

# Biological effects of pheromones on sexual behavior of bulls

## Summary

Pheromones are chemical substances, which are excreted from the body, and of which one individual can smell the scent of the second individual of the same species, which cause a specific reaction. This particular reaction may be a manifestation of a specific behavior or the initiation of a physiological process, usually by changing the level of activity of the hormones. Pheromones in this way play a role in reproductive physiology, they can affect the appearance of estrus in females, alter reproductive behaviors and reactivity of individuals of the opposite sex, influence the successful development of the fetus during pregnancy, and more. In addition, biostimulatory effect of pheromones has been observed in cattle, especially in terms of the positive effect of the presence of breeding bulls on ovarian activity in cows, and the success or failure of the conception.

The main goal of this study was to test the effectiveness of two molecules of pheromones identified in the urine of cows in estrus at stimulating sexual function and increasing reproductive capacity of bulls. The trial lasted four weeks and the bulls were divided into two groups (experimental and control) with ten bulls. For this experiment we used an intranasal spray comprising the pheromone molecules. We tested the effectiveness of pheromones primarily on semen quality parameters but also on libido and sexual behavior of bulls.

**Keywords:** pheromones, semen quality parameters, the sexual behavior of bulls

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## Introduction

Chemical means of communication is the oldest way of exchanging information among animals. Semiochemicals are substances that take part in this communication. They are divided into two classes, the first allelochemicals that are used for communication between individuals of different species. Subclass allelochemicals are kairomones- used for prey detection and identification of victims, allomones - used to attract prey victims, and synomones- allelochemicals of mutual benefit in the communication between two different species. In the second class are chemical substances that are used to exchange information between individuals of the same species, and their subclass consisting of pheromones. Pheromones are chemical substances that are excreted from the body and one individual whose scent can feel another individual of the same species, in that cause a specific reaction. They are sometimes classified as ectohormones and chemical messages are transmitted outside the body. This specific reaction may be the expression of a particular form of behavior or play a particular physiological process, usually by changing the level of activity the hormone.

Pheromones are divided by function of parts of the pole, alarm, fear pheromones, pheromones, which are used for marking paths of movement, marking territory, soothing, relaxing pheromones and other. They are excreted from the body of animals in several ways: urine, estrogen mucus, feces, sebum, sweat, saliva, milk, exhaled air, and is thought to be secreted, and other ways that are not yet discovered. It can be said that signaling pheromones are our olfactory genetic fingerprint. Some butterfly species males can smell females from a distance up to 10km.

The composition of the pheromones may be saturated carbonic

acid, steroids, aldehydes, ketones, alcohols or other compounds. For example: Androstenol and androstenone in boars saliva as sexual attractants. Pheromones are either volatile or soluble substances and due to the sensitive cell (receptor) by inhalation or physical contact. They can be attached to other molecules or carrier, for example, or be transformed by bacteria before they become pheromones.

Pheromones also serve to identify the herd, the herd, pack, social status of other individuals, age, sex and reproductive status within the same social group. Precisely in pheromone established the first social bond between mother and cubs (usually fathers are not able to). The so-called primer pheromones of mammals are caused by hormonal changes that affect the appearance of estrus in females, modify the full cycle of breeding cows, changing reproductive behaviors and reactivity of individuals of the opposite sex. Additionally affect the successful development of the fetus during pregnancy

The Sensory organ for the vomeronasal pheromone, or Jacobson organ whose function is effective in younger than in older animals. For better reception and guidance of volatile pheromone molecules in the air to Vomeronasal organs animals use Flehmen reaction, which is characterized by specific movements of the upper and lower lips and nostrils (Figure 1 & Figure 2). Pheromones cause a change in the current activity of the hypothalamus and amygdala.

Additional olfactory system consists of the vomeronasal organ (VNO), additional olfactory bulb (AOB), nuclei in the forebrain and connecting neurons. Sensory epithelium vomeronasal organs consist of two different groups of receptors, V1Ra and V2Rs. Information from V1Rs is transmitted to the front while the additional olfactory bulb of V2Rs transferred to the rear. This information is then merged into overlapping projections to the nucleus of the amygdala.<sup>1</sup>



**Figure 1** Flehmen reaction in different animal species.



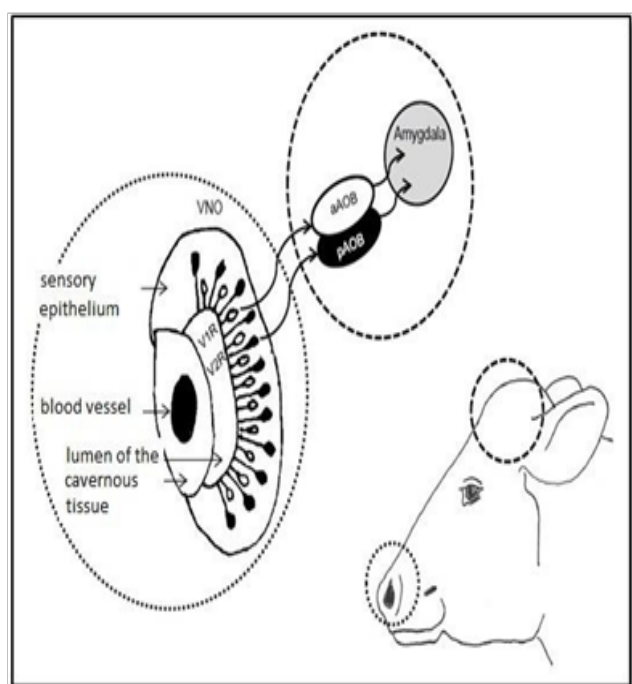
**Figure 2** Flehmen reaction in heifers.

Synthetic analogues of pheromones were until recently the most practiced in disinfection and for monitoring the size of the population of harmful insects and for preventing their reproduction. In domestic animals structural analogues of pheromones are used in reproduction. For earlier estrus manifestation gilts used pheromone boars, cows, sows and sheep pheromones which also apply for synchronizing ovulation. Pheromone represents the use of structural synthetic analogues of pheromones in therapy already manifested, and actual or potential behavioral disorders in domestic animals.

By binding to lipocalins (protein receptors in the vomeronasal organ of pheromones) pheromones cause changes in activity limbic system. The zootechnical purposes, pheromones are mostly applied in cattle and pig breeding. On the market there are already commercial preparations based on structural analogues of pheromones, which have been successfully used in the prevention and treatment of various disorders. Pheromones have a role in bio-stimulation in animal reproduction. It is known that communication pheromones play an important role in the behavior of mammals and reproductive processes. The mere presence of a boar at the time of insemination sperm transport and ovulation, and thus the presence vasectomies bull was observed that accelerates puberty in heifers and early resumption of ovarian activity in cows after calving. Understanding the role of pheromones can have potentially economic significance. It is possible and improvement of fertility by means of a pheromone. On some farms used nasal rings with substances containing pheromones. These

substances are urine and mucus from cows at the height of their estrous cycle.

Pheromones are substances that are used in communication between individuals of the same species. The word pheromone comes from the Greek word pherein, for transmitting and hormone for arousal. Most mammals have two olfactory system, the main olfactory system and additional olfactory system. The main olfactory system involves the olfactory epithelium of the nasal cavity, the main olfactory bulb, different centers of the front part of the brain and the neurons that connect these structures (Figure 3). Vomeronasal organ and olfactory bulb make additional extra olfactory system together with their nuclei and neurons in the forebrain.<sup>2</sup>



**Figure 3** Main olfactory system.

Pheromones affect the reproduction of many mammals, including domestic animals and humans. The works have shown that they can, in addition to many other things, to influence, to encourage the attractiveness of opposite poles and aggression between males, accelerate puberty, shortening the period of anoestrus, change estrous cycle, inducing mating behavior in both sexes and others.<sup>3,4</sup>

Great attention has been paid to the impact of male to female in cattle. Izard & Vandenberg<sup>5</sup> have reported that heifers that before puberty oronasal receive treatment with urine bulls reach puberty earlier than when receiving control treatment with water. Heifers treated with urine bull calf earlier and have a shorter season than the control heifers calving, but the level of pregnancy is the same for both groups.

This biostimulatory effect on ovarian activity was also observed in zebu cows<sup>6</sup> and beef breeds of cows in lactation, where the presence of a bull shortened postpartum anestrus. This was confirmed by Berardinelli & Joshi,<sup>7</sup> who found that exposure to a bull or its excretory products accelerate the continuation of luteal activity after calving. However, they did not notice the positive effect of the presence of the bull on postpartum anestrus.<sup>8</sup>

Biostimulatory effect seems to be higher when the bulls changed during the experiment, compared with continuous use of the same 23 bulls. There seems to be a reverse linear relationship between daily life the presence of a bull and the interval from calving or the beginning of a bull to the continued presence of ovarian activity.<sup>9,10</sup> Other authors emphasize the influence of pheromones on the development or reproductive physiology, including full cycle in females, as well as the success or failure of conception.<sup>11,12</sup>

## Materials and methods

The trial involved a total of 20 bulls of different breeds of A.I. Station Krnjaca. The bulls were aged 1.5 to 10years, and they are all kept in the same conditions as food and accommodation. The main objective of this study was to test the effectiveness of two molecules of pheromones identified in the urine of cows in estrus on stimulation of sexual function bull and increase reproductive capacity bullish. The trial lasted four weeks and bulls were divided into two groups (experimental and control) with ten bulls. For this experiment we used an intranasal spray-known French manufacturer of equipment and resources used in the reproduction of animals, which contains molecules of pheromone. We tested the effectiveness of pheromones on libido, sexual behavior bull, as well as changes in semen production. The manufacturer states that the target group of the bulls during puberty, young people or adults who have problems in sexual behavior (libido) as a result of over-exploitation (saturation), or lack of interest, or at the bulls in which the problem of another kind (jealousy, changing environment or the person who takes the semen, changes in pressure or temperature of the artificial vagina, etc.). Not for use in bulls who have physical problems compatible with their

reproductive potential (too old bulls, with acute or chronic problems, etc.).

The spray is sprayed directly in the experiment in the bull's nostrils, twice on each side, a distance of about 20 cm. The spray is used as soon as the bull enters the room for taking semen or within 20 min. before jumping on the phantom. The dose was not increased, as there may be counter-receptor saturation or nasal mucous membrane, also was not needed nor the habituation. Semen samples from bulls are usually taken - two times a week, the semen were tested in a standard way. In preparing the bull to jump, after application of the pheromone spray was studied to see whether it is to be a change in the time from preparation to ejaculation. In the laboratory were compared semen from the experimental and control groups in the following parameters and volume, concentration and the percentage of live and progressively motile sperm before and after freezing.

## Results and discussion

The processed data are presented in Tables 1–3. As seen in the Table, the volume of ejaculate in the experimental group ranged from 4.7ml to 7.0ml with an average of 5.81ml in the control group ranged from 4.6ml to 6.2ml with an average of 5, 32ml. In the experimental group in which the pheromone is used, the volume of ejaculate was an average of 0.49ml greater than in the control group, but this difference was not statistically significant ( $p>0.05$ ). When it comes to the number of spermatozooids in 1ml of ejaculate, concentration, it is moving in the experimental group from 1.20 to 1.79billion/ml (mean value of 1.49billion/ml), while the control group had an average of 1.33billion ml (1.06 to 1,56billion/ml). The difference of 0.16billion/ml in favor of the experimental group was statistically significant ( $p<0.05$ ).<sup>13–17</sup>

**Table 1** Average values of parameters of semen of bulls-experimental group

Bull	Volume (ml)	Concentration (mlrd/ml)	% of live and progressively motile sperm (before freezing)	% of live and progressively motile sperm(after freezing)
1	5,5	1,38	81,33	61,24
2	4,9	1,79	83,65	60,20
3	6,6	1,44	82,55	64,74
4	6,1	1,39	80,27	62,15
5	7,0	1,58	80,71	61,96
6	4,9	1,20	82,50	60,88
7	5,2	1,69	82,80	62,01
8	5,0	1,24	83,13	60,53
9	5,5	1,55	84,17	64,18
10	4,7	1,64	82,37	61,32
x	5,81	1,49	82,35	61,92

**Table 2** Average values of parameters of semen of bulls-control group

Bull	Volume (ml)	Concentration (mlrd/ml)	% of live and progressively motile sperm (before freezing)	% of live and progressively motile sperm (after freezing)
1	5,8	1,52	80,75	60,12
2	4,7	1,22	82,70	61,36
3	6,1	1,16	84,39	63,77
4	5,5	1,36	81,73	61,02
5	4,9	1,12	84,11	63,69
6	6,2	1,06	83,41	62,17
7	5,3	1,36	83,28	60,41
8	4,6	1,44	82,96	61,87
9	5,1	1,49	83,31	62,33
10	5,0	1,56	80,22	59,78
x	5,32	1,33	82,69	61,65

**Table 3** Differences in the values of the parameters of sperm bulls between experimental and control groups

	N	Volume(ml)	Concentration (mlrd/ml)	% of live and progressively motile sperm (before freezing)	% of live and progressively motile sperm (after freezing)
Experimental	10	5,81	1,49	82,35	61,92
Control	10	5,32	1,33	82,69	61,65
<b>Difference</b>		<b>+ 0,49</b>	<b>+ 0,16</b>	<b>- 0,34</b>	<b>+ 0,27</b>

Analysis of the results relating to the percentage of live and progressively motile sperm, have been observed following differences in mean. In the experimental group of bulls given percentage was 0.34% lower before freezing compared to the control. The difference in the percentage of live and progressively moving after freezing is 0.27% higher in the group with pheromone before jumping in respect of bulls which are not applied. This difference is not statistically significant ( $p > 0.05$ ).<sup>18,19</sup>

In addition to those presented and statistically analyzed results when preparing bulls both groups for taking ejaculate observed the behavior of bulls, and the reaction time after the bull approaching phantom to ejaculation. Although time is not measured, it seems that the bulls of the experimental group after the preparation of pheromones needed less time to ejaculation in relation to the bulls of the control group.

## Conclusion

Confirmed the biological effects of pheromones - molecules isolated from breeding cows in estrus, breeding bulls given in terms of increasing the number of sperm in the ejaculate. Registered an increase ejaculate volume and the percentage of live and progressively motile sperm after nasal spray pheromones to application, but it is not statistically significant. It has been observed in bulls from the experimental group and shortening response times after approaching

phantom and to decrease the transformation before ejaculation, which needs further investigation, since in addition to saving time, this reduction of time spent in the room for taking semen may also limit the risk of injuries related to the handling of the bulls.

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## Conflict of interest

Author declares that there is no conflict of interest.

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