Development of sexual behaviour in ram lambs and its correlation to serum testosterone

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SUMMARY

The drive to mate females is variable among the rams and can have a major impact on sheep production. Androgen testosterone may be a key mediator within the expression of various morphological and behavioural traits in mammals, but the factors underlying individual variation in circulating testosterone levels are poorly understood.

The aim of this study was to investigate the development of sexual behaviour patterns in ram lambs as well as the role of testosterone in the expression of their libido.

Research was carried out on the sheep farm of the Institute for Animal Husbandry, Belgrade, Serbia. The study included 20 crossbred ram lambs (autochthonous breed Pirot Pramenka x Merinolandschaf x Ile de France). All ram lambs used in the study were of same age and rearing conditions. They were weaned at 2 months of age and kept indoors from birth throughout the whole study. Animals were introduced in the study at the age of 3 months and the trial was completed at the rams' age of 17 months. Blood samples for testosterone levels were taken bimonthly (at the age of 3, 5, 7, 9, 11, 13, 15 and 17 months), as well as were male-female and male-male interactions recorded.

Male-female interactions included the following elements of behaviour: nosing (or anogenital sniffing), pawing, flehmen response, attempted mounts. Also, duration of all male activities directed towards ewe was recorded (male-female interactions in total). Male-male interactions involved the frequency of male-male mounts.

Performed investigations showed that sexual behaviour of rams was age dependent, but poorly correlated to serum testosterone. The average serum testosterone levels ranged from 1.83-13.28 ng/mL, and were age dependant ($P<0.05$).

Male-female oriented behaviour developed linearly with age, while male-male specific behaviour was characterized by high intensity in young age and then pronounced variability in later test periods. None of the studied behavioural interactions were highly correlated to serum testosterone. These findings support standpoint of more than one factor influencing development of sexual behaviour of ram lambs.

KEY WORDS

Rams, sexual behaviour, testosterone, correlative relationships.

INTRODUCTION

The desire to mate is variable among the rams and can have a major impact on sheep production, especially when only one ram is used for reproduction in the flock. Libido or sexual desire refers to sexual motivation and is manifested through certain forms of behaviour such as: searching for sheep, detection of sheep in the oestrus, courting and mating. Rams exhibit a wide range of different libido levels, from none to extremely aggressive, which is focused solely on the search and mating of female animals, while sacrificing all other needs, such as food, water and rest[1]. There are several stereotyped forms of behaviour that the ram can exhibit just before the first mount, which are defined as courtship behaviour. These include anogenital sniffing, ewe's flunk nudging, impatient foot stomping, lifting the head and neck while simultaneously raising the upper lip as a reaction to the smell of the ovine urine, called flehmen response, as well as emitting low-pitched 'gargling' vocalizations[2, 3].

Mating behaviour and libido of rams can be estimated through so called serving capacity tests in which rams are exposed to ewes confined to a pen of limited size[4]. These tests are also known as pen tests and usually imply using females in heat and are repeated in order for rams to be assigned with a serving capacity score, which is the average number of ejaculations each ram achieves over repeated 30 minutes testing periods. Sexual behaviour can be very variable among rams and while some become immediately attracted by present female and begin courtship even if female is out of heat, others never approach ewes or take a long time before they do. These strong individual variations in sexual behaviour of rams are not completely understood.

Testosterone as main androgen was often considered as a predominant for expressing and maintaining libido in rams as reported in previous studies of various authors[3, 5, 6]. However, many

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other studies showed no significant correlation between androgen levels and sexual behaviour in rams[7,8]. Testosterone may be a key mediator in the expression of various morphological and behavioural traits in mammals, but the factors underlying individual variation in circulating testosterone levels are not completely understood[9].

Understanding the scheme of sexual behaviour of rams is not only important from the aspect of selection of quality males, but also in order to determine the best male to female rations for mating.

The aim of this study was to investigate the development of sexual behaviour patterns in ram lambs as well as the role of testosterone in the expression of their libido.

MATERIAL AND METHODS

Investigations were carried out on an experimental sheep farm of the Institute for Animal Husbandry, Belgrade. The study included 20 crossbred rams (autochthonous breed Pirot Pramenka x Merinolandschaf x Ile de France). All ram lambs used in the study were of same age and conditions. Prior to weaning, male lambs were kept with their mothers and, starting from the second week of age, additionally fed with alfalfa hay and concentrate (160 g protein/kg plus vitamins and minerals) *ad libitum*. Lambs were weaned at the age of 2 months and were kept in the barn system in one group until the end of the study. The post weaning diet was based on the use of alfalfa hay and concentrates. Animals were introduced in the study at the age of 3 months and the trial was completed at the rams' age of 17 months.

Blood samples for testosterone level were collected every two months (at the age of 3, 5, 7, 9, 11, 13, 15 and 17 months, respectively) from the jugular vein in the morning period from 8 to 9 am. After one hour, blood samples were centrifuged, and the blood serum was extracted, which was then used for the analysis. Analysis was performed by radioimmunoassay (RAİ) method using a commercial kit (Immulite® total testosterone, Siemens healthcare diagnostic inc., UK) for animal use.

The aspects of sexual behaviour of rams were studied through male-female and male-male interactions every two months, i.e. at the age of rams of 3, 5, 7, 9, 11, 13 and 15 months.

Male-female interactions were monitored individually by introducing a ram into a pen with non-oestrous restrained ewe for 20 minutes[10]. These interactions included the following elements of behaviour: 1) the incidence of direct contact by sniffing anogenital region of ewe (nosing), 2) the frequency of impatient foot stomping of the ram (pawing), 3) the frequency of the manifestation of the flehmen response (upper lip response), 4) the frequency of attempted mounts, 5) duration of all activities directed towards ewe (male-female interactions in total). All interactions were tracked by directly observing and recording all the relevant activities, as well as by video recording.

Male-male interactions were monitored in a group box for 8 hours per day by video recording, and the frequency of male-male mounts was recorded.

Statistical analysis of the experimental data was performed using the statistical package IBM SPSS Statistics 21. Statistical significance of differences of all examined parameters were determined by means of the one way ANOVA, followed by the Tukey HSD test. The relative dependence of the traits was determined by Pearson's correlation coefficient, whose significance was tested. Analyses were performed for the significance levels of 5% and 1%, and the results are presented as mean ± SEM. Animal experimentation was conducted within standard ethical norms.

RESULTS

Testosterone concentration changed with rams' age and also showed high variability throughout the study period (Figure 1), which was previously investigated by Maksimović et al.[11]. The average values of the testosterone level in the blood serum of the rams ranged from 1.83 ng/ml at three months of age to 13.28 ng/ml at the end of the test period (when rams were at the age of 17 months), which was significant at $P<0.05$.

Table 1 shows mean values, variability and statistical difference of analysed sexual activity patterns expressed through male-female interactions in different ages of rams.

Incidence of all analysed interactions between rams and ewe was elevated with age of rams, and this was statistically significant ($P<0.05$). Frequency of these interactions rose linearly from third to ninth month of age, after which it showed variability in terms of declining and rising throughout rest of study period. The most frequent male-female interactions in forms of nosing, pawing and mounts were observed in rams at the age of 13 months, as well as the longest duration of all activities directed towards ewe.

Male-male interactions analysed through frequency of attempted mounts in different age of rams are given in Table 2. From the data presented it can be seen that a total of 175 male-male mounts were registered for the whole study period with

Table 1 - Mean ± SEM of analysed male-female interactions.

Age of rams (months)	Nosing (frequency)*	Pawing (frequency)*	Flehmen (frequency)*	Mounts (frequency)*	Total male-female interactions (minutes)*
3	$3.05^{d} \pm 0.56$	$0.10^{d} \pm 0.10$	$0.15^{d} \pm 0.10$	$0.00^{e} \pm 0.00$	$0.51^{d} \pm 0.15$
5	$3.35^{d} \pm 0.52$	$0.25^{d} \pm 0.25$	$0.25^{cd} \pm 0.09$	$0.10^{de} \pm 0.10$	$0.81^{d} \pm 0.20$
7	$8.20^{c} \pm 1.44$	$4.35^{bc} \pm 1.85$	$1.05^{abc} \pm 0.32$	$6.00^{bc} \pm 3.31$	$4.48^{c} \pm 1.12$
9	$13.30^{ab} \pm 1.61$	$5.30^{bc} \pm 1.87$	$2.40^{a} \pm 0.69$	$9.55^{bc} \pm 4.22$	$8.14^{b} \pm 1.27$
11	$9.20^{bc} \pm 1.07$	$2.20^{cd} \pm 1.39$	$1.80^{ab} \pm 0.61$	$2.00^{cd} \pm 1.56$	$4.38^{c} \pm 0.60$
13	$15.60^{a} \pm 1.65$	$20.10^{a} \pm 3.82$	$1.50^{ab} \pm 0.38$	$15.45^{a} \pm 3.18$	$12.66^{a} \pm 1.02$
15	$9.20^{bc} \pm 1.21$	$12.35^{b} \pm 3.80$	$0.75^{bcd} \pm 0.35$	$11.15^{ab} \pm 3.28$	$6.62^{bc} \pm 1.01$

* $P<0.05$; ^{a,b,c,d} - column means with different letters differ significantly.

Table 2 - Mean ± SEM of analysed male-male interactions.

Age of rams (months)	No of mounts/ram	Mounts (sum)
3	$0.1^{c} \pm 0.1$	2
5	$3.2^{a} \pm 1.29$	66
7	$1.75^{a} \pm 0.88$	35
9	$0.25^{bc} \pm 0.20$	5
11	$0.9^{abc} \pm 0.69$	18
13	$1.55^{ab} \pm 0.75$	31
15	$0.9^{abc} \pm 0.61$	18

* P<0.05; ^{a,b,c} - column means with different letters differ significantly.

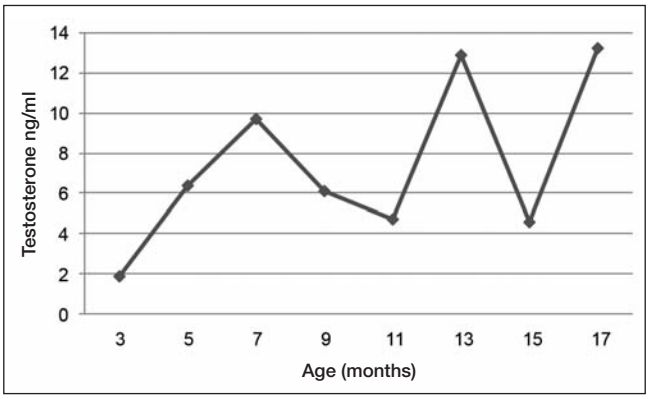

Figure 1 - Serum testosterone levels in rams depending on the age.

Table 3 - Correlation coefficients between serum testosterone and sexual behaviour activities.

Parameters	Nosing	Pawing	Flehmen	Male-female mounts	Male-female interactions in total (duration)	Male-male mounts
Testosterone	0.35**	0.19*	0.05	0.12	0.29**	0.18*

* P<0.05; ** P<0.01.

average number of mounts per ram ranging from 0.1 to 3.2. The highest activity was recorded at the age of 5 months. The determined frequencies of male-male mounts were significantly dependent on the age (P<0.05), and they showed significant variability throughout the test period.

Table 3 shows correlation coefficients determined between testosterone concentration and sexual activity patterns of rams. The obtained results of the analysed correlations between testosterone concentration and the aspects of sexual behaviour of rams showed a weak to moderate relationship. Weakest link was between testosterone and flehmen response and the strongest one was with nosing behaviour, which was also statistically significant (P<0.01).

DISCUSSION

The concept of sexual behaviour of male individuals is quite complex, both in its basis, in terms of differentiation, as well as in terms of the way and the strength of expression. As it was pointed out, individual differences in libido and sexual preferences that exist among rams are not completely understood. When exposed to ewe, most rams will exhibit courtship behaviour usually consisted of anogenital sniffing, ewe's flunk nudging, impatient foot stomping and flehmen response, followed by completed mounts. In contrast, a small percentage of rams develop a same-sex preference for other rams even when raised with females[12, 13]. In current study, all of the examined aspects of sexual behaviour showed almost an identical trend, with the increase in value during the period from 3 to 9 months of rams' age, followed by subsequent periods of lower and higher activities, with a very significant jump of activity at the age of 13 months, when the highest values of these parameters were determined. This is in agreement with the research by Ungerfeld and Gonzalez-Pensado[10], who monitored the development and manifestation of sexual behaviour of rams from 6 weeks to 9 months of age, in which it is emphasized that the frequency of expressing sexual behaviour toward non-oestrous ewe progressively increased with age.

Observations of sexual activity patterns of individual rams showed that not all of them exhibited all forms of courting and mating behaviour, but all of them engaged in some form of sexual interaction at some point even though the female used were out of heat.

Male-male interactions that are expressed in the context of sexual behaviour are quite common among domestic animals. Rams begin to develop and manifest this kind of sexual behaviour early, even within the first 10 weeks of life[10], which is characterized by mounting attempts and simulation of copular behaviour that is directed at other males in the group.

A total of 175 male-male mounts were registered for the whole study, with most frequent interactions being recorded in 5 months old lambs. The determined values were statistically significant (P<0.05) depending on the age, but showed significant variability during the entire test period. The very number of identified interactions itself is difficult to compare with the number determined in other studies, due to the difference in the number of animals, the length of the tests and the calculation methodology, but also because of very few such studies. However, this study is in agreement with previous findings of Ungerfeld et al.[14] who determined intensive male-male interactions in ram lambs age 5 to 6 months. This is also consistent with the findings of Grubb[15], who reported intense courting behaviour among the rams when they were about six months old. Nonetheless, although this study showed a significant effect of the age, it cannot be concluded that there was a linear trend in increasing these activities with age, which is contrary to the findings of Ungerfeld and Gonzalez-Pensado[10]. Grubb[16] reported that among wild Soay male lambs, male-male mounts were more frequently observed than male-female

mounts. Some authors proposed that this kind of behaviour is related to establishment of social hierarchy[15, 17]. Some consider that rams mount opportunistically, e.g., if one ram is eating at the feeder or drinking, the other ram will try to mount him, or when rams are clogged in the crowd while moving, they will mount the individuals in front of them[3,18]. However, there is little information about this form of behaviour and its physiological significance.

The results obtained in this study showed that sexual behaviour of rams wasn't directly related to serum testosterone. Tested correlations between testosterone concentrations and aspects of sexual behaviour were generally weak to moderate. Even though some of these correlations were statistically significant, the strength of established correlative relationships is not convincing enough to support the distinct testosterone-dependent nature of rams' sexual behaviour. This is in agreement with other work, which reported that there is no correlation between plasma testosterone and libido[19, 20]. It is likely that in the basis of male reproductive behaviour, testosterone has a significant place, but that it is not solely responsible for its expression, i.e. that there are other systems and mechanisms involved whose role cannot be ignored or excluded. As proposed by some authors, sexual behaviour displayed by adult rams requires minimum concentrations of circulating testosterone[21, 22], so when this minimum requirement is met sexual performance won't be directly related to testosterone concentrations.

Roselli et al.,[23] consider that hormones themselves are important but not directly responsible for the regulation of sexual behaviour, but that a significant role in this process has a nervous system, more specifically a region that includes a medial preoptic area/anterior hypothalamus (MPOA/AH). The MPOA/AH comprises a steroid-sensitive brain region that contains high concentrations of androgen and oestrogen receptors[24]. Roselli et al.,[25] explain the hypothesis that a critical period exists in early perinatal life during which circulating testosterone produced from the foetal testis masculinises and defeminises both the neuroendocrine and behavioural potential of the brain.

CONCLUSIONS

Performed investigations concluded that sexual behaviour of rams was age dependent, but poorly correlated to serum testosterone. Male-female oriented behaviour developed linearly with age, while male-male specific behaviour was characterized by high intensity in young age and then pronounced variability in later test periods. None of the studied behavioural interactions were highly correlated to serum testosterone. These findings support standpoint of more than one factor influencing development of sexual behaviour of ram lambs.

DISCLOSURE STATEMENT

No conflict of interest was reported by the authors.

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