the impact of amniotic fluid olfactory stimulation on salivary oxytocin (sOXT) in Labrador Retriever. Dogs were divided into a control group (CG, n = 9) receiving water, and a treatment group (TG, n = 8) receiving amniotic fluid. Saliva samples were collected from each dog before (T0) and 10 minutes after administration (T1). Multiparous bitches had significantly higher sOXT levels (mean ± S.D. = 114.1 ± 16.2 pg/ml) than primiparous ones (mean ± S.D. = 93.6 ± 16.38 pg/ml) at T0 (t = 2.56; p = 0.021). However, no significant differences in sOXT concentrations were found between CG and TG at either time point, nor within groups between T0 and T1. Although not statistically significant, yellow-coated dams showed higher sOXT levels (108.1 ± 20.22 pg/ml) compared to chocolate (100.6 ± 22.98 pg/ml) and black (106.2 ± 17.45 pg/ml) ones. A positive correlation was observed between age and sOXT at T0 (r = 0.445; p = 0.035). These results suggest that amniotic fluid alone does not increase sOXT and is likely insufficient to induce maternal behavior in anestrus dogs.

Key Words: amniotic fluid, canine, dog, maternal behavior, olfaction, salivary oxytocin.

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Introduction

Domestic dogs are mono-estrous, non-seasonal spontaneous ovulators with a luteal phase followed by an obligate anestrus lasting 8–40 weeks. Poorly understood environmental interactions (such as pheromones and possibly photoperiod) and a potential endogenous circannual cycle (sensitive to dopaminergic, serotonergic, and/or opioid pathways) are responsible for terminating anestrus (Concannon, 2011). During anestrus, serum estradiol levels are reported to be generally low (5–10 pg/ml) and serum progesterone remains below 1 ng/ml. Despite the pulsatile nature, basal LH levels are also low, while FSH levels are high (Concannon, 2009).

Maternal behavior is regulated in two phases: the onset of maternal behavior, and its maintenance. The hormonal stimulus responsible for the onset of maternal behavior is not sustained postpartum; in fact, ovarian, adrenocortical, and pituitary hormones have been shown to act as modulators, rather than essential factors in maintaining postpartum maternal behavior (Rosenblatt and Siegel, 1981). Despite ongoing debate regarding the hormonal regulation of maternal behavior, the role of oxytocin (OXT) in both the onset and maintenance of mothering is widely accepted (Lezama-García *et al.*, 2019a). In dogs, the only correlational study on salivary OXT (sOXT) and maternal care suggests a weak hormone-behavior relationship (Ogi, Mariti, *et al.*, 2021). However, another correlation study on polymorphic variants of the oxytocin receptor (*OXTR*) gene suggests that the presence of the A allele in rs8679684 SNP may positively influence maternal behavior (Ogi et al., 2021).

Instead, sensory stimulation and psychological processes, such as pup stimulation, appear to

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play a crucial role in regulating postpartum maternal behavior (Rosenblatt and Siegel, 1981). Stress or pathological conditions in the newborn may affect the mother's response to them (Lee et al., 1974). Stress can also have a direct impact on the maternal oxytocinergic system (Ogi et al., 2020) potentially influencing maternal behavior, albeit to a limited extent (Ogi et al., 2021), which in turn may affect the behavior of the offspring (Guardini *et al.*, 2017).

Pup stimulation appears to play a pivotal role not only in the maintenance but also in the onset of maternal behavior. In "naive" female mice, that had never previously encountered pups, except for their own littermates, a rapid onset of maternal behavior (in less than five minutes) has been observed following pup exposure (Noirot, 1972). Studies have also been found that ovariectomized female mice (Gandelman, 1973) and ewes (Poindron and Le Neindre, 1980), as well as hypophysectomized-ovariectomized female rats, can exhibit maternal behaviors after exposure to pups (Rosenblatt, 1967).

One form of sensory stimulation that can trigger the onset of maternal behavior is olfactory stimulation through amniotic fluids. Indeed, most mammal parturient females instinctively lick their offspring, ingesting the amniotic fluids (Collias, 1956; Underwood, 1979; Levy, Poindron and Le Neindre, 1983; Lidfors, 1996; Klopfer and Gamble, 2010), a behavior correlated with the mother-infant bond (Lévy et al., 2004). More specifically, olfactory attraction to amniotic fluid is crucial for mother-infant recognition in both human and non-human mammals (Schaal and Marlier, 1998). Lévy and colleagues conducted several studies on this topic in ewes, demonstrating the central role of the olfactory system in maternal acceptance or rejection of newborns (Levy, et al., 1983; Levy and Poindron, 1987; Lévy et al., 1995).

According to Dunbar and colleagues, lactating dogs are attracted to the amniotic fluid on the newborns for up to the 30th day after parturition (Dunbar, et al., 1981). Indeed, in their simultaneous-choice retrieval test conducted on three purebred female lactating beagles it was observed that the dams "were strongly attracted towards amniotic fluids" (Dunbar, et al., 1981). Moreover, according to the findings of Abitbol and Inglis (Abitbol and Inglis, 1997), who showed maternal rejection of bathed neonates, the presence of amniotic fluid would result in a necessary condition for mothering even in lactating dams.

In particular, licking behavior toward pups is clearly triggered by the presence of amniotic fluid and/or bodily secretions on the newborns (Abitbol and Inglis, 1997). This crucial behavior is not only fundamental for maternal acceptance, but it also stimulates newborn breathing and meconium elimination (Lezama-García et al., 2019b; Santos, et al., 2020).

It is important to remember the significance of licking in maternal behavior and its impact on puppy development. As demonstrated by previous research (Guardini et al., 2016, 2017), this behavior facilitates the transition from the primary attachment bond with the mother to the establishment of a strong bond with human caregivers (Mariti et al., 2020).

Olfactory cues are also pivotal for the young. Already in the seventies, a study provided evidence for olfactory attraction in newborn albino rats to the chemosensory properties of maternal amniotic fluids. The attachment of newborns to their mother's nipples was eliminated by washing the nipples and attachment was reinstated only when the washed nipples were painted with a distillate of the nipple wash, parturient-mother's saliva, or amniotic fluids (Teicher and Blass, 1977).

Thanks to the research on this topic, we can state that the mother-infant olfactory recognition is essential in mammals for both providing care (epimeletic behavior) and seeking care (et-epimeletic behavior). Olfactory attraction to and recognition of amniotic fluid also play a role in human parenting (Vaglio, 2009). Mothers and fathers can recognize the amniotic fluid of their own newborn (Schaal and Marlier, 1998) while neonates, in turn, can recognize their mother's scent (Varendi, et al., 1994). They are not only attracted to (Klaus, 1998) nut also soothed by (Varendi et al., 1998) the distinctive olfactory signature of their mother's amniotic fluid. Prenatal olfactory learning has also been described in domestic dogs (Wells and Hepper, 2006).

Conversely, research on the olfactory regulation of maternal behavior in dogs is limited, and to the authors' knowledge, no information is available on the potential correlation between sensory stimulation and hormonal processes underlying the onset of mothering in this species. Given the extraordinary sense of smell in dogs and its crucial role in guiding their behavioral expression, the present study aims to explore the possible correlation between olfactory stimulation from amniotic fluid and salivary oxytocin (sOXT) in anestrus female Labrador Retriever.

Materials and Methods

Salivary oxytocin (sOXT) concentrations were measured in 10 pluriparous (mean age ± S.D. = 73 ± 19 months) and 7 primiparous (mean age ± S.D. = 31 ± 5 months) healthy anestrous female Labrador Retriever. All dams, which used to be handled and well socialized, were raised by the same breeder.

The entire study was conducted at the breeder's facility, in a controlled room familiar to the subjects. Before each test, the dogs were allowed to explore the room freely for 5 min.

To assess the effect of amniotic fluid on salivary oxytocin levels, the dogs were divided into two groups: a control group consisting of 9 dogs (CG) which received water, and a treatment group consisting of 8 dogs (TG) which received amniotic fluid (Table 1). Fluid administration and saliva collection were performed by two experienced veterinarians.

Table 1. Subjects. The coat color, age, number of parturitions, and the group – control group (CG) or treated group (TG) – of the 17 dams involved in the present study.

Dog (n°)	Coat	Age (months)	Parturitions (n°)	Group
1	Yellow	62	2	CG
2	Yellow	96	4	CG
3	Black	80	4	CG
4	Chocolate	50	3	CG
5	Yellow	101	3	CG
6	Chocolate	38	1	CG
7	Chocolate	26	1	CG
8	Black	31	1	CG
9	Yellow	26	1	CG
10	Chocolate	60	3	TG
11	Black	45	2	TG
12	Black	84	3	TG
13	Yellow	81	2	TG
14	Black	69	4	TG
15	Yellow	36	1	TG
16	Black	31	1	TG
17	Yellow	26	1	TG

The pull of amniotic fluid administered to the dogs was collected from three different natural deliveries of healthy dams and it was immediately frozen at -18°C in 1 ml doses. Before being presented to the subjects, each dose of amniotic fluid and each portion of water were brought to body temperature (38°C).

For the TG a pipette, containing 250 µl of amniotic fluid, was presented to the dogs, allowing them to sniff it. After five seconds of smelling, the liquid was gently applied to their lips to induce ingestion and stimulate the vomeronasal organ (VMO). Conversely, 250 µl of water was administered to the CG using the same procedure.

Two saliva samples were collected from each dog: the first one immediately before the test (T0) and the second one 10 minutes after fluid administration (T1). Sample collection followed the methodology described in our previous studies [5,6,8] and sOXT concentrations were measured using the same validated ELISA Kit® (Item #500440 – Cayman Chemical, Ann Arbor, MI, USA). Statistical analyses were performed using GraphPad Prism 9 (Graph-Pad Software, San Diego, CA, USA). Data normality was assessed with the Kolmogorov-Smirnov test, followed a two-way analysis of variance with correlation analysis conducted using Pearson's test.

Results

Data analysis revealed significantly ($t = 2.56$; $p = 0.021$) higher sOXT concentrations in multiparous bitches (mean ± S.D. = 114.1 ± 16.2 pg/ml) compared to primiparous ones at T0 (mean ± S.D. = 93.6± 16.38 pg/ml) (Fig. 1).

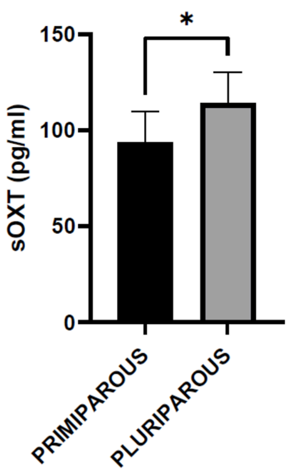

Figure 1. Salivary oxytocin concentrations (mean ± S.D.) in multiparous and primiparous bitches at T0. * ≤0.05.

No significant difference was found between sOXT concentration of CG (mean ± SD = 100.5 ± 19.02 pg/ml) and TG (mean ± SD = 111.5 ± 18.11 pg/ml) at T0. Similarly, after treatment (T1), no significant difference was found between CG (mean ± SD = 93.72 ± 34.76 pg/ml) and TG (mean ± SD = 98.62 ± 26.40 pg/ml). Moreover, no significant difference was in sOXT concentrations were detected between T0 and T1 within either CG or TG.

Although not statistically significant, difference was found in the pre-treatment sOXT concentrations among dams depending on coat colors, yellow-coated bitches had higher sOXT levels (108.1 ± 20.22 pg/ml) compared to chocolate (100.6 ± 22.98 pg/ml) and black (106.2 ± 17.45 pg/ml) bitches.

Additionally, a positive correlation was found between the ages of dams and their pre-treatment salivary oxytocin concentrations (r= 0.445; p = 0.035) (Fig. 2).

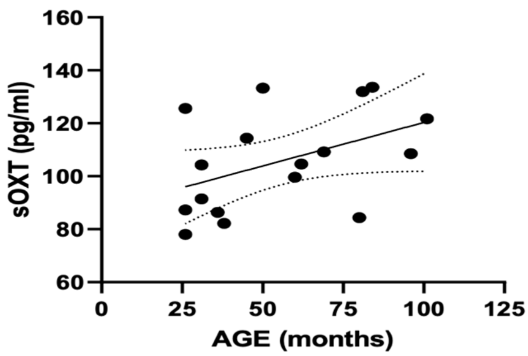

Figure 2. Correlation between sOXY (pg/ml) at T0 and dog age (months).

Discussion

This study aimed to clarify whether stimulation of both the main and accessory olfactory system could be involved in the secretion of oxytocin, an important hormone in the expression of maternal behavior, contrary to what has been demonstrated by previous research in rodents (Lévy et al., 2004).

Parturient females typically consume their own newborns' fluids and lick the vaginal excretions of other parturient females (Levy et al., 1983). Additionally, administration of human amniotic fluid has become widely discussed due to its therapeutic potential as a suppressor of cytokine activation, which may help reduce the severity of COVID-19 infection (Mitrani et al., 2021).

Measuring peripheral OXT in saliva is a low-stress sampling method, and sOXT appears to better reflect central OXT levels compared to plasma (Martin et al., 2018). Moreover, the slightly longer half-life of sOXT could mitigate the impact of pulsatile release of this hormone (Higuchi et al., 1983; Huffmeijer et al., 2012). The specific timing and half-life of sOXT in dogs are still undefined. However, as we reported in a previous study (Ogi *et al.*, 2020), an increase of this hormone in saliva is detectable after 5 min from a positive stimulus as also shown by MacLean et al. (MacLean et al., 2017). Conversely, the potential decrease in OXT levels in response to negative stimuli is still unknown and requires further investigation (Ogi et al., 2020).

It is well established that first-time motherhood leads to behavioral and hormonal changes that can significantly influence subsequent reproductive experiences (Pawluski et al., 2006). Reproductive experience differentially affects spatial reference and working memory performance in mother (Pawluski, Walker and Galea, 2006). Indeed, primiparous dams have been found to be more anxious compared to multiparous ones (Ogi and Gazzano, 2024). Moreover, plasma OXT levels were found to be significantly higher in multiparous dams than in primiparous dams in rodents (Lopatina *et al.*, 2012). The higher amount of sOXT levels observed in multiparous dams suggest that the physiological changes occurring after the first birth may persist throughout the entire estrous cycle of female dog, including the anestrus phase. Due to the limited literature available on this topic in dogs, comparing these findings with previous studies and formulating consistent hypothesis remains challenging. Furthermore, to the author's knowledge there are no significant findings concerning the correlation between oxytocin levels and age in dogs, but our study suggests a link between these two parameters at least in dams. This observation would be confirmed by a study conducted on Rhesus Macaques in which it has been found that cerebrospinal fluid OXT levels were positively correlated with age in adult females but negatively correlated with age in infants [43], suggesting that the correlation with age could be in turn dependent on the number of births. From a genetic perspective, the presence of the A allele in the rs8679684 SNP is correlated with higher levels of sOXT levels and appears to have a positive influence on maternal behavior of lactating Labrador Retriever dogs [6]. Consequently, our findings on oxytocin levels in relation to coat color, although not statistically significant, could have a genetic basis. However, the potential correlation between sOXT and coat color requires further investigation to develop a well-founded thesis on this topic. In dogs (Kim et al., 2010) as well as in cats (Stelow et al., 2016; Wilhelmy et al., 2016) behavior appears to be linked to different coat color patterns. According to our findings, olfactory stimulation with amniotic fluid is not sufficient to induce a significant variation of sOXT levels in anestrus female dogs. One possible explanation is that the amount of liquid provided was not sufficient to alter OXT levels. However, given the high sense of smell in dogs, we are confident that the stimulation was adequate, particularly as it was reinforced by oral administration. Another possible reason for these results could be the necessity of additional pup-relates stimuli, such as visual and auditory cues (Kendrick, 1994; Keller et al., 2003). Moreover, the limited sample size may have contributed to the lack of significant results. However, the homogeneity of the sample (same breed, same breeder) likely helped minimize this potential bias.

Contrary to initial expectation, our findings revealed a slight reduction in sOXT levels following both water and amniotic fluid administration. This suggests that the decrease in oxytocin could be linked to potential stress induced by handling (Ogi et al., 2020). However, given the expertise of the veterinarians administering the fluid and the dams' habituation of being handled, this hypothesis seems unlikely. Instead, we propose that the hormonal response observed was primarily caused by the amniotic fluid itself.

While the role of the olfactory system in promoting epimeletic behavior in parturient mammals is well established, little is known about the potential avoidance effect of amniotic fluid olfactory stimulation in nonparturient females. Unlike mice, where even nonparturient females exhibit maternal responsiveness to young, most mammals tend to avoid neonates when they are neither pregnant nor lactating, largely due to olfactory cues. For instance, virgin female hamster often cannibalizes unrelated newborns but, after a lesion to the olfactory system, they tend to accept and retrieve them (Marques, 1979). Similar behaviors have been observed in rats, rabbits, and sheep (Lévy et al., 2004). Moreover, the oxytocinergic system itself seems to be negatively influenced by olfaction. In fact, research has shown that only by eliminating the inhibitory effect of olfactory cues, can the intracerebroventricular oxytocin infusion successfully stimulate the epimeletic behavior (Wamboldt and Insel, 1987).

Accordingly, we can tentatively assume that the slight reduction in sOXT levels was induced by a repulsion toward the amniotic fluid. Indeed, the sOXT decrease was more pronounced in the TG than in the CG at T1. This hypothesis aligns with the findings of Lévy et al. (Levy et al., 1983), who observed that ewes exhibit an aversion to amniotic fluid throughout most of the tested periods, except for a brief interval around parturition. When not focused on giving birth or nursing, olfactory cues may exert an inhibitory effect on maternal responsiveness (Lévy et al., 2004). This phenomenon could be linked to olfactory modifications occurring during pregnancy (Corona and Lévy, 2015). In humans, in fact, studies have demonstrated olfactory impairment during gestation due to hormonal changes (Dikici et al., 2017; Albaugh et al., 2022). However, to the authors' knowledge, no correlational studies has been conducted to explore this topic in dogs, and the impact of hormonal changes on the olfaction in this species remains unknow.

Further research is needed to investigate the effect of amniotic fluid olfactory stimulation on salivary oxytocin levels in pregnant female dogs, particularly using amniotic fluid collected from non-alien pups. Such studies would help deepen our understanding of this intriguing and little-explored topic.

Conclusions

The present findings suggest that administering a small amount of amniotic fluid collected from alien newborn pups is not sufficient to stimulate an increase in sOXT and it is likely not an adequate stimulus to induce any maternal behavior in anestrus dogs. Although the small sample size involved in the present study may have contributed to the lack of significant results, it could be hypothesized that the changes in estradiol and progesterone levels during pregnancy, or pseudo pregnancy, are necessary to stimulate attraction towards amniotic fluid and enhance OXT release.

References

Abitbol, M.L. and Inglis, S.R. (1997) 'Role of Amniotic Fluid in Newborn Acceptance and Bonding in Canines', *Journal of Maternal-Fetal and Neonatal Medicine*, 6(1), pp. 49–52. Available at: https://doi.org/10.3109/14767059709161952.

Albaugh, S.L. *et al.* (2022) 'Olfaction in pregnancy: systematic review and meta-analysis', *Chemical Senses*, 47(October), pp. 1–13. Available at: https://doi.org/10.1093/chemse/bjac035.

Collias, N.E. (1956) 'The Analysis of Socialization in Sheep and Goats', *Ecology*, 37(2), pp. 228–239. Available at: https://doi.org/10.2307/1933135.

Concannon, P.W. (2009) 'Endocrinologic control of normal canine ovarian function', *Reproduction in Domestic Animals*, 44(SUPPL. 2), pp. 3–15. Available at: https://doi.org/10.1111/j.1439-0531.2009.01414.x.

Concannon, P.W. (2011) 'Reproductive cycles of the domestic bitch', *Animal Reproduction Science*, 124(3–4), pp. 200–210. Available at: https://doi.org/10.1016/j.anireprosci.2010.08.028.

Corona, R. and Lévy, F. (2015) 'Chemical olfactory signals and parenthood in mammals', *Hormones and Behavior*, 68, pp. 77–90. Available at: https://doi.org/10.1016/j.yhbeh.2014.06.018.

Dikici, O. *et al.* (2017) 'Effects of pregnancy on olfaction', *ENT Updates*, 7(2), pp. 104–107. Available at: https://doi.org/10.2399/jmu.2017002009.

Dunbar, I., Ranson, E. and Buehler, M. (1981) 'Pup retrieval and maternal attraction to canine amniotic fluids', *Behavioural Processes*, 6(3), pp. 249–260. Available at: https://doi.org/10.1016/0376-6357(81)90004-8.

Gandelman, R. (1973) 'The ontogeny of maternal responsiveness in female Rockland-Swiss albino mice', *Hormones and behavior*, 4(3), pp. 257–268.

Guardini, G. *et al.* (2016) 'Influence of morning maternal care on the behavioural responses of 8-week-old Beagle puppies to new environmental and social stimuli', *Applied Animal Behaviour Science*, 181, pp. 137–144. Available at: https://doi.org/10.1016/j.applanim.2016.05.006.

Guardini, G. *et al.* (2017) 'Influence of Maternal Care on Behavioural Development of Domestic Dogs (Canis Familiaris) Living in a Home Environment', *Animals*, 7(12), p. 93. Available at: https://doi.org/10.3390/ani7120093.

HIGUCHI, T. *et al.* (1983) 'Pulsatile secretion of prolactin and oxytocin during nursing in the lactating rat.', *Endocrinologia Japonica*, 30(3), pp. 353–359. Available at: https://doi.org/10.1507/endocrj1954.30.353.

Huffmeijer, R. *et al.* (2012) 'Salivary levels of oxytocin remain elevated for more than two hours after intranasal oxytocin administration', *Neuro endocrinology letters*, 33(1), pp. 21–25.

Keller, M. *et al.* (2003) 'Maternal experience influences the establishment of visual/auditory, but not olfactory recognition of the newborn lamb by ewes at parturition', *Developmental Psychobiology: The Journal of the International Society for Developmental Psychobiology*, 43(3), pp. 167–176.

Kendrick, K.M. (1994) 'Neurobiological correlates of visual and olfactory recognition in sheep', *Behavioural Processes*, 33(1–2), pp. 89–111.

Kim, Y.K. *et al.* (2010) 'Behavioural reactivity of the Korean native Jindo dog varies with coat colour', *Behavioural Processes*, 84(2), pp. 568–572. Available at: https://doi.org/10.1016/j.beproc.2010.02.012.

Klaus, M. (1998) 'Mother and Infant: Early Emotional Ties', *Pediatrics*, 102(Supplement_E1), pp. 1244–1246. Available at: https://doi.org/10.1542/peds.102.SE1.1244.

Klopfer, P.H. and Gamble, J. (2010) 'Maternal "Imprinting" in Goats: The Role of Chemical Senses', *Zeitschrift für Tierpsychologie*, 23(5), pp. 588–592. Available at: https://doi.org/10.1111/j.1439-0310.1966.tb01615.x.

Lee, M.H.S. and Williams, D.I. (1974) 'Changes in licking behaviour of rat mother following handling of young', *Animal Behaviour*, 22(3), pp. 679–681. Available at: https://doi.org/10.1016/S0003-3472(74)80016-3.

Lévy, F. *et al.* (1995) 'Involvement of the main but not the accessory olfactory system in maternal behavior of primiparous and multiparous ewes', *Physiology & Behavior*, 57(1), pp. 97–104. Available at: https://doi.org/10.1016/0031-9384(94)00200-O.

Lévy, F., Keller, M. and Poindron, P. (2004) 'Olfactory regulation of maternal behavior in mammals', *Hormones and Behavior*, 46(3), pp. 284–302. Available at: https://doi.org/10.1016/j.yhbeh.2004.02.005.

Levy, F. and Poindron, P. (1987) 'The importance of amniotic fluids for the establishment of maternal behaviour in experienced and inexperienced ewes', *Animal Behaviour*, 35(4), pp. 1188–1192. Available at: https://doi.org/10.1016/S0003-3472(87)80175-6.

Levy, F., Poindron, P. and Le Neindre, P. (1983) 'Attraction and repulsion by amniotic fluids and their olfactory control in the ewe around parturition', *Physiology & Behavior*, 31(5), pp. 687–692. Available at: https://doi.org/10.1016/S0031-9384(83)80004-3.

Lezama-García, K. *et al.* (2019a) 'Maternal behaviour in domestic dogs', *International Journal of Veterinary Science and Medicine*, 7(1), pp. 20–30. Available at: https://doi.org/10.1080/23144599.2019.1641899.

Lezama-García, K. *et al.* (2019b) 'Maternal behaviour in domestic dogs', *International Journal of Veterinary Science and Medicine*, 7(1), pp. 20–30. Available at: https://doi.org/10.1080/23144599.2019.1641899.

Lidfors, L.M. (1996) 'Behavioural effects of separating the dairy calf immediately or 4 days post-partum', *Applied Animal Behaviour Science*, 49(3), pp. 269–283. Available at: https://doi.org/10.1016/0168-1591(96)01053-2.

Lopatina, O. *et al.* (2012) 'The roles of oxytocin and CD38 in social or parental behaviors', *Frontiers in Neuroscience* [Preprint]. Available at: https://doi.org/10.3389/fnins.2012.00182.

MacLean, E.L. *et al.* (2017) 'Effects of affiliative human-animal interaction on dog salivary and plasma oxytocin and vasopressin', *Frontiers in Psychology*, 8(SEP), pp. 1–9. Available at: https://doi.org/10.3389/fpsyg.2017.01606.

Mariti, C. *et al.* (2020) 'Does attachment to man already exist in 2 months old normally raised dog puppies? A pilot study', *Dog Behavior*, 6(1), pp. 1–11. Available at: https://doi.org/10.4454/db.v6i1.96.

Marques, D.M. (1979) 'Roles of the main olfactory and vomeronasal systems in the response of the female hamster to young', *Behavioral and neural biology*, 26(3), pp. 311–329.

Martin, J. *et al.* (2018) 'Oxytocin levels in saliva correlate better than plasma levels with concentrations in the cerebrospinal fluid of patients in neurocritical care', *Journal of Neuroendocrinology*, 30(5), p. e12596. Available at: https://doi.org/10.1111/jne.12596.

Mitrani, M.I. *et al.* (2021) 'Case Report: Administration of Amniotic Fluid-Derived Nanoparticles in Three Severely Ill COVID-19 Patients', *Frontiers in Medicine*, 8, p. 242. Available at: https://doi.org/10.3389/fmed.2021.583842.

Noirot, E. (1972) 'The Onset of Maternal Behavior in Rats, Hamsters, and Mice A Selective Review', in *Advances in the Study of Behavior*, pp. 107–145. Available at: https://doi.org/10.1016/S0065-3454(08)60008-X.

Ogi, A. *et al.* (2020) 'Effects of Stroking on Salivary Oxytocin and Cortisol in Guide Dogs: Preliminary Results', *Animals*, 10(4), p. 708. Available at: https://doi.org/10.3390/ani10040708.

Ogi, A., Naef, V., *et al.* (2021) 'Oxytocin Receptor Gene Polymorphism in Lactating Dogs', *Animals*, 11(11), pp. 1–10. Available at: https://doi.org/10.3390/ani11113099.

Ogi, A., Mariti, C., *et al.* (2021) 'The Influence of Oxytocin on Maternal Care in Lactating Dogs', *Animals*, 11(4), p. 1130. Available at: https://doi.org/10.3390/ani11041130.

Ogi, A. and Gazzano, A. (2024) 'Maternal Behavior in Domestic Dogs', *Advances in Small Animal Care*, 5(1), pp. 1–7. Available at: https://doi.org/10.1016/J.YASA.2024.06.001.

Parker, K.J. *et al.* (2010) 'Effects of age on cerebrospinal fluid oxytocin levels in free-ranging adult female and infant rhesus macaques', *Behavioral Neuroscience*, 124(3), pp. 428–433. Available at: https://doi.org/10.1037/a0019576.

Pawluski, J.L., Walker, S.K. and Galea, L.A.M. (2006) 'Reproductive experience differentially affects spatial reference and working memory performance in the mother', *Hormones and Behavior*, 49(2), pp. 143–149. Available at: https://doi.org/10.1016/j.yhbeh.2005.05.016.

Poindron, P. and Le Neindre, P. (1980) 'Endocrine and sensory regulation of maternal behavior in the ewe', in *Advances in the Study of Behavior*. Elsevier, pp. 75–119.

Rosenblatt, J.S. (1967) 'Nonhormonal Basis of Maternal Behavior in the Rat', *Science*, 156(3781), pp. 1512–1514. Available at: https://doi.org/10.1126/science.156.3781.1512.

Rosenblatt, J.S. and Siegel, H.I. (1981) 'Factors governing the onset and maintenance of maternal behavior among nonprimate mammals', in *Parental care in mammals*. Springer, pp. 13–76.

Santos, N.R., Beck, A. and Fontbonne, A. (2020) 'A review of maternal behaviour in dogs and potential areas for further research', *Journal of Small Animal Practice*, 61(2), pp. 85–92. Available at: https://doi.org/10.1111/jsap.13085.

Schaal, B. and Marlier, L. (1998) 'Maternal and Paternal Perception of Individual Odor Signatures in Human Amniotic Fluid – Potential Role in Early Bonding?', *Neonatology*, 74(4), pp. 266–273. Available at: https://doi.org/10.1159/000014033.

Stelow, E.A., Bain, M.J. and Kass, P.H. (2016) 'The Relationship Between Coat Color and Aggressive Behaviors in the Domestic Cat', *Journal of Applied Animal Welfare Science*, 19(1), pp. 1–15. Available at: https://doi.org/10.1080/10888705.2015.1081820.

Teicher, M.H. and Blass, E.M. (1977) 'First Suckling Response of the Newborn Albino Rat: The Roles of Olfaction and Amniotic Fluid', *Science*, 198(4317), pp. 635–636. Available at: https://doi.org/10.1126/science.918660.

Underwood, R. (1979) 'Mother-Infant Relationships and Behavioral Ontogeny in the Common Eland (Taurotragus-Oryx-Oryx)', *South African Journal of Wildlife Research*, 9(1–2), pp. 27–45.

Vaglio, S. (2009) 'Chemical communication and mother-infant recognition', *Communicative & Integrative Biology*, 2(3), pp. 279–281. Available at: https://doi.org/10.4161/cib.2.3.8227.

Varendi, H. *et al.* (1998) 'Soothing effect of amniotic fluid smell in newborn infants', *Early Human Development*, 51(1), pp. 47–55. Available at: https://doi.org/10.1016/S0378-3782(97)00082-0.

Varendi, H., Porter, R.H. and Winberg, J. (1994) 'Does the newborn baby find the nipple by smell?', *The Lancet*, 344(8928), pp. 989–990. Available at: https://doi.org/10.1016/S0140-6736(94)91645-4.

Wamboldt, M.Z. and Insel, T.R. (1987) 'The ability of oxytocin to induce short latency maternal behavior is dependent on peripheral anosmia.', *Behavioral Neuroscience*, 101(3), p. 439.

Wells, D.L. and Hepper, P.G. (2006) 'Prenatal olfactory learning in the domestic dog', *Animal Behaviour*, 72(3), pp. 681–686. Available at: https://doi.org/10.1016/j.anbehav.2005.12.008.

Wilhelmy, J. *et al.* (2016) 'Behavioral associations with breed, coat type, and eye color in single-breed cats', *Journal of Veterinary Behavior*, 13, pp. 80–87. Available at: https://doi.org/10.1016/j.jveb.2016.03.009.

Effetto della stimolazione olfattiva con liquido amniotico sulle cagne in anestro

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Sintesi

È noto che il liquido amniotico attrae i mammiferi intorno al parto, ma il suo impatto ormonale attraverso la stimolazione sensoriale rimane poco chiaro. Questo studio ha esaminato l'impatto della stimolazione olfattiva del liquido amniotico sull'ossitocina salivare (sOXT) nel Labrador Retriever. I cani sono stati divisi in un gruppo di controllo (CG, n = 9) che ha ricevuto acqua e un gruppo di trattamento (TG, n = 8) che ha ricevuto liquido amniotico. Campioni di saliva sono stati raccolti da ciascun cane prima (T0) e 10 minuti dopo la somministrazione (T1). Le cagne pluripare avevano livelli di sOXT significativamente più alti (media ± DS = 114,1 ± 16,2 pg/ml) rispetto a quelle primipare (media ± DS = 93,6 ± 16,38 pg/ml) a T0 (t = 2,56; p = 0,021). Tuttavia, non sono state riscontrate differenze significative nelle concentrazioni di sOXT tra CG e TG in entrambi i punti temporali, né all'interno dei gruppi tra T0 e T1. Sebbene non statisticamente significative, le madri con mantello giallo hanno mostrato livelli di sOXT più elevati (108,1 ± 20,22 pg/ml) rispetto a quelle color cioccolato (100,6 ± 22,98 pg/ml) e nere (106,2 ± 17,45 pg/ml). È stata osservata una correlazione positiva tra età e sOXT a T0 (r = 0,445; p = 0,035). Questi risultati suggeriscono che il liquido amniotico da solo non aumenta la sOXT ed è probabilmente insufficiente per indurre il comportamento materno nelle cagne in anestro.