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Observation on Possibility to Identify by the Stud Dogs the Signs of the Fertile Period in Bitches

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Abstract: According to the reports of other researchers, some dogs are able to determine not just if the bitch is in estrus but if she is at the optimal time for breeding. The aim of the study was to evaluate if this thesis is true. Nineteen stud dogs of different breeds and thirty five bitches were used. Bitches were presented to the males at the beginning of estrus, confirmed by the low level of progesterone (<2 ng mL^{-1}) and at the time considered as a optimal for breeding (10-15 ng mL^{-1}). The males reaction to the female was recorded. The seven males confirmed their ability to recognize the best time for breeding in the bitches. They were ready to mate only the females being at the optimal period for conception. The average age and experience of these dogs were significantly higher than the age and experience of the dogs not presenting those capabilities. That could suggest the meaning of learning in process of developing such a skills.

Key words: Dogs, pheromones, estrus detection, conception, bitches, optimal

INTRODUCTION

The influence of the volatile chemicals on human and animal behavior is well documented and used to be applied in practice (Fatjo, 2010; Graham *et al*., 2005; Havlicek, 2011; Levy *et al*., 2004; Nishimura *et al*., 1991; Pageat and Gaultier, 2003; Pickel *et al*., 2004). In bitches during the estrus, the pheromones present in their vaginal discharge and urine cause the female to become attractive to the male dogs. Adult male dogs stimulated by those chemical signals show readiness for mating.

According to the reports of other researchers and many breeders, some dogs are able to determine not just if the bitch is in estrus but if she is at the optimal time for breeding (Kustritz, 2005).

Wierzbowski also described the meaning of sense of smell for the sexual behavior of the animals suggested that the scent signals in females whose estrus lasted for a relatively long time, changed as they approached the time of ovulation. The aim of the studies was to verify those reports and find out if the phenomenon of the identification of the optimal time for breeding by some dogs can be truly observed.

MATERIALS AND METHODS

The animals: During the experiment, nine German Shepherd stud dogs, four Golden Retrievers, two Beagles and one Bavarian Mountain dog, Sharpay dog and Great Dane at age 2-7 years were examined (Table 1). Thirty five

Table 1: The age, breeding experience and maintenance system of the stud dogs

			Maintenance system	
Breed	Age (years)	Breeding experience (No. of mating/year)*	Kept in kennel with females	Kept in separation from females
German shepherd	5.0	90**	+	-
Shetland sheepdog	6.5	10	-	+
German shepherd	2.0	4	+	-
German shepherd	2.0	3	+	-
German shepherd	3.0	6	-	+
Geat dane	6.0	7	+	-
German shepherd	2.5	6	-	+
Sharpay dog	6.0	6	+	-
Golden retriever	3.0	4	+	-
German shepherd	5.0	90	-	+
German shepherd	3.0	7	-	+
Beagle	2.5	7	+	-
German shepherd	7.0	38	+	-
German shepherd	5.5	43	+	-
Beagle	3.0	5	+	-
Golden retriever	5.0	6	-	+
Golden retriever	6.0	5	-	+
Golden retriever	5.5	6	-	+
Bavarian mountain dog	7.0	6	-	+

*In the year of experiment; **In Germany the stud dogs of German Shepherd breed are allowed up to a maximum of 90 mating per calendar year (According to SV rules http://www.vonjagenstadt.com/SVRules.html)

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bitches of different breeds were used. We focused on the young (2-3 years old) females to minimize the effect of dominance that is reported as a potential reason for problems with the mating and which could be the cause of false negative reaction (Mertens, 2006). Too nervous or aggressive bitches was not included in the experiment. The bitches used in experiment were checked for the signs of estrus based on the history taken from the owners and the clinical examination.

The exact detection of the stage of estrus cycle was determined by clinical examination and laboratory tests. During clinical examination the presence and the character of discharge, the presence and the quality of vulva oedema, the tolerance reflex and the electrical resistance of vaginal mucous were evaluated.

Laboratory tests consisted of the vaginal cytology and the progesterone concentration analysis in the peripheral blood. Furthermore, the vaginal cytology was performed and the presence, appearance and degree of cornification of the epithelial cells were evaluated (Kustritz, 2006). Progesterone concentration were determined by validated RIA.

Blood sampling, progesterone assay and timing of mating: About 8 mL of blood were taken by venipuncture from the cephalic vein and into heparinised tubes. Plasma was separated 60 min after blood taking by centrifugation for 15 min at 2000×g.

Progesterone concentration was determined on the same day with the use of a commercial Radioimmunoassay (RIA) kit (Progesterone Coat-a-Count kit, Diagnostic Products Corporation, Los Angeles, CA, USA), validated for dog plasma (Srikandakumar *et al*., 1986). Blood samples were taken from every bitch at 48-72 h intervals starting from early proestrus onwards. Bitches were mated when plasma progesterone concentration reached values from 10-15 ng mL^{-1}.

The experiment procedures: The experiment was carried out in the five German Shepherd's kennels that the male dogs came from, located near Wroclaw, Poland and in Germany in two kennels breeding the Golden Retriever and Bavarian Mountain dog in one kennel breeding Beagles, Sharpey dog and Great Dane. The experience procedures took place in the habitual residence of the stud dog.

The bitches were presented to the males two times in different stages of the estrus cycle. Each dog was presented with three different bitches, 2 times during the cycle. Some bitches were presented to the different males during the same heat (usually when they come from the same kennel). The presentation of the female to the male started from 6 days of proestrus cycle (the 1st day of proestrus was defined as the day when the first signs of vaginal bleeding were observed by the owner). While the progesterone concentration was <2 ng mL^{-1}. After the progesterone concentration in blood increased >2 ng mL^{-1}, the bitch was presented to the stud dog only in the time considered as a optimal for mating.

According to the literature data and the clinical observation, the best time for mating correlates with the level of progesterone between 10-15 ng mL^{-1} (Johnston *et al*., 2001; Manothaiudom *et al*., 1995). The reaction of the stud dogs to the female was analyzed, refer to the modified method proposed by Goodwin *et al*. (1979). Shortly before the exposition, a bitch was let free on a restricted area where she could urinate and the male could observe her behaviour.

After it, the male was allowed for the free contact with the bitch. To avoid aversive reaction of the female that may be observed in the proestrus and which could influence the male behaviour, the bitches in all cases were gently kept by the owners during the procedures of testing. During this contact, male reaction to the female was recorded based on the following criteria:

Reaction type I: The male was immediately interested in the bitch, after short contact the male mounted or tried to mount the female.

Reaction type II: The male approached the female, sniffed and licked the vulva but then showed little interest in the bitch and he did not tried to mount her. The reaction of the male was compared to previously obtained results of laboratory tests. Also the detailed evaluation of the male behavior during the tests were conducted (Table 2).

The study was approved by the Local Ethical Committee for the Affairs of Experiments on Animals of the Wroclaw University of Environmental and Life Sciences.

Statistical analysis: Obtained data (percentage of the reaction typy I or II test of attractiveness) were evaluated with the U-Mann-Whitney test with the significance level $p = 0.05$. The age of the stud dogs and the experience (measured by the average number of mating per year) was compared with the use of U-Mann-Whitney test ($p<0.01$).

Table 2: The reaction of the male during the contact with the females being in optimal (A2, B2, C2) and not optimal (A1, B1, C1) time for mating (level of progesterone >10 or <2 ng mL^{-1}, respectively)

	Male behavior																							
	Sniffing						Licking the vulva						Pawing						Mounting					
Female/ males	A1	A2	B1	B2	C1	C2*	A1	A2	B1	B2	C1	C2	A1	A2	B1	B2	C1	C2	A1	A2	B1	B2	C1	C2
I**	P (P***)	P	P	P	P	P	P	P	P	P	P	P	N (N****)	P	N	P	N	P	N	P	N	P	N	P
II	P	P	P	P	P	P	P	P	N	P	P	P	N	P	N	P	N	P	N	P	N	P	N	P
III	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
IV	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
V	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	N	P	P	P	P	P	N	P
VI	P	P	P	P	P	P	P	P	P	P	P	P	N	P	N	P	N	P	N	P	N	P	N	P
VII	P	P	P	P	P	P	P	P	P	P	P	P	N	P	P	P	P	P	N	P	P	P	P	P
VIII	P	P	P	P	P	P	P	P	P	P	P	P	N	P	P	P	N	P	N	P	N	P	N	P
IX	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
X	P	P	P	P	P	P	P	P	P	P	P	P	N	P	N	P	N	P	N	P	N	P	N	P
XI	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
XII	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
XIII	P	P	P	P	P	P	P	P	P	P	P	P	P	P	N	P	N	P	N	P	N	P	N	P
XIV	P	P	P	P	P	P	P	P	P	P	P	P	N	P	N	P	N	P	N	P	N	P	N	P
XV	P	P	P	P	P	P	P	P	P	P	P	P	N	P	P	P	P	P	N	P	P	P	P	P
XVI	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
XVII	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
XVIII	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
XIX	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P

*(A1, A2, B1, B2...) females. Females A-C in most cases were different for different males; **(I, II, III,...) male dogs; ***(P) Positive-behaviour observed ****(N) Negative-the lack of the behaviour

RESULTS AND DISCUSSION

Researchers found that seven, out of nineteen examined dogs (males no 1, 2, 6, 8 ,10, 13, 14) showed the ability to recognize the best time for breeding in the bitches. Those dogs showed the type I reaction only with bitches being in optimal time for breeding and in other cases they did not mounted the females (reaction type II). The other dogs in the most cases were deeply interested and earnestly attempted to cover bitches, regardless they were in the optimal time for mating or not (reaction type I). Figure 1 shows the results as a percentage of reaction (type I or II) depending on the level of progesterone in bitches presented to the stud dogs.

Researchers found that average age of the dogs presenting mentioned capabilities was significantly higher, than the age of the males not presenting those capabilities (5.8±0.7 and 3.6±1.5, repetitively). Moreover, the experience in mating was the significant factor distinguishing both groups (the mean number of mating was 40.5±36 and 5.4±1.2 for the dogs detecting and not detecting the optimal time for mating). We did not find any relationship between the kennel facilities of the stud dogs (kept together or in separation from the bitches).

Semiochemical substances emitted into the environment can modify the behavior of individuals receiving them in a variety of ways (Brennan and Keverne, 2001; Graham *et al*., 2005; Jezierski, 1992; Levy *et al*., 2004; Pageat and Gaultier, 2003; Tod *et al*., 2005; Vazquez and Orihuela, 2001). In reproduction, the

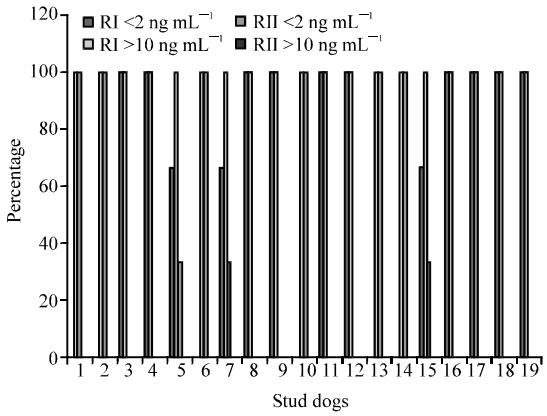

Fig. 1: Results as a percentage of reaction (type I or II), depending on the level of progesterone in bitches presented to the stud dogs. RI = Reaction type I, RII = Reaction type II

observation of the male behavior during the contact with the female suspected to be in estrus is the simplest and most popular method for the estrus detection which is routinely used as a breeding procedures in many species including the farm animal like cows or sheep (Nishimura *et al*., 1991; Stevens *et al*., 1982; Ungerfeld *et al*., 2006). Because of the short time of estrus duration (18-36 h), the estrus detection system based on the olfactory stimulus in many species is sufficient. In contrast in dogs and also in horses where the estrus period is considerable longer (in bitches heat takes about 18-20 days), just the detection of the estrus

is not sufficient to predict the fertile period (Kustritz, 2005). In those species, the more specific and adequate methods for prediction of the optimal time for breeding (vaginal cytology, concentration of hormones in blood, ultrasound examination of ovarian activity) are routinely used (Johnston *et al*., 2001; Kustritz, 2006).

Pheromones are chemical signals usually consisting of a few compounds (Gelez and Fabre-Nys, 2006; Pageat and Gaultier, 2003). Goodwin *et al*. (1979) indicates as the main bitche's pheromone, the methyl parahydroxybenzoate ($CH_3(C_6H_4(OH)COO)$). However, those researchers clearly state that there are many more unidentified substances present in the vaginal discharge during the estrus which purpose and function are still unknown. Moreover, the pheromones secreted in the anal glands could carry important information in the context of sexual attractiveness (Pickel *et al*., 2004). It cannot be excluded that simultaneously with the changes in hormone concentration (decrease of estrogens and increase of progesterone), the changes in concentration or/and composition of some substances secreted to the environment as chemical signals occurs. These changes are consistent with the knowledge that the concentration of sex hormones correlate and has a direct impact on the presence or absence of specific volatile compounds including pheromones (Gawienowski *et al*., 1976; Goodwin *et al*., 1979; Raymer *et al*., 1986).

According to some breeder's suggestions, the abilities of some stud dogs to detect the most fertile period in female during the estrus is related to the maintenance system: those abilities should be shown by males kept in the kennel with the females. In the experiment, data examination did not confirm these assumptions (Table 1).

The ability to distinguish the particular phases during the bitch's heat seems to be very useful for the leading stud dogs which are usually intensively used. According to the observation that ability is typical for some experienced stud dogs while inexperienced, younger males did not show this abilities.

Question that arise is if that what we observed are innate or learned skills. That would confirm the importance of learning in process of pheromone detection as suggested by some researchers who investigated the influence of the pheromones (also the sex pheromones) on the animal behavior (Chanvallon and Fabre-Nys, 2009; Gelez and Fabre-Nys, 2004; Lanuza *et al*., 2011). Although, pheromones, according to the definition should cause the preprogrammed effect responses which are minimally influenced by experience (Karlson and Luscher, 1959), the modern, current and based on the latest research model of the semiochemicals activity takes into account also the importance of the learning process (Chanvallon and Fabre-Nys, 2009; Fatjo, 2010; Gelez and Fabre-Nys, 2004; Hosokawa and Chiba, 2005; Lanuza *et al*., 2011). Gelez and Fabre-Nys (2004) concluded that in mammals, some sexual pheromones, particularly those eliciting behavioral responses, require learning processing to be effective. Lanuza *et al*. (2011) which investigated the neural circuits allowing the transfer of chemosensory information to the brain reward system indicated the importance of convergence of the main and accessory olfactory bulbs in the medial amygdale, anterior cortical amygdale and the cortex-amygdala transition zone for the animals response to the chemical signals including pheromones. In their opinion, these pathways may constitute the neural substrate of innate responses to attractive (sexual pheromones) and learned responses to associated chemosensory stimuli (Lanuza *et al*., 2011). The genetic predisposition as an important factor conditioning the development of these capabilities was also considered by many researchers (Lesniak *et al*., 2008; Maejima *et al*., 2007; Mundy, 2006).

CONCLUSION

Researchers found that some experienced stud dogs are able to determine the most fertile period during the bitches' heat. Additional studies are indicated to explain the phenomenon: whether the ability to determine the most fertile period is caused by changes in concentration of specific pheromone, like methyl parahydroxybenzoate ($CH_3(C_6H_4(OH)COO)$) (Goodwin *et al*., 1979) or by the changes in composition of more complex mixture consisted of several substances which are characteristic for the specific parts of the estrus. Currently, we have a number of sophisticated methods as vaginal cytology and measurement of hormones concentration in the blood allowing to determine the most fertile period of the cycle in bitches.

However, none of them refers directly to the senses used in nature by the male dog. Taking into account that the pheromones which are often the hormones metabolites (in the past called also exohormones) can reflect the hormonal status of the organism, we concluded that development of the method based on the detection of characteristic chemical substances present in vaginal environment (urine and vaginal discharge) during the fertile period could be in the future, the valuable tool in the veterinary practice.

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