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REGULAR ARTICLES

Grouping previously unknown bucks is a stressor with negative effects on reproduction

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Abstract Grouping previously unknown animals produces social stress, which might have negative effects on reproduction. The aims of the experiment were to determine if grouping unknown bucks (1) triggers a stress response and produces changes in body weight; (2) affects scrotal circumference, testosterone concentration, and semen quality; and (3) has differential effects between resident and introduced bucks. One group of nine Saanen bucks was transported and introduced (introduced bucks, group IG) to a group of eight Saanen bucks (resident bucks, group RG). On day 0, cortisol concentration and rectal temperature were determined, and from day −7 to day 29, body weight, scrotal circumference, and testosterone concentration were recorded and semen quality was determined. The stress response was different between groups: on several moments on day 0, resident bucks had greater cortisol concentration ($P<0.0001$), while introduced bucks had higher rectal temperature ($P=0.02$). Body weight decreased similarly in both groups from day −7 to day 2 ($P<0.0001$), but on day 29, IG bucks were lighter than RG bucks ($P=0.05$). Also, the reproductive response differed between groups: introduced bucks had lower scrotal circumference ($P<0.01$), lower testosterone concentration ($P=0.02$), and lower percentage of motile spermatozoa in the ejaculate ($P=0.05$). It was concluded that grouping unknown bucks was stressful and negatively affected the reproduction, being more serious for the introduced than the resident bucks.

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Introduction

In livestock production systems, it is common to join groups of animals from different origins, according to their age, body weight and body condition, and reproductive or productive traits (Veissier et al. 2001; Bøe and Færevik 2003; Fernández et al. 2007). Grouping unknown animals produces an increase of aggressiveness with negative effects in welfare and in health (Patt et al. 2013). For example, the Council of Europe (1988) recommended not introducing bulls to a previously established group. However, there is scarce scientific information that validates this recommendation in goats.

Grouping unknown animals results in an increase of agonistic behavior to establish new hierarchical relationships in the group (Andersen et al. 2011), producing social instability (see review: Estevez et al. 2007) and social stress (dairy cows: Schirmann et al. 2011; steers: Gupta et al. 2005; lambs: Miranda de la Lama et al. 2012). As a consequence, there is an increase on the release of glucocorticoids, impairing reproductive functions through inhibiting GnRH and thus gonadotropin secretion, and the synthesis of sexual steroids (see review: Ferin 2006).

In most trials that studied consequences of social mixing, animals were grouped in places previously unknown by all the animals (beef cows: Mench et al. 1990; calves: Veissier et al. 2001; steers: Gupta et al. 2005). This avoids the possible advantages of resident individuals in relation to those that are introduced (Stricklin et al. 1980). For example, those female goats introduced to an established group had greater cortisol concentration and spend more time lying and less time feeding than the resident individuals of the group (Patt et al. 2012).

Considering all this information, our hypothesis were that (1) grouping previously unknown bucks triggers a stress response, (2) testosterone concentration and semen quality are negatively affected by grouping, and (3) the negative effects of grouping on reproductive traits are greater in introduced than in resident animals. Therefore, the aims of the experiment were to determine if grouping previously unknown bucks (1) triggers a stress response and affects body weight, (2) affects reproductive traits (scrotal circumference, testosterone concentration, and semen quality), and (3) provokes different responses in resident and introduced bucks.

Material and methods

Animals and management

The experiment was performed in a humid subtropical zone of South America (Uruguay; 35° S), at the Campo Experimental N° 2 of the Facultad de Veterinaria, during June–July (winter). All animal management was approved by the Comisión de Experimentación en el Uso de Animales of the Facultad de Veterinaria. During all the experiments, animals were fed with alfalfa hay and concentrated, receiving the same amount of the same food/buck/day, and had free access to water.

We used 17 yearling Saanen bucks that were hornless and had been separated from their dams during the first 24 h after birth, being in a single group until 20 days old. Then, individuals were assigned to two groups: resident and introduced group. The resident group lived with females until they were 10 months old. The other group was moved to a similar paddock about 42 km away. This experiment was performed when the bucks were 1 year old, and the groups were joined (day 0) (group RG: n=8, body weight 40.8±2.7 kg, and group IG: n=9, body weight 39.1±1.2 kg).

On day 0 at hour 0, animals were grouped. Introduced bucks were previously transported (transport time approximately 1 h) and then introduced to the pen where the RG bucks were housed. Immediately before day 0 hour 0, blood samples were collected and cortisol and testosterone concentration were determined. During 29 days after grouping, testosterone concentration and rectal temperature were determined, body weight and scrotal circumference were recorded, and semen quality was determined.

Body weight, scrotal circumference, and rectal temperature

Animals were weighed, and their scrotal circumference was measured on the morning (0800 hours) of day −7, 2, 5, 8, 13, 22, and 29. Rectal temperature was measured with a digital thermometer on day 0 at −60, 0, 30, 60, 120, 180, 240, and 300 min.

Blood collection and hormonal determinations

Blood samples (5 mL) were collected by jugular venipuncture on day 0, at −60, 0, 30, 60, 90, 120, 180, 240, and 300 min. Two samples per day (1000 and 1700 hours) were collected on day −7 and on day 2 to 6, 8, 9, 12, 13, 15, and 19. Samples were allowed to clot for 60 min at room temperature before being centrifuged for 20 min, and serum was stored at −20 °C until hormonal measurement.

Cortisol and testosterone serum concentration were measured at the Laboratorio de Técnicas Nucleares, Facultad de Veterinaria, Montevideo, Uruguay, by radioimmunoassay with Coat-A-Count solid-phase kits (TKCO2, TKTT5, Diagnostic Products Corporation, Siemens, Los Angeles, CA, USA) specific for each hormone. Cortisol was measured only in samples obtained on day 0, and testosterone in samples taken on day −7 and day 0 at −60 min, and all samples taken from day 2 to day 19. The detection limit of the assay for cortisol was 10.5 nmol/L, and the intra-assay coefficient of variation was 13.3 %. The detection limit of the assay for testosterone was 0.23 nmol/L, and the intra-assay coefficient of variation was 10.9 %.

For testosterone concentration, data were analyzed and are presented as the mean of the serum concentration of the samples obtained in the morning and in the afternoon.

Semen characteristics

Semen was obtained by electroejaculation on day −7, 2, 5, 8, 13, 22, and 29. The penis was grasped and held at the end of a glass vessel previously warmed up to 37 °C. Electrical stimulation (8 V) was applied for intervals of 3 s and alternated with rest periods of similar duration until ejaculation. Mass motility (scale 0 to 5) was determined according to Evans and Maxwell (1987). Sperm concentration, total number of spermatozoa (sperm concentration × volume), and total number of motile spermatozoa (individual motility × total number of spermatozoa) in the ejaculate were calculated.

Statistical analysis

Data were compared with ANOVA for repeated measures. The statistical model included the effect of group, time, and the interaction between group and time. Values obtained before grouping (day −7) were included in the model as a covariable. For cortisol concentration, rectal temperature, testosterone concentration, and all the seminal characteristics, the co-variable had no effect and was removed from the analysis. An ANOVA for repeated measures was also used to compare body weight and scrotal circumference recorded on days 0 and 2. All the seminal characteristics were square root transformed, and testosterone concentration was log (x+ 1)

transformed before performing the statistical analysis (normality was tested by Shapiro-Wilk test).

Results

Body weight, cortisol concentration, and rectal temperature

Body weight decreased similarly in both groups from day −7 to day 2 (39.9±1.4 and 38.0±1.3, respectively, $P<0.0001$). From day 2 to day 13 and from day 23 to day 29, body weight increased in both groups ($P<0.0001$). Resident bucks were heavier than IG bucks on day 29 ($P=0.05$) (Fig. 1a).

Cortisol concentration was similar for both groups and increased since −60 to 300 min ($P<0.0001$). At the moment of grouping (hour 0), cortisol concentration was greater in IG than in RG bucks ($P<0.0001$); however, at 60, 90, and 180 min, cortisol concentration was greater in RG than in IG bucks ($P<0.0001$) (Fig. 2a).

Rectal temperature increased similarly in both groups and reached the highest value at 30 and 60 min ($P<0.0001$). At 0, 30, 60, 90, and 120 min, rectal temperature was greater in IG than in RG ($P=0.02$) (Fig. 2b).

Scrotal circumference, testosterone concentration, and semen characteristics

Scrotal circumference was lower for IG than for RG bucks (22.8±0.1 vs. 23.1±0.2, respectively, $P=0.01$) and decreased from day 5 to day 13, then increased to values similar to the initial ones on day 22 ($P=0.01$) (Fig. 1b).

Testosterone concentration decreased similarly in both groups after grouping ($P<0.0001$). On day 0 and from day 13 to the end of the experiment (day 29), testosterone concentration was lower in IG than in RG ($P=0.02$) (Fig. 3).

As sperm concentration and total number of spermatozoa in the ejaculate were not different between groups, data are presented pooled. Both decreased after grouping ($P=0.003$ and $P=0.004$, respectively) (Fig. 4a and b, respectively). Total number of motile spermatozoa in the ejaculate decreased from day 2 to day 29 ($P=0.003$) and was lower in IG than in RG bucks on day 2 and 29 ($P=0.002$ and $P=0.05$, respectively) (Fig. 4c). Mass motility decreased on day 5 (day −7, 1.3±0.2,

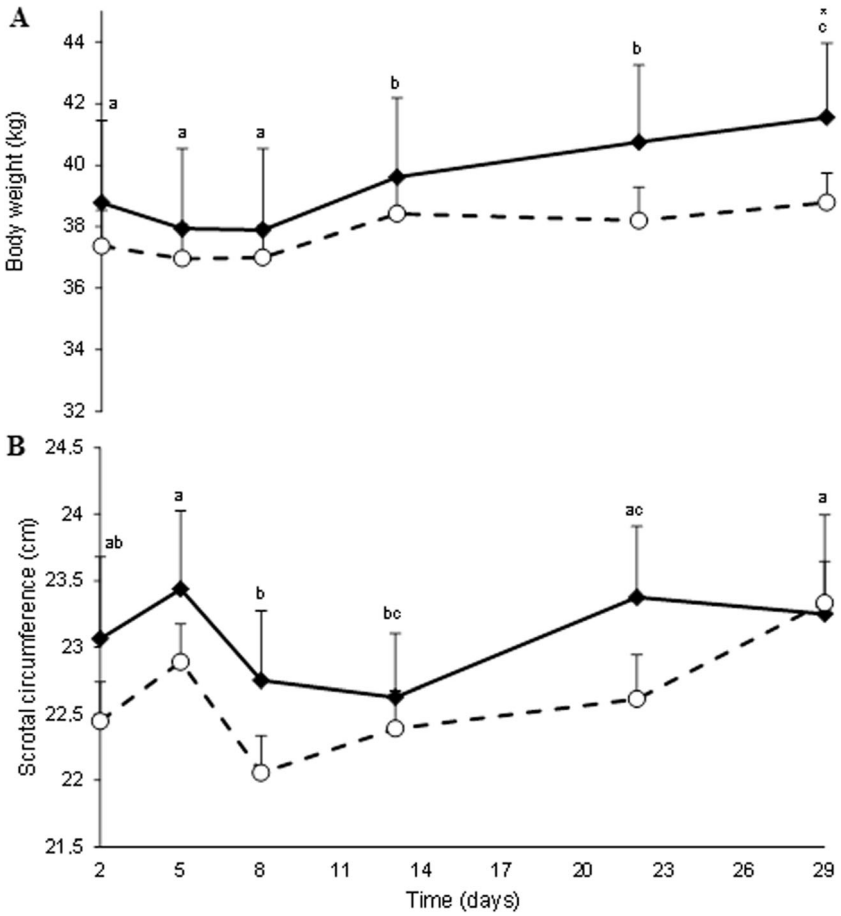

Fig. 1 **a** Body weight, **b** scrotal circumference (mean±SEM) for introduced (*circles in dashed line*, $n=9$) and resident bucks (*diamonds in solid line*, $n=8$). Different *letters* represent differences between times ($P<0.05$), and differences between groups are shown as * ($P<0.05$)

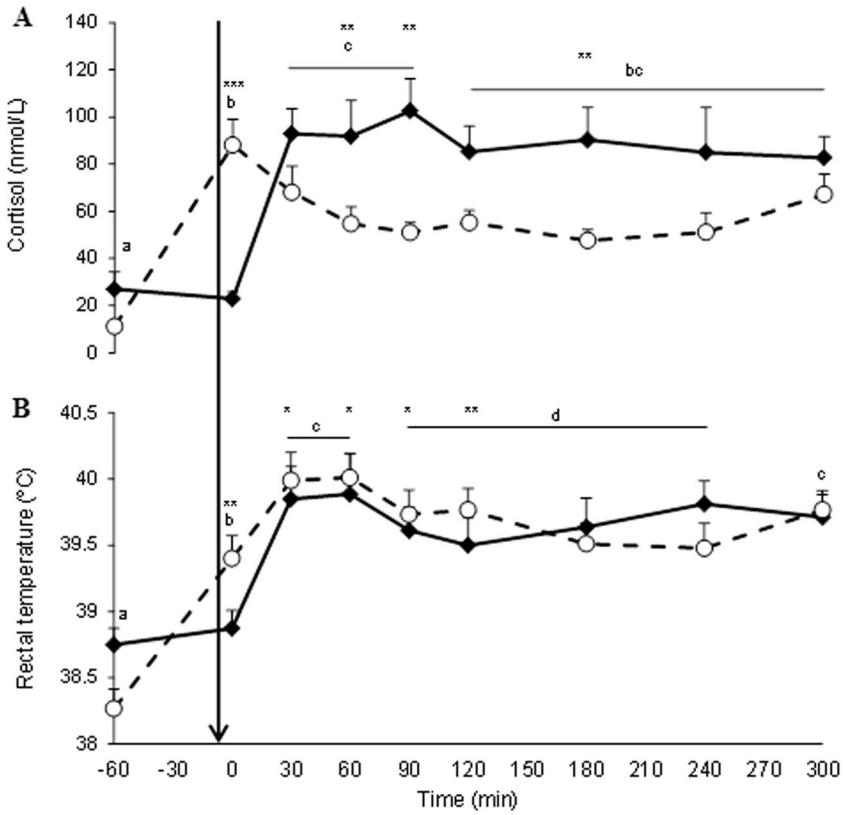

Fig. 2 **a** Cortisol serum concentration, **b** rectal temperature (mean±SEM) for introduced (*circles in dashed line*, $n=9$) and resident bucks (*diamonds in solid line*, $n=8$). Different *letters* represent differences between times ($P<0.05$) and differences between groups are shown as * ($P<0.05$), ** ($P<0.01$), and *** ($P<0.0001$). *Arrow* shows the moment immediately before grouping

vs. day 5, 0.6±0.3, $P=0.008$) and then increased to values similar to the initial ones.

Discussion

Grouping bucks triggered an important stress response, evidenced by the increases in cortisol concentration and rectal temperature and also impaired reproductive traits. Although there are previous studies that investigated the effects of grouping animals on some other productive traits and animal health (sows: Arey and Edwards 1998; lactating ewes: Sevi et al. 2001; calves: Veissier et al. 2001; heifers and dairy cows: Bøe and Færevik 2003), our results expand the knowledge to a wider view, as it was demonstrated that grouping previously unknown bucks affected the main reproductive parameters, and this happened in both groups of animals, those that were moved and those that remained in their paddock.

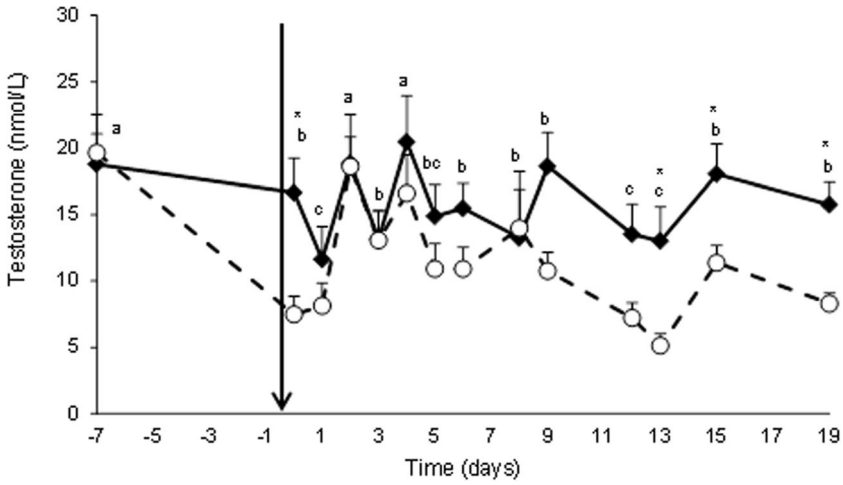

Fig. 3 Testosterone serum concentration (mean±SEM) for introduced (*circles in dashed line*, $n=9$) and resident bucks (*diamonds in solid line*, $n=8$). Different *letters* represent differences between times ($P<0.05$) and differences between groups are shown as * ($P<0.05$). *Arrow* shows the moment immediately before grouping

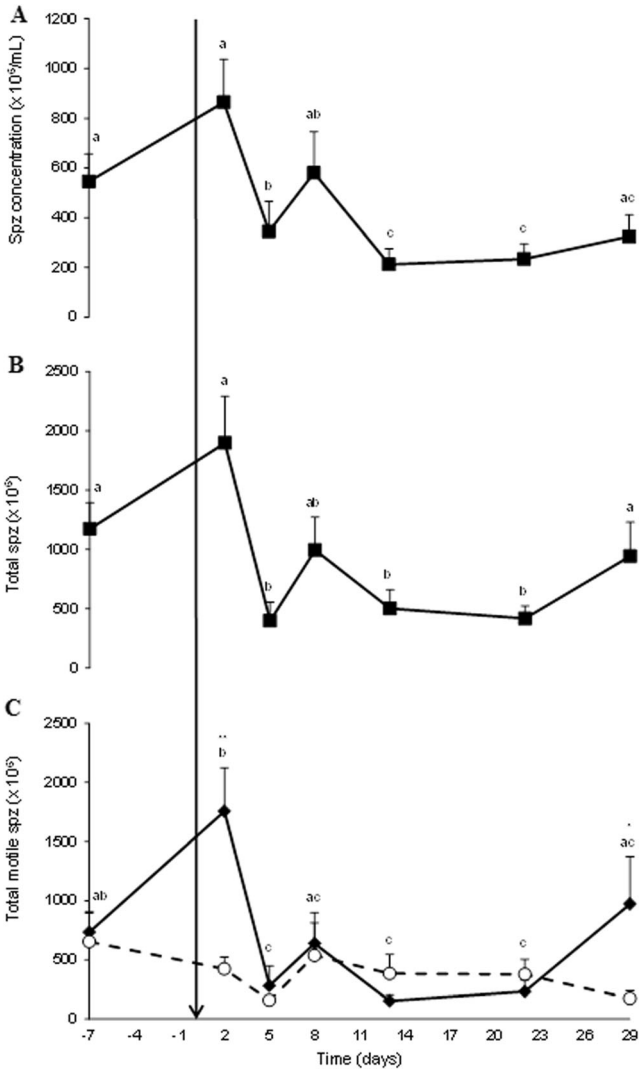

Fig. 4 Seminal characteristics (mean±SEM) for introduced (*circles in dashed line*, n=9) and resident bucks (*diamonds in solid line*, n=8). **a** Seminal of seminal concentration. **b** Total number of spermatozoa in the ejaculate. **c** Total motile spermatozoa in the ejaculate. **a**, **b** Data are shown grouped (*squares in solid line*). Different *letters* represent differences between times (P<0.05) and differences between groups are shown as * (P<0.05) and ** (P<0.01). *Arrow* shows the moment immediately before grouping

Immediately before grouping, introduced bucks had greater cortisol concentration than resident bucks, probably as a consequence of previous transportation (Kadim et al. 2010, 2014). Therefore, after grouping, it was not possible to discriminate the relative impact of transport and grouping on cortisol response. However, after grouping, resident animals reached greater cortisol concentration than introduced bucks. It is possible that joining represented a stronger stressful event for resident bucks since they had a greater cortisol response in those animals, even though the introduced ones were previously transported. It could be assumed that the introduced animals remained stressed although their cortisol concentrations declined, as high cortisol concentration is not maintained during long periods even if the stressor is still acting (see review: Sapolsky et al. 2000). It would be interesting to determine if the stress response differs in resident and introduced individuals without the effects of a previous stressor as happened with transportation.

Reproduction was negatively affected by grouping, which was evidenced by decrease in scrotal circumference, testosterone concentration, and semen quality. It has been previously reported that testosterone concentration decreases in rams exposed to both acute (Damián and Ungerfeld 2011) and chronic (Lacuesta and Ungerfeld 2012) stress situations. In this sense, as blood cortisol concentration is closely related with cortisol semen concentration (Graves and Eiler 1979) and glucocorticoids negatively affect semen quality, the initial cortisol increase may be the cause of semen quality deterioration. In agreement, it has been demonstrated that treatment with dexamethasone, a synthetic glucocorticoid, leads to a decline in testosterone concentration and impairs semen parameters in bulls (Barth and Bowman 1994) and rams (Gür et al. 2005). Overall, our data expand previous observations of stress effects on testosterone secretion and semen quality to the effects of social mixing in bucks.

Introduced bucks had lower body weight, and this could be related to possible increased physical activity. These bucks could be exposed to a greater social destabilization than resident bucks as they were also transported and exposed to a novel housing area. In this sense, it is possible that social instability affected the introduced bucks to a major extent and therefore could lead them to more intense physical activity and to greater energy costs and thus to lower body weight (Raab et al. 1986).

Although both groups were stressed by grouping, the negative effects on reproductive traits (scrotal circumference, testosterone concentration, and motile spermatozoa in the ejaculate) were more serious in the introduced bucks. This could indicate that resident and introduced animals were differently stressed and had a different reproductive response to stress.

Overall, it was concluded that grouping previously unknown male goats triggered a stress response and decreased body weight. Although the reproductive traits were negatively affected in both groups, the effect was more serious in the introduced than in the resident bucks.

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Conflict of interest The authors have no conflict of interest.

Animal rights The experiment was approved by the ethical committee Comisión de Experimentación en el Uso de Animales of the Facultad de Veterinaria and has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

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