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Competition for oestrous ewes between rams reared by their mothers or artificially reared: Effects on sexual behaviour and testosterone and cortisol serum concentrations

J.P. Damián [a, *], M.J. Hötzel [b], G. Banchero [c], R. Ungerfeld [d]

[a] Departamento de Biología Molecular y Celular, Facultad de Veterinaria, Universidad de la República, Montevideo, Uruguay
[b] Laboratório de Etologia Aplicada e Bem-Estar Animal, Departamento de Zootecnia e Desenvolvimento Rural, Universidade Federal de Santa Catarina, Florianópolis, Brazil
[c] Unidad de Ovinos, INIA "La Estanzuela", Colonia, Uruguay
[d] Departamento de Fisiología, Facultad de Veterinaria, Universidad de la República, Montevideo, Uruguay

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ABSTRACT

The objective of this study was to determine how the social competition for an oestrous ewe affects the sexual behaviour and the endocrine response in two groups of rams, one reared by their mothers and another artificially reared. Thus, we compared the sexual behaviour and testosterone and cortisol changes in each group of rams in competitive and non-competitive tests, both during the first and second breeding seasons. Two groups of rams were: 1) artificially reared lambs, separated from their dams 24 −36 h after birth (Week 0) and artificially fed with sheep milk until 10 weeks of age (group AR, n = 14); and 2) lambs reared by their dams until 10 weeks of age (group DR, n = 13). Rams were subjected to non-competitive and competitive tests for an oestrous ewe during their first and second breeding seasons, when they were 8 and 20 months old, respectively. Sexual behaviours toward an oestrous ewe were recorded during 20 min and the testosterone and cortisol concentrations were determined in serum samples collected immediately before the test, and 20, 40 and 60 min after it. During the first breeding season, the number of flehmen decreased in DR rams, and the number of flehmen and ano-genital sniffings also decreased in DR rams, but the frequency of some copulatory behaviours increased (matings and ejaculation/total mounts in DR rams, and total mounts in AR rams) in competitive tests. During the second breeding season, competition caused a decrease in the number of all the recorded behaviours (courtship and copulation) with the exception of flehmen in AR rams; however, in DR rams only the number of the copulatory behaviours decreased under competition. Competition did not affect the endocrine response during the first breeding season. During the second breeding season, while testosterone concentrations were greater in non-competitive than in competitive tests at 60 min (P = 0.0008) in AR rams, in DR rams it tended to be greater (P = 0.09). Competition did not affect cortisol concentrations in any group or season, but in all tests the concentrations increased at the end of the test (P < 0.05). In conclusion, the lack of the mother during rearing negatively affected the sexual motivation and the testosterone response of rams to oestrous ewes in competitive tests, effects that were more evident when adults. Neither the absence of the mother during rearing nor competition for oestrous ewes affected the stress response (evidenced by increase in cortisol concentration) in rams during both seasons.

1. Introduction

The sexual behaviour of adult rams can be affected by the social environment that they had during rearing [1,2]. Maternal behaviour is essential for the care and survival of the offspring, thus contributing to the future reproductive success [1,3,4]. We recently reported that rams that were reared by their dams display better sexual behaviour toward oestrous ewes during their development and adult life than rams that were reared artificially [5,6]. In those

* Corresponding author.
E-mail address: jpablodamian@gmail.com (J.P. Damián).

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studies the sexual behaviour of each male was studied in individual tests with an oestrous ewe. However, the social environment is very different in productive breeding practices than that of individual sexual tests, as rams commonly compete for the access to oestrous ewes. In general rams reduce the display of sexual behaviours for an oestrus ewe in competition with other males [7–9]. Therefore, the competitive tests provide the opportunity to study how these rams cope with the presence of other rams in sexual tests; which may be useful to understand clearly what happens in competitive breeding services. In this work we proposed studying the ability of rams that were reared by their mothers or rams reared artificially in competition for an oestrus ewe.

Mating and exposure to oestrous ewes stimulate LH, testosterone [10,11] and cortisol [12,13] secretion in rams. In addition, competitive interactions act as stressors, inducing an increase in serum cortisol [14]. Conditions during rearing may also affect rams' stress responses; for example, rams reared by their mothers are more stressed by social isolation than rams artificially reared [15,16]. Therefore, although the exposure to oestrous ewes may increase rams' testosterone serum concentrations, the increase in cortisol may interfere with testosterone secretion [17,18]. Considering that the stress response varies according to how rams were reared [15,16], the testosterone response to oestrous ewes may also vary. In short, unlike that which occurs in individual sexual tests, competition is an additional stressor, and therefore the competitive situation itself can affect the sexual and endocrine responses differently according to how the rams were reared.

We hypothesized that competition for an oestrous ewe has more negative effects on the sexual activity and the endocrine response after exposure to oestrous ewes in rams reared by their dams than in rams artificially reared. The objective of this study was to determine how the social competition for an oestrous ewe affects the sexual behaviour and the endocrine response in two groups of rams, one reared by their mothers and another artificially reared. Thus, we compared the sexual behaviour and testosterone and cortisol changes in each group of rams in competitive and non-competitive tests, both during the first and second breeding seasons.

2. Materials and methods

All the experimental procedures were approved by the Comisión Honoraria de Experimentación Animal (CHEA) of the Universidad de la República (PI No. 25/10).

2.1. Location, animals and management

The study was conducted at the Unidad Experimental de Ovinos, INIA La Estanzuela, Uruguay (34°19′ S) with the same animals used by Damián et al. [5,19]. In summary, 27 Polwarth rams born as singletons (Week 0) were tagged, and both members of the dyad registered immediately after birth. Lambs were assigned to two experimental groups: 1) artificially reared lambs were separated from their dams 24–36 h after birth (group AR, n = 14); 2) lambs reared by their dams remained with their dams until Week 10 (group DR, n = 13). Ewes from the same flock were milked, and milk was provided to AR lambs by artificial teats in a similar frequency to natural suckling. Four adult ewes bearing their single lambs were kept with the AR group during the lactation period to avoid the potential confounding effect of the absence of adult ewes in this group. All lambs received solid ration from the third week of age, grazed on improved pastures, and had free access to water. At Week 10, DR lambs were weaned and milk provision to AR lambs was ended, and the ewe-lamb dyads kept with AR lambs were moved out. The two groups of rams remained in paddocks separated by an electric fence.

The present study was carried out during the first and second breeding seasons (April 2012 and 2013, mid-autumn), when the rams were 8 and 20 months old, respectively. Prior to April 2012 the lambs were individually tested with oestrous ewes as part of another study [5].

2.2. Sexual behaviour during non-competitive and competitive tests

Rams were subjected to non-competitive (individual) sexual behaviour tests, and one week later to competitive tests. All tests were performed in a 5 m x 5 m pen using one ewe induced into oestrus with a hormonal treatment [5–6 days of intravaginal sponges impregnated with medroxi-progesterone acetate, and i.m. injections 1.5 mg of oestradiol benzoate every 12 h (Bioestrogen, Biogénesis Bagó, Buenos Aires, Argentina)]. In the competitive tests, two rams, one from each group (paired according to their body weight at that moment [first breeding season: AR: 40.5 ± 0.7 kg and DR: 39.5 ± 0.9 kg, second breeding season: AR: 49.3 ± 1.0 and DR: 48.4 ± 1.5 kg]), entered into the pen simultaneously. The number of ano-genital sniffings, lateral approaches, flehmen, mounting attempts, mounts (without ejaculation), matings (mounts with ejaculation) were recorded during 20 min, and the ejaculation/total mounts ratio was calculated.

2.3. Blood samples and determinations of testosterone and cortisol concentrations

Blood samples were collected by jugular venipuncture immediately before (time zero = 0), and 20 min (when the test ended), 40 min and 60 min after each test. Samples were allowed to clot for 1 h at room temperature, centrifuged (2500 × g for 15 min), and stored at −20 °C. Serum testosterone and cortisol concentrations were measured at the Laboratorio de Técnicas Nucleares (Facultad de Veterinaria, Universidad de la República, Uruguay) by radioimmunoassay, using a solid phase kit (DPC; Siemens, Los Angeles, CA, USA). The detection limit of the assay for testosterone was 0.17 nmol/L and for cortisol 8.1 nmol/L. The intra-assay and inter-assay coefficients of variation for testosterone and cortisol were all below 8%.

2.4. Statistical analysis

Data collected in each breeding season were analysed separately. The number of each sexual behaviour in the competitive and non-competitive tests in rams from each group were compared by the Wilcoxon test. Testosterone and cortisol serum concentrations between both tests in each group of rams were compared with an ANOVA for repeated measurements. The statistical model included the type of test (competitive and non-competitive tests), time, and the interaction between type of test and time as fixed effects. Post-hoc comparisons were performed with least significant difference. Differences were considered significant when $P < 0.05$; data are presented as mean ± SEM.

3. Results

The effect of competition on sexual behaviours of DR and AR rams during the first breeding season is presented in Table 1. During the first breeding season, the number of flehmen decreased and the number of ano-genital sniffings tended to decrease in DR rams in competitive tests. However the ejaculation/total mounts ratio increased and the number of matings tended to increase in DR rams (Table 1). In AR rams, the number of flehmen tended to decrease and the total number of mounts tended to increase in competitive