Maternal behaviour in domestic dogs: a comparison between primiparous and multiparous dogs

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Abstract: Maternal care was assessed in 10 litters from different breeds of domestic dog living in a home environment. Subjects were divided into two groups; a primiparous group (5 mothers, 32.4±22.3 months old; 5.0±1.6 puppies per litter) and a multiparous group (5 mothers, 74.4±10.0 months old, 6.6 ±1.7 puppies per litter). Mothers and puppies were videoed for 15 minutes each day, in the morning, from day 1 to 21 after the birth.

Videos were analysed on a second-by-second basis, observing each mother-puppy couple and using an ethogram of mother-puppy interaction behaviours adapted from Rheingold (1963).

The median total amount each interaction behaviour (during the 21 day period) was compared between primiparous and multiparous groups (Mann Whitney test). Levels of each interaction behaviour were also compared between the groups at three timepoints (day 1, 10 & 19) to investigate changes in maternal behaviour during the three weeks of observation (Mann Whitney test). Within-group changes in interaction behaviours were evaluated for the same time points (Wilcoxon signed-rank test).

Total contact between mother and pup was significantly higher during the 21 day period in multiparous mothers, and remained consistent between timepoints. In primiparous mothers there was a statistically significant increase in contact over time that, at day 19, led to a statistically higher value compared to multiparous mothers ($p=0.042$; $U=283.000$). Primiparous mothers showed a significant increase in nursing behaviour during the analysed period, and when compared with multiparous mothers, were found to spend significantly more time nursing their puppies at day 19 ($p=0.002$;$U=212.000$). The amount of licking of the anogenital area was found to be significantly higher in primiparous mothers over the 21-day period, and increased significantly from day 1 to day 19. When compared with multiparous mothers, primiparous mothers were found to spend significantly more time licking the anogenital area of their puppies at day 19 ($p=0.001$; $U=199.500$).

In summary, in primiparous mothers there was a marked increase in licking the anogenital area, nursing and staying in contact with the puppies from day 1 to 21, which led at the end of the third week to a higher quantity of maternal care compared to multiparous mothers; whilst in multiparous mothers there was a tendency towards constant maternal care. These preliminary results suggest interesting differences in maternal behaviour between primiparous and multiparous bitches.

Key Words: puppy, dog, maternal care.

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1. Introduction

Maternal care giving is a behavioural pattern that is crucial to the survival of offspring in most mammal species (Blaustein, 2007). Expression of maternal behaviour involves a complex interaction between internal and external sensory cues, multiple endocrine systems and the brain (Champagne et al., 2003; Blaustein, 2007). Early mother-infant interactions have a strong influence on the development of the infant's future emotional reactivity and social behaviour (Bowlby, 1969; Mogi et al., 2011; Numan & Insel, 2003; Masís-Calvo et al., 2013). Bowlby, one of the most famous researchers of attachment, describes the mother's love in infancy and childhood as being as important for mental health as vitamins and proteins are for physical health (Ridenour, 1952).

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Differences in stress response, maternal care and its effects on behavioural development of offspring between primiparous and multiparous mothers have been investigated in many mammalian species. In both primates and rodents, the decreased mother-infant contact resulting from extended periods of maternal separation, contributes to increased fearfulness, inappropriate social behaviour, and impaired cognitive development of the offspring (Suomi, 1997; Trickett & McBride-Chang, 1995). Moreover, in the rat, variations in maternal behaviour in terms of mother body contact and amount of licking received by the pups in the nest, produce important long-term behavioural and physiological consequences, in terms of regulation of hypothalamic-pituitary-adrenal axis (HPA) activity, spatial learning and memory (Barha et al., 2007; Toki et al., 2007), brain morphology (Bagot et al., 2009; Champagne et al., 2008), neurotransmitter content in several brain structures (Zhang et al., 2005; Champagne et al., 2004; Masís-Calvo et al., 2013), which may remain until adulthood and can be transmitted to the following generations (Rosenzweig, 1984; Meaney, 2001; Francis et al., 2002; Champagne et al., 2003; Poindron, 2005; Schechter et al., 2012).

It would be expected that rearing experience might have an impact on future maternal competence and efficiency during subsequent breeding episodes because the experienced mother ought to have learned how to best handle her infant (Numan & Insel, 2003; Poindron, 2005), but results from literature are inconsistent.

Some studies have confirmed an association between maternal experience and rearing performance. An improvement in maternal behaviour after the first parturition is well documented in ewes (Poindron, 2005; Houpt, 2011). Human mothers are generally less anxious with their second-born than with their firstborn (Lasko, 1952; Mcarthur, 1956; Mitchell & Stevens, 1969; Jacob & Moss, 1976). Litter survival and weight gain has been observed to improve significantly with experience and increasing parity in some species (Myers & Master, 1983; Wright & Bell, 1978), such as in *Peromyscus polionotus* (Margulis et al., 2005), in rats (Cohen & Bridges, 1981), in mice (Brown et al., 1999), in tamarins (Tardif et al., 1984), in gerbils (Salo & French, 1989), in vervet monkeys (Fairbanks, 1990), and in prairie voles (Wang & Novak, 1994). Retrieval behaviour (Carlier & Noirot, 1965; Swanson & Campbell, 1979; Bienest-Noirot, 1961), and time spent in the whelping box (Wang e Nowak, 1994), also seem to improve significantly with experience in rodents. Even motherless monkeys that behave inappropriately towards their first baby appear to behave normally towards the second one (Ruppenthal et al., 1976; Kemps et al., 1989).

However, other studies in rodents and monkeys have shown that experience of motherhood has no effect on maternal behaviour or on the mother-infant relationship (Moltz & Robbins, 1965; Beach & Jaynes, 1965a and 1965b; Schino et al., 1995). Swanson & Campbell (1979) compared the maternal behaviour of female golden hamsters during their first and second litters and found that the composite measures of maternal behaviour (e.g. amount of nursing and nest building) did not differ between the two groups.

There has been very little investigation to describe and evaluate the effects of maternal care in domestic dogs in the first post-partum weeks (Scott, 1950; Scott & Marston, 1950; James, 1952; Rheingold, 1963). With few exceptions (Pal, 2005), most studies have been conducted under kennel or laboratory conditions, and without focusing on the reproductive experience, or parity, of the subjects. Previous researchers have shown that immediately following parturition bitches display a novel repertoire of pup-directed maternal behaviours, comprising; a nursing posture that facilitates and directs suckling to the mammary zone, body/genital licking of pups and retrieving pups to the nest (Rosenblatt, 1975; Rosenblatt et al., 1988; Macbeth & Luine, 2010). However, the effect of maternal experience, acquired during an initial reproductive episode, on competence and efficiency of canine maternal behaviour during subsequent reproductive cycle has not been investigated.

The aim of this research was to analyse maternal care in family dogs and investigate whether there are differences in maternal care given to the offspring between primiparous and multiparous mothers.

2. Subjects, materials and methods

2.1. *Subjects*

Ten litters of puppies, of different pedigree and mixed-breeds, were recruited from Italian professional and non-professional dog breeders, giving a total of 10 mothers and 58 puppies (table 1).

The mean age of primiparous mothers was 32.4 ± 22.3 months and that of multiparous mothers 74.4 ± 10.0 months.

The mean number of puppies per litter was 5.0 ± 1.6 for primiparous mothers and 6.6 ± 1.7 for multiparous mothers, with a mean number of male puppies per litter of 3.6 ± 1.14 for primiparous and 2.6 ± 1.34 for multiparous mothers. The mean number of female puppies per litter was 1.4 ± 0.9 for primiparous and 4 ± 2.64 for multiparous mothers.

In order to gather information on each bitch and her offspring, breeders were asked to complete a questionnaire at the commencement of the study (including mother signalment, provenance -private or professional breeder- parity of mother, litter information such as number and sex of puppies, number of puppies died at birth or during nursing and weight of puppies during the first 21 days after the birth).

Table 1. Characteristics of the 10 litters included in the research.

litter	mother	breed	age (months)	n. parity	males	females	provenance
1	1	a	18	1	3	2	PB
2	2	b	72	1	4	0	PB
3	3	c	24	1	2	1	NPB
4	4	b	72	3	4	3	PB
5	5	d	60	3	2	3	PB
6	6	b	84	2	4	1	PB
7	7	e	72	3	2	5	PB
8	8	f	24	1	5	2	PB
9	9	g	84	3	1	8	PB
10	10	h	24	1	4	2	NPB

Legend: a = Weimaraner, b = Belgian Shepherd Groenendael, c = Cross-breed, d = Short Haired Dachshund, e = German Shepherd, f = Labrador retriever, g = Boxer, h = Border collie; PB = professional breeder, NPB = not professional breeder. In lilac, the primiparous mother.

2.2. *Mother-litter interaction*

On every day from day 1 to 21, a 15-minute video recording was made of each mother with her puppies (to include the neonatal and transitional period). Puppies were identified with different satin coloured ribbons (fig. 1), which had to remain on the puppy throughout the 21 day period. Videos were made by the breeder in the morning, when the mother returned to the whelping box after the first exit of the day to urinate and defecate. Two videocameras were used (Sony HDR-CX240EB) mounted on tripods (Manfrotto MKC3-H01), and oriented toward opposite sides of the whelping box to ensure that the whole area was captured.

Fig. 1. Mother with her puppies wearing coloured ribbons (Litter 1-Weimaraner).

2.3. *Ethogram*

To assess maternal care given to each puppy and the behaviour of each single puppy, an ethogram from Rheingold (1963) was used (table 2).

The ethogram includes mother-puppy interactions (contact mother-pup, licking, licking anogenital, nursing), which are specific to a mother-puppy dyad.

Table 2. Ethogram from Rheingold (1963).

Behaviour	Definition
Contact mother-pup (contact)	Physical contact of any sort between mother and pups in the whelping box.
Nursing (nursing)	Composed of several components: active suckling, finding a nipple, maintaining the grasp, pushing in of forelegs, pulling up of head, and disposing of rear legs.
Licking (licking)	Each occurrence of the mother's licking of the pup other than in the anogenital area.
Licking anogenital area (licking-ag)	Each occurrence of the mother's licking the perineal region (areas of anus, urinar opening, belly and rear).

2.4. *Statistical analysis*

Mother-puppy interaction behaviours were recorded on a second-by-second basis throughout each video (continuous recording). To evaluate overall between-group differences in maternal behaviour, the total amount of each mother-puppy interaction behaviour was calculated for each puppy over the 21-day observation period (21-day-sum) and the Mann-Whitney test was used to compare totals between primiparous and multiparous mothers.

Three sample days were chosen (days 1, 10 & 19) as timepoints to compare mother-puppy interaction behaviours between primiparous and multiparous mothers at different stages of development. The total amount of each behaviour was calculated for each day. The Mann Whitney test was used to compare primiparous to multiparous mothers and the Wilcoxon signed-rank test was used to compare different timepoints within the same group of mothers.

3. Results

The mean number of puppies that died at birth for primiparous and multiparous mother was 0.6 ± 0.9.

No one puppy died during nursing in the litters of primiparous mothers while 0.1 ± 0.31 died in those of multiparous.

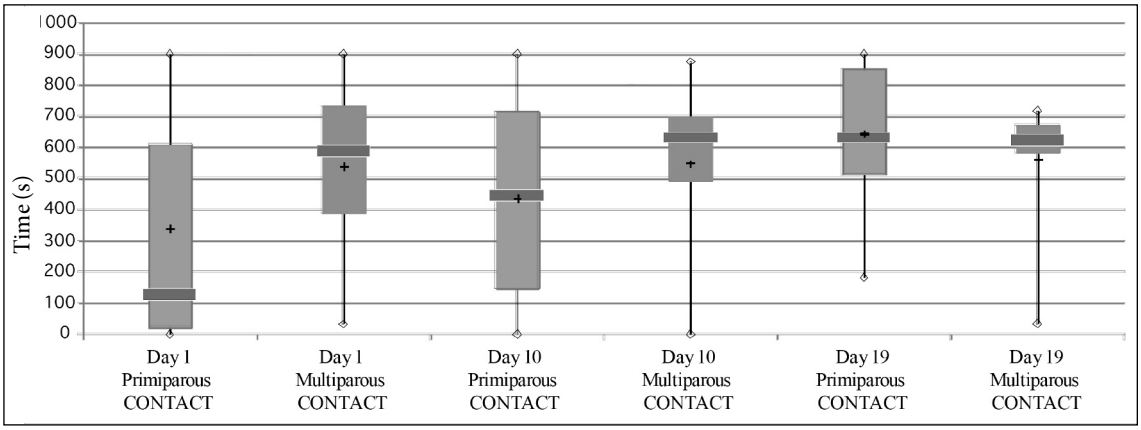
Table 3 presents results for those 21-day-sum variables that were statistically different between primiparous and multiparous mothers. Total levels of licking and licking-ag behaviours were significantly higher in the primiparous mothers (respectively U=275.500; p=0.031; U=190.500, p=0.001), while contact was statistically higher in the multiparous mothers (U=273.000; p=0.028). There was no difference in nursing behaviour.

Diagram 1 shows that when comparing successive timepoints (day 1, 10 & 19), levels of contact were consistently high in multiparous mothers throughout the analysed period, while in primiparous mothers there was a statistically significant increase that, at day 19, led to a statistically higher value compared to multiparous mothers (p=0.042; U=283.000).

Table 3. Comparison of median total amount of each mother-puppy interaction behaviour that significantly differed between primiparous and multiparous mothers.

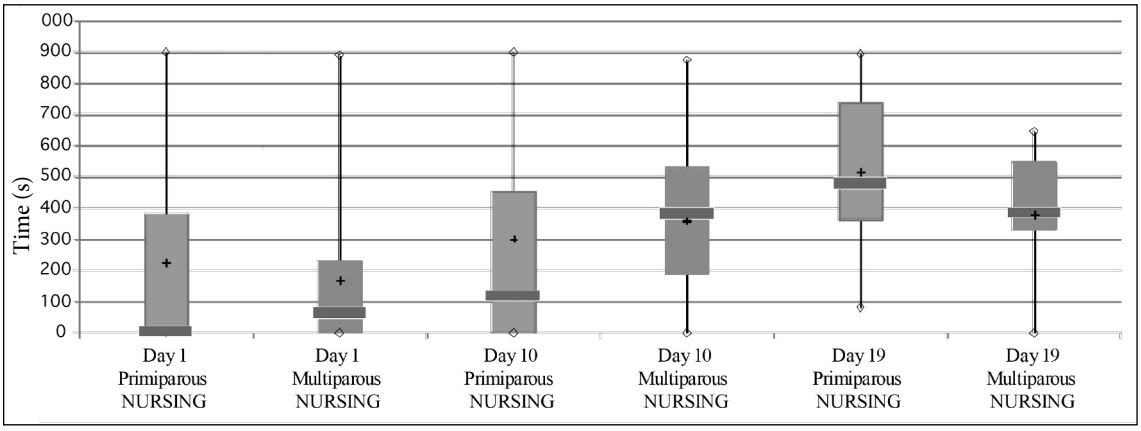
Behaviour	Group	median	U Mann-Whitney	(p)
contact	PRIMIPAROUS	455.62	273.000	0.028
	MULTIPAROUS	584.21		
licking	PRIMIPAROUS	2.10	275.500	0.031
	MULTIPAROUS	0.71		
licking-ag.	PRIMIPAROUS	38.65	190.500	0.001
	MULTIPAROUS	16.26		

Diagram 1. Box and whisker plot showing the amount of contact behaviour at each of the three timepoints for primiparous and multiparous bitches (boxes extend from the 1st to the 3rd quartiles, the median is indicated by the red line and whiskers show the maximum and minimum values).



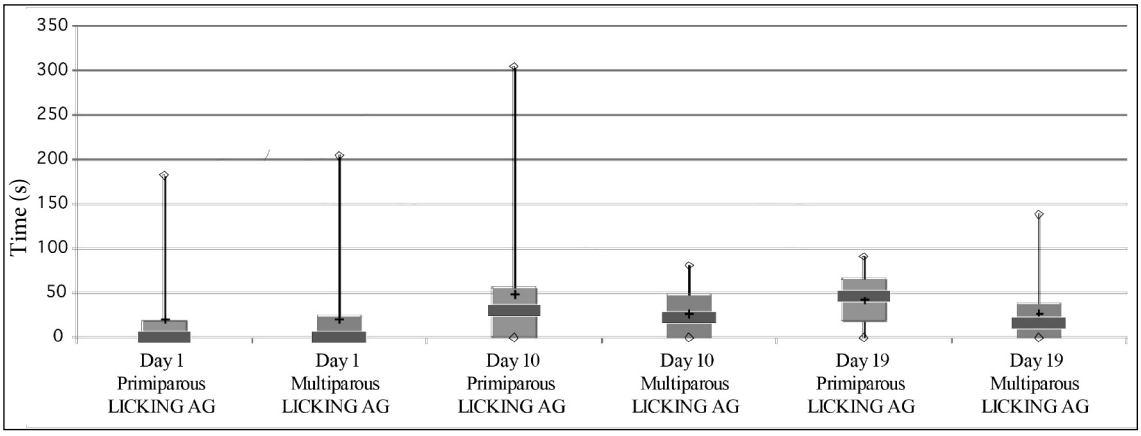
Day 10-day vs. day 1 (p=0.017); Day 19-day vs. day 1 (p=0.001); Day 19-day vs. day 10 (p=0.001). Primiparous-Multiparous: Day 19 → p=0.042; U=283.000.

Diagram 2. Box and whisker plot showing the amount of nursing behaviour at each of the three timepoints for primiparous and multiparous bitches (boxes extend from the 1st to the 3rd quartiles, the median is indicated by the red line and whiskers show the maximum and minimum values).



Day 10-day vs. day 1 (p=0.017); Day 19-day vs. day 1 (p=0.001); Day 19-day vs. day 10 (p=0.001). Primiparous-Multiparous: Day 19 → p=0.002; U=212.000.

Diagram 3. Box and whisker plot showing the amount of licking-ag behaviour at each of the three timepoints for primiparous and multiparous bitches (boxes extend from the 1st to the 3rd quartiles, the median is indicated by the red line and whiskers show the maximum and minimum values).



Primiparous: Day 19 vs. day 1 (p=0.007). Primiparous-Multiparous: Day 19 → p=0.001; U=199.500.

Despite the fact that there was no significant difference between primiparous and multiparous mothers with respect to total nursing, primiparous mothers showed a significant increase in nursing behaviour across the three timepoints (see diagram 2). Also, compared with multiparous mothers, primiparous mothers were found to spend significantly more time nursing their puppies at day 19 (p=0.002; U=212.000).

In primiparous mothers there was a significant increase in licking-ag between day 1 and 19 (see diagram 3). Compared with multiparous mothers, primiparous mothers were found to spend significantly more time licking the anogenital area of their puppies at day 19 (p=0.001; U=199.500).

4. Discussion

There is little published work on maternal care in domestic dogs during the first weeks post-partum, apart from some in lab dogs (Rheingold et al., 1963) and in free ranging dogs (Pal, 2005), and no study so far has focussed on the parity of the subjects.

Our preliminary results highlight interesting differences in maternal care between primiparous and multiparous family dogs that have not previously been presented in the literature.

We observed two different trends: in multiparous mothers there is a tendency towards constant maternal care in terms of licking, nursing and staying in contact with the puppies, whilst in primiparous mothers there is a marked increase in these behaviours throughout period from day 1 to 21, which leads at the end of the third week to a higher quantity of maternal care compared to multiparous mothers.

As a result of the limited literature available in dogs on this topic, it is difficult to compare these results with those from past work. Apart from the general lack of research into the effects of maternal experience, the differences in methodology and study population (lab dogs or free ranging dogs vs. companion dogs) between studies complicates any comparison. Pal (2005) noted a gradual decrease in maternal care in free ranging dogs from the first week post-partum. Rheingold (1963) observed that from the second week contact, nursing and licking began to decrease. However when

the puppies acquired better vision and locomotor skills during the transitional period, they approached the mother more often, and consequently there was a slightly increase in contact, nursing and licking.

There are a number of ways to explain the different trends we observed for primiparous and multiparous mothers.

Facilitation learning (Lorenz, 1978) may explain the increase in maternal behaviour in primiparous mothers; through mere repetition a behaviour becomes more prominent in the individual's repertoire. In the multiparous mothers maternal behaviour is more consistent as it has already been learnt during previous maternal experiences. In rodents a similar pattern was observed in retrieving behaviour, which improves during the first lactation period and this improvement is maintained throughout the later lactation period (Bienest-Noirot, 1961).

It is also possible that primiparous mothers are more anxious than multiparous ones. This has been reported in humans (Lasko, 1952; Mcarthur, 1956; Mitchell & Stevens, 1969; Jacob & Moss, 1976), non-human primates (Kemps & Timmermans, 1984), and golden hamsters (Swanson & Campbell, 1979), in which primiparous are generally more protective and infant-directed, because of the over-attention aroused by the novelty of having offspring. HPA activation in rodents and women is reduced with multiple pregnancies, as multiparous but not primiparous mothers have a decreased cortisol plasma level in response to social and emotional stressors (Tu et al., 2006; Figueira et al., 2008; Pawluski et al., 2009). It is possible that the increase in exploration by puppies during the transitional period might generate more anxiety in primiparous mothers who will tend to lick and retrieve the puppies more often, in order to maintain proximity and contact with them. In order to verify this hypothesis it would be necessary to take specific behavioural and physiological measures of anxiety that were not part of this study.

Differences in maternal behaviour in primiparous and multiparous mothers could be also related to oxytocin release, which is essential to initiate and promote adequate maternal care. Through suckling, the infant exerts a direct influence on maternal physiology, stimulating oxytocin release not only into the peripheral circulation to promote milk let-down, but also in the brain (Mogi et al., 2011). Over time, repeated oxytocin release in primiparous mothers may facilitate mother-infant bonding, leading to an increase in maternal care shown toward the puppies. Interestingly, in women plasma levels of oxytocin during breastfeeding have been found to be positively correlated with multiparity (Lucas et al., 1980; Sibolboro Mezzacappa & Endicott, 2007), and higher plasma oxytocin levels during the first postpartum month correlate with higher levels of maternal postpartum behaviours (Feldman et al., 2007). Moreover, increases in oxytocin receptor mRNA in the paraventricular nuclei of the hypothalamus was observed only in multiparous ewes (Broad et al., 1999).

It appears that parity of the mother influences maternal care in dogs and may have important effects on the behavioural development of the puppy. More research is needed in order to confirm our findings and to find possible correlations between the amount of maternal care and behaviour of older puppies.

Other factors need to be investigated, as they may affect maternal care, as demonstrated in several mammalian species: litter size (Seitz, 1958; Grota & Ader, 1969; Priestnall, 1972; Deviterne & Desor, 1990; Tanaka, 1998; Guerra & De O Nunes, 2001; Dimitsantos et al., 2007), gender composition of the litter (Moore & Morelli, 1979; Moore & Chadwick-Dias, 1986; Alleva et al., 1989), age of the mother (Schino et al., 1995), weight of puppies (Deviterne et al., 1990), season of birth (Wilsson & Sundgren, 1998; van der Waaij et al., 2008), breed, temperament and personality, or type of breeding, and individual variability (Champagne et al., 2003; Fairbanks, 1996).

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Il comportamento materno nel cane domestico: confronto tra cagne primipare e pluripare

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Sintesi

Le cure materne sono state valutate in 10 cucciolate di differenti razze di cani che vivevano in un ambiente familiare. I soggetti sono stati divisi in due gruppi: il gruppo delle primipare composto da 5 femmine (in media 32,4±22,3 mesi d'età e 5,0±1,6 cuccioli per nidiata) ed il gruppo delle pluripare composto da altre 5 femmine (in media 74,4±10,0 mesi d'età e 6,6±1,7 cuccioli per nidiata). Le madri ed i cuccioli sono stati videoregistrati per 15 minuti, ogni giorno, al mattino, dal giorno del parto al 21° giorno.

I filmati sono stati analizzati interamente, secondo per secondo, osservando ogni coppia madre cucciolo ed usando, per la valutazione del comportamento, un etogramma riportato da Rheingold (1963) e modificato per l'attuale ricerca.

Comportamento	**Definizione**
Contatto madre-cucciolo	Contatto fisico di ogni tipo tra la cagna ed il cucciolo nella cassa parto.
Nursing	Composto da alcuni comportamenti: suzione attiva del capezzolo, ricerca del capezzolo, mantenimento della presa della mammella con la zampa, spinta con la zampa sulla mammella, spinta con la testa sulla mammella, posizione con le zampe posteriori distese.
Leccamento	Ogni evento di leccamento della madre nei confronti del cucciolo, ad eccezione del leccamento ano-genitale
Leccamento ano-genitale	Comportamento di leccamento della madre indirizzato verso la regione anale o ombelicale o vulvare del cucciolo.

È stato effettuato un confronto, attraverso il test statistico di Mann Withney, della quantità totale media di ogni comportamento di interazione (durante i 21 giorni) tra le cagne primipare e quelle pluripare.

I livelli di ogni interazione sono stati anche comparati nei due gruppi in 3 diversi momenti (giorno 1, 10 e 19) per verificare eventuali cambiamenti del comportamento materno durante le 3 settimane di osservazione.

Il contatto totale tra madre e cucciolo è stato significativamente più alto durante i 21 giorni nelle madri pluripare, mentre nelle primipare si nota un continuo incremento di tale comportamento che al giorno 19 supera in quantità quello espresso dalle pluripare ($p=0,042$; $U=283,000$).

Per quanto riguarda il nursing, le primipare mostrano un significativo aumento di tale comportamento durante il periodo analizzato. Anche in questo caso al giorno 19 esse lo manifestano in modo più prolungato rispetto alle pluripare ($p=0,002$; $U=212,000$).

Il leccamento ano-genitale è più prolungato nelle primipare rispetto alle pluripare per tutti i 21 giorni esaminati ed aumenta significativamente dal giorno 1 al 19.

In conclusione, nelle cagne primipare si nota un marcato incremento dei comportamenti considerati dal 1° al 21° giorno.

Una possibile spiegazione può risiedere nel fenomeno della Facilitazione, una forma di apprendimento non associativo che consiste nel miglioramento di un comportamento in seguito alla sua ripetizione. Inoltre non si può escludere che le madri primipare siano più ansiose e siano quindi più protettive nei confronti dei cuccioli.

Anche i fattori ormonali devono essere presi in considerazione: è possibile che nelle cagne primipare il rilascio di ossitocina sia ridotto e quindi provochi un ridotto comportamento materno.