



## Ovine Research

# Effect of glutamate and/or testosterone administration on appetitive and consummatory sexual behaviors in pubertal rams and their influence on the reproductive performance of nulliparous anovulatory ewes



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## ABSTRACT

We tested whether glutamate and/or testosterone administration to pubertal Dorper rams would increase their appetitive sexual behavior (ASB) and consummatory sexual behavior (CSB). We then evaluated their ability to induce the sexual response of nulliparous-anestrous ewes during early spring (26° N). Dorper rams ( $n = 20$ , 11 months old) were distributed in four groups homogeneous regarding live weight ([LW],  $55 \pm 0.09$  kg), body condition score ( $3.2 \pm 0.1$  units), and scrotal circumference ( $30 \pm 0.3$  cm) and received the following treatments of: 1) intramuscular glutamate (GG);  $7 \text{ mg kg}^{-1}$  LW of glutamate (every 3 days  $\times$  28 days; iv), 2) testosterone treated (GT); 1 ml of saline (every 3 days  $\times$  15 days; iv) + 25 mg of testosterone (every 3 days  $\times$  15 days; im), 3) glutamate + testosterone (GGT);  $7 \text{ mg kg}^{-1}$  LW of glutamate (every 3 days  $\times$  28 days; iv) + 25 mg testosterone (every 3 days  $\times$  15 days; im), and 4) GC; 1 mL of saline (every 3 days  $\times$  28 days; iv). Thereafter, nulliparous-anestrous Dorper ewes ( $n = 80$ ; LW  $42.2 \pm 2.3$  kg, body condition score  $3 \pm 0.09$  units) were exposed to these treated males. On male-to-female contact, a sexual behavioral test (2 hours  $\times$  2 days) was conducted to evaluate the ASB and CSB frequencies. Afterward, the female reproductive performance (interval to estrus, estrus response, ovulation response, ovulation rate, and pregnancy rate) was quantified. Although the GGT-males had the best ASB and ASB+CSB ( $P < 0.05$ ), the GT-males had the best CSB ( $P < 0.05$ ). The estrus response, ovulation response, and ovulation rate did not differ ( $P < 0.05$ ) among groups, yet, interval to estrus and pregnancy rate favored ( $P < 0.05$ ) to the ewes-GGT rams. Our results revealed that the ASB and CSB of young Dorper rams were improved by frequent GGT administration. Moreover, the shortest onset to estrus and the largest pregnancy rate were observed in those ewes exposed to the GGT-treated pubertal rams. Such results are encouraging from a comparative sexual behavior standpoint, while may hold potential translational applications.

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## Introduction

In small ruminants, mainly under temperate and subtropical conditions, a seasonal pattern of reproductive function has been reported, being more accentuated as genotypes originate from northern latitudes (Goodman et al., 2010). Such reproductive behavior generates a defined pattern of ovarian activity with most

ovulatory cycles occurring in the autumn and winter (breeding season) and a resting reproductive period during spring and summer (anestrous season) (Fabre-Nys et al., 2016; Goodman et al., 2010). One of the main strategies to counter this paucity in the reproductive activity (Abecia et al., 2015; Menassol et al., 2012) is the introduction of a sexually active males to the female's herd (Fabre-Nys et al., 2015; Hawken & Martin 2012).

However, the endocrine response of anestrus ewes to the exposure to males depends on the intensity and quality of the ram stimulus on the anestrous females (Ungerfeld et al., 2014; Veliz et al., 2006). According to Perkins & Fitzgerald (1994), a large percentage of anestrous ewes exposed to rams with high libido ovulated (95%) regarding rams with low libido (78%). Recently, Tejada et al. (2017) reported that Dorper rams treated with exogenous testosterone increased both the appetitive sexual behavior (ASB) and consummatory sexual behavior (CSB) when exposed to anovulatory ewes during the anestrous season. Interestingly, they also reported increases in estrus activity, up to 85%, as well as an augmented ovulation percentage, up to 80%, once the anestrous ewes were exposed to these rams, which were previously exposed to the “female effect”. Moreover, adult Dorper rams treated with intramuscular glutamate + testosterone (GGT) showed the highest ( $P < 0.05$ ) ASB + CSB frequencies followed by those rams only treated with intramuscular glutamate (GG). When exposed to nulliparous-anovulatory ewes, the GGT rams induced not only the largest ovulatory activity and fastest estrus response but also the greatest pregnancy rate ( $P < 0.05$ ) (Calderon-Leyva et al., 2018).

Certainly, in males, glutamate, the main neurotransmitter in the central nervous system, participates in the expression of sexual behavior because of its action on GnRH neurons (Iremonger et al., 2010; Meza-Herrera, 2012). This interesting excitatory amino acid increases the neural activity that facilitates penis erection and mating behavior (Melis et al., 2004) not only in male rats (Dominguez et al., 2006; Li et al., 2013; Olney et al., 1976) but also in hamsters (Ebling et al., 1998). In addition, in mice, after 2 hours of being treated subcutaneously with glutamate, the baseline serum testosterone levels increased from about 1–4 ng/mL in 3 hours and to 5 ng/mL in 6 hours (Olney et al., 1976). Building in these previous findings, we tested the hypothesis that frequent treatments with glutamate intravenous and/or testosterone intramuscular in young Dorper rams would increase their sexual behavior, and if these treated rams would be able to induce reactivation of the ovarian function through the “male effect” in nulliparous-anestrous Dorper ewes during the anestrous season.

## Material and methods

### General

All the methods and management of the experimental units used in this study were in strict accordance with accepted guidelines for ethical use, care, and welfare of animals in research at international (FASS, 2010) and national (NAM, 2002) levels, with institutional approval reference number UAAAN-UL/14-510-400-2.

### Location, environmental conditions, animals, and management

The research was conducted from April to June, in Northern Mexico (Coahuila; 26° 04' N and 103° 26' W, 1,120 m. altitude) under natural light conditions. In the study area, the mean annual rainfall and mean annual temperature were 258 mm and 24°C, respectively, with maximum and minimum temperatures of 41°C and –1°C (summer and winter, respectively). Moreover, the specific environmental conditions observed during the experimental period are presented in Figure 1 (temperature (°C) and temperature-humidity index [THI], units) and Figure 2 (precipitation [mm] % photoperiod [h]). Pubertal Dorper rams and nulliparous Dorper ewes were raised and maintained in a special production unit linked to a Holstein Dairy Cow intensive production system. The animals were fed twice a day (1200 and 1800); the diet was based on 60% sorghum silage and 40% concentrate (14% CP), and a mixture of macrominerals. Ewes and rams had *ad libitum* access to the food, clean water, mineral salts, and shades. The health status of all the experimental units was supervised by an experienced veterinarian during the whole experimental period. Besides, efforts were made to minimize any possible discomfort in the experimental units.

### Pubertal Dorper rams

Pubertal rams ( $n = 20$ ; 11 months old) were homogeneously distributed in four groups ( $n = 5$ , each) in terms of live weight (LW), body condition score, and scrotal circumference: LW =  $55 \pm 0.9$  kg, body condition score =  $3.2 \pm 0.10$  units, scrotal circumference =  $30 \pm 0.38$  cm. On March 26, each group received one of the following treatments: 1) GG; intravenous injection of 7 mg kg<sup>-1</sup> LW of glutamate (Merck-C5H9NO4-art-101791, every 3 days  $\times$  28 days), 2) Testosterone treated (GT); intramuscular injection of 25 mg of testosterone propionate intramuscularly (Testosterone 50, Lab Brovel Mexico City) every 3 days  $\times$  15 days, and then iv injection of

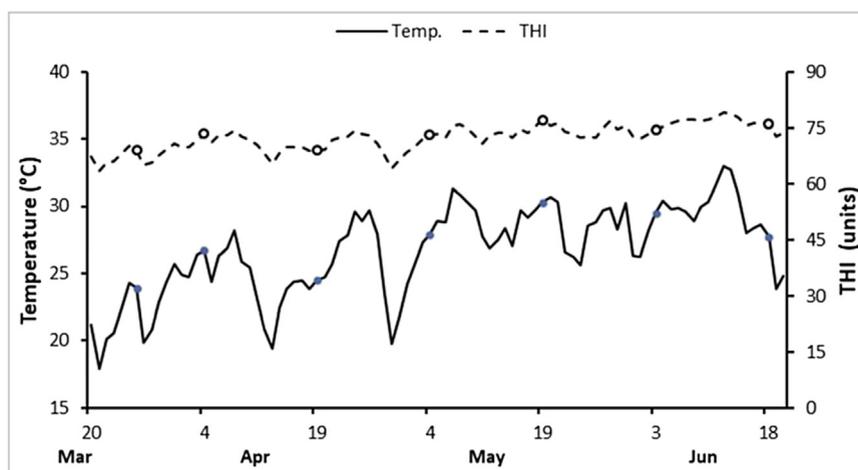


Figure 1. Average temperature (°C) and temperature-humidity index ([THI], units) throughout the experimental period.

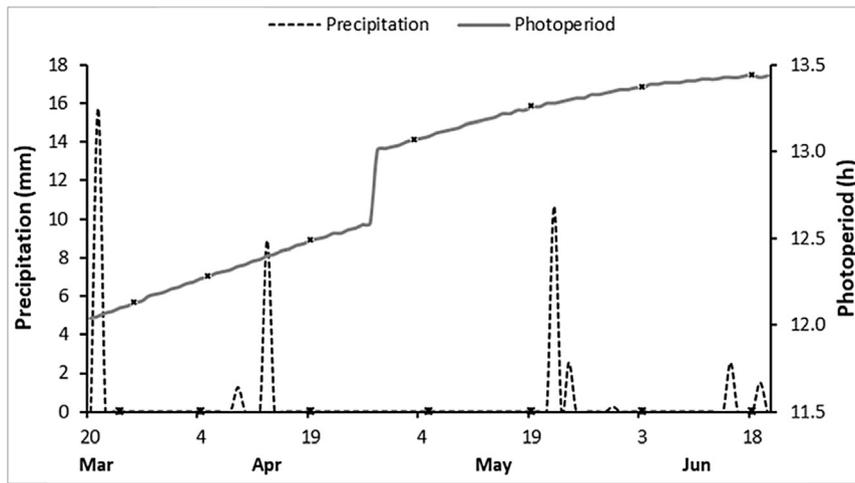


Figure 2. Average precipitation (mm) and photoperiod (h) throughout the experimental period.

1 mL of saline every 3 days  $\times$  15 days, 3) GGT; intravenous injection of 7 mg kg<sup>-1</sup> LW of glutamate (every 3 days  $\times$  28 days) + intramuscular injection of 25 mg testosterone propionate (every 3 days  $\times$  15 days), and 4) GC; intravenous injection of 1 mL of physiological saline solution (every 3 days  $\times$  28 days).

#### Nulliparous Dorper ewes

Nulliparous Dorper ewes (n = 129; 7–8 months) underwent two transrectal ultrasounds to evaluate the status of the ovary (Tejada et al., 2017); ultrasound was performed on days 14 and 7 before mating by using an Aloka SSD-500 with 7.5-Mhz transducer (Aloka; Corometrics Medical Systems Inc., Wallingford, CT). Although 100 ewes (77.5%) of the original population were confirmed as anovulatory, 29 ewes (22.5%) were found to have luteal bodies, so they were considered cyclical and eliminated from the study. Once the anovulation status was confirmed, a total of 80 ewes were randomly selected and were completely isolated from the rams contact. Ewes received an injection of 60 mg of progesterone im (Progesterone, Lab Brovel, Mexico City), divided as 20 mg on days 6, 4, and 2 before to be exposed to the treated males, to avoid the short cycles, and that the first ovulation was accompanied by estrus (Chemineau et al., 2006).

#### In search of the male effect

The ewes were divided into four homogeneous groups in terms of weight and body condition ( $42.2 \pm 2.3$  kg and  $3 \pm 0.09$  units; respectively) 24 hours before putting them in contact with the rams and were placed in separate pens (4  $\times$  3 m) at a distance of more than 100 m from each other. On day 0 after the treatment period of the rams (April 26), each group of ewes were introduced four rams belonging to the same treatment, which were rotated within treatment (two by two) every 24 hours  $\times$  20 days.

#### Sexual behavior of rams: response variables and treatment design

On males-females contact, the first 2 hours (0800 to 1000) and during first 2 days of contact, ram sexual behavior tests were performed (Carlussio et al., 2013; Guillen-Muñoz et al., 2016). In these tests, the ASB (anogenital sniffing, approximation, kicking, vocalization, and flehmen), the CSB (attempted mounts and complete mounts), and the indicators of sexual rest ([ISR]; aggressions, distractions, attempt of escape, and isolation of foot) were registered. Treatment design consisted of a 2  $\times$  2 factorial arrangement of treatments with two levels of glutamate (with and without) and two levels of testosterone (with and without). On day 0 of the

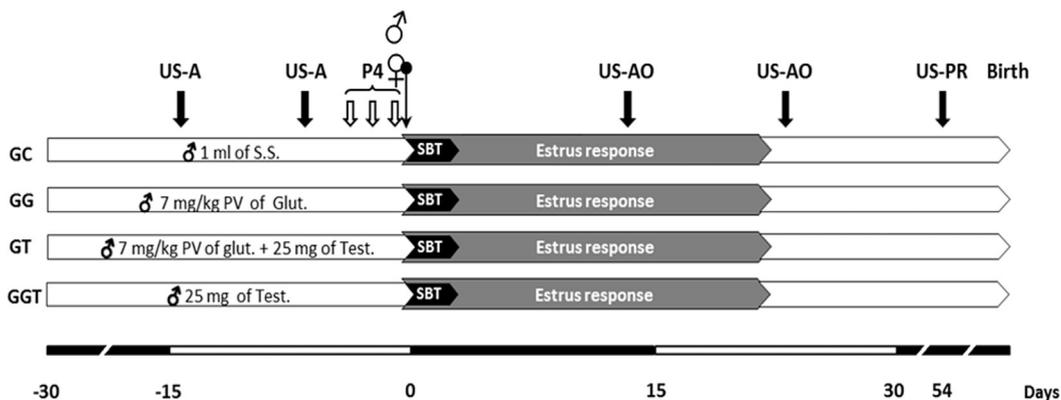


Figure 3. Experimental design of Dorper rams treated with glutamate (GG), testosterone (GT), glutamate + testosterone (GGT), and saline solution (GC) and later exposed to anovulatory-nulliparous Dorper ewes groups. US-A, ultrasound for detected anovulation at day 14 and 7 before contact with rams; P4, application of 60 mg of progesterone divided into three doses of 20 mg each and supplied 6, 4 and 2 days before contact with the treated rams; SBT 4 $\delta$ :20 $\eta$ , tests of sexual behavior with exposure of four rams with the same treatment to 20 anovulatory-nulliparous sheep, two rams the first 2 hours of contact with the ewes and the first 2 hours of the following day. Estrus response, Monitoring of estrous activity for 20 days every 12 hours; US-AO, Ultrasound to detect ovulatory activity on days 6, 13, and 23 after the introduction of rams to ewes; US-PR, ultrasound to determine implantation rate on days 54 after introduction of rams.

experimental breeding, four rams from each experimental group were randomly selected and immediately introduced to pens with anovulatory-nulliparous ewes (i.e., 4 males: 19–20 females) to evaluate the male effect. The records for the ASB, CSB, and ISR frequencies have been previously described (Calderon-Leyva et al., 2018). Therefore, the effect to treat the males with glutamate, testosterone, and the interaction with respect to the control group, on the sexual behavior of rams exposed anovulatory-nulliparous Dorper ewes, was evaluated (Figure 3).

#### Sexual and reproductive performance of ewes: evaluated response variables

The sexual activity of the females was monitored from day 0 to day 20, and the following response variables were determined: *Interval of estrus initiation*, through direct observation of the groups twice a day (0800 and 1800 hours), considering the time elapsed since the contact ram-ewe until the first mating occurred. *Estrus response*, the number of ewes showing standing estrus was recorded, considering the ewe's identification number that remained still and allowed to be mounted by the ram (Perkins & Roselli, 2007). *Ovulation response*, was determined at 6, 13, and 23 days after the introduction of the rams, considering the number of ewes with the presence of CL in any ovary determined throughout transrectal real-time ultrasonographic scan by using an Aloka SSD-500 with 7.5-Mhz transducer (Aloka; Corometrics Medical Systems Inc., Wallingford, CT), as outlined by Viñoles et al. (2004). *Ovulatory rate*, the total number corpora lutea observed throughout transrectal ultrasonographic scanning and divided by the total number of ewes which ovulated. *Pregnancy rate*, determined by counting the number of embryos observed in the uterine cavity throughout transrectal ultrasonography at day 54 after the introduction of the rams. Figure 3 shows a schematic representation of the experimental protocol.

#### Statistical analyses

Regarding the male sexual behavior, data were analyzed through an ANOVA for a completely random design having a 2 × 2 factorial arrangement of treatments (i.e., glutamate, testosterone, and their interaction); previously data of sexual behavior were log transformed because they did not present a normal distribution. In the case of a significant treatment effect, mean separations were achieved using the PDIFF option of the GLM procedure. Count variable distribution regarding the anestrus ewe's reproductive outcomes once exposed to the male effect (i.e., estrus induction, estrus onset interval, ovulation rate, and pregnancy) between main effects were compared using the CATMOD procedure of SAS to determine the possible effect of the treated males on the reproductive response of the previously anovulatory Dorper ewes. Because a glutamate × testosterone interaction was observed, both regarding the sexual test and the reproductive outcomes of the nulliparous ewes, single main effects for both treatment levels are presented. Results are presented as nontransformed means ± s.e.m., and a difference of  $P < 0.05$  was considered as significant. All the analyses were computed using the procedures of SAS (version 9.2; SAS Inst. Inc. Cary, NC, USA).

#### Results

Table 1 summarizes the sexual behavior of young rams during the 2 days × 2 hours exposure to nulliparous-anovulatory Dorper ewes. The highest frequencies in ASB were shown by GGT (38%) followed by GG (25%), GC (21%), and GT (16%); significant differences ( $P < 0.05$ ) were observed among groups. On the other hand,

**Table 1**

Frequencies for appetitive and consummatory sexual behaviors, and indicators of sexual rest (ASB, CSB, and ISR, respectively) in young Dorper rams treated with glutamate (GG), testosterone (GT), glutamate + testosterone (GGT), and saline (GC) and subsequently exposed to anovulatory-nulliparous Dorper ewes groups (2 days × 2 hours) to test the sexual behavior under the natural anestrus season (April-May, 26° North)

Rams (n)	GG	GT	GGT	GC	SEM
	4	4	4	4	
ASB (n)	1230 <sup>b</sup>	782 <sup>d</sup>	1866 <sup>a</sup>	1024 <sup>c</sup>	232
CSB (n)	113 <sup>b</sup>	197 <sup>a</sup>	122 <sup>b</sup>	151 <sup>ab</sup>	19
ASB and CSB (n)	1343 <sup>b</sup>	979 <sup>d</sup>	1988 <sup>a</sup>	1175 <sup>c</sup>	219
ISR (n)	22 <sup>b</sup>	52 <sup>a</sup>	11 <sup>b</sup>	12 <sup>b</sup>	10

SEM: Most conservative standard error of the mean is presented.

Note: Frequencies for ASB, CSB, ASB + CSB, and ISR are the summation of behavioral events per male within treatment; to test differences among treatments, frequencies were compared according to the procedure proposed by Bedos et al. 2012.

<sup>a, b, c</sup> Values with different superscript within response variable and among treatments, differ ( $P < 0.05$ ).

the GT-males had the highest CSB performance (34%;  $P < 0.05$ ) regarding the GC (26%), GGT (21%), and GG (19%) groups, yet, with no differences observed among the last three experimental groups ( $P > 0.05$ ). When adding the ASB plus CSB frequencies, differences ( $P < 0.05$ ) among the experimental groups were observed: GGT (35%), GG (24%), GC (22%), and GT (19%). In relation to the ISR, the highest values were shown by the GT (54%;  $P < 0.05$ ) compared to the GG (23%), GC (12%), and GGT (11%) groups, without observing differences between these last three groups ( $P > 0.05$ ).

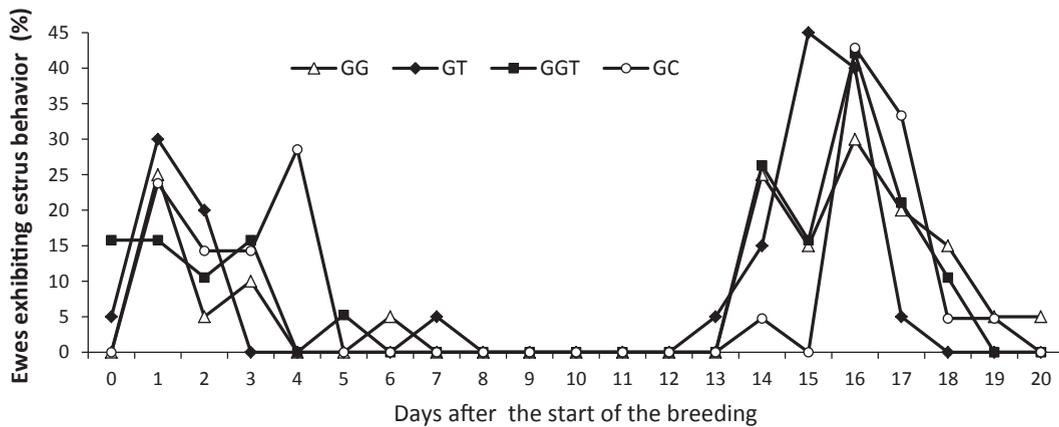
The sexual and reproductive response of ewes exposed to the treated rams appear in Table 2. No differences ( $P > 0.05$ ) among treatments were observed regarding estrus response, ovulation response, and ovulation rate (general averages:  $16 \pm 0.4$ ,  $19.5 \pm 0.25$ , and  $1.25 \pm 0.04$ , respectively) during the entire experimental period (day 0 to day 20). The estrus onset interval did not differ ( $P > 0.05$ ) among treatments during the first 10 days of exposure to males (general average  $47.8 \pm 3$  hours). Yet, this trend changed during the following 10 days (day 11 to day 20;  $P < 0.05$ ), observing

**Table 2**

Sexual and reproductive outcomes of anovulatory-nulliparous Dorper ewes once being exposed to the stimulus of young rams previously treated with glutamate (GG), testosterone (GT), glutamate + testosterone (GGT), and saline (GC) under the natural anestrus season (April-May, 26° North)

Ewes (n)	GG	GT	GGT	GC
	20	20	19	21
Estrus response (n)				
Day 0 to 10	7/20 <sup>a</sup>	6/20 <sup>a</sup>	10/19 <sup>a</sup>	6/21 <sup>a</sup>
Day 11 to 20	12/20 <sup>a</sup>	12/20 <sup>a</sup>	11/19 <sup>a</sup>	11/21 <sup>a</sup>
Day 0 to 20	15/20 <sup>a</sup>	16/20 <sup>a</sup>	17/19 <sup>a</sup>	16/21 <sup>a</sup>
Repeated estrus	4/15 <sup>a</sup>	2/16 <sup>b</sup>	4/17 <sup>a</sup>	1/16 <sup>b</sup>
Ovulation response (n)				
Day 0 to 10	18/20 <sup>a</sup>	18/20 <sup>a</sup>	17/19 <sup>a</sup>	18/21 <sup>a</sup>
Day 11 to 20	12/20 <sup>a</sup>	13/20 <sup>a</sup>	12/19 <sup>a</sup>	11/21 <sup>a</sup>
Day 0 to 20	19/20 <sup>a</sup>	20/20 <sup>a</sup>	19/19 <sup>a</sup>	20/21 <sup>a</sup>
Silent ovulations				
Day 0 to 10	11/20 <sup>a</sup>	12/20 <sup>a</sup>	7/19 <sup>a</sup>	12/21 <sup>a</sup>
Day 11 to 20	0/20 <sup>a</sup>	1/20 <sup>a</sup>	0/19 <sup>a</sup>	0/21 <sup>a</sup>
Interval to estrus onset (h)				
Day 0 to 10	53 ± 17 <sup>a</sup>	50 ± 24 <sup>a</sup>	48 ± 11 <sup>a</sup>	40 ± 7 <sup>a</sup>
Day 11 to 20	370 ± 12 <sup>ab</sup>	352 ± 6 <sup>b</sup>	359 ± 8 <sup>b</sup>	385 ± 7 <sup>a</sup>
Ovulation rate				
Day 0 to 10	1.4 ± 0.21 <sup>a</sup>	1.4 ± 0.16 <sup>a</sup>	1.2 ± 0.15 <sup>a</sup>	1.1 ± 0.13 <sup>a</sup>
Day 11 to 20	1.2 ± 0.13 <sup>a</sup>	1.2 ± 0.11 <sup>a</sup>	1.3 ± 0.17 <sup>a</sup>	1.2 ± 0.11 <sup>a</sup>
Pregnancy rate (n)				
Day 54	15/20 <sup>a</sup>	16/20 <sup>a</sup>	17/19 <sup>a</sup>	16/21 <sup>a</sup>

<sup>a, b, c</sup> Values with different superscript within response variable and among treatments, differ ( $P < 0.05$ ).



**Figure 4.** Percentage of ewes exhibiting estrus behavior when exposed during an experimental breeding (20 days) to Dorper rams treated with glutamate (GG), testosterone (GT), glutamate + testosterone (GGT), and saline (GC) under the natural anestrus season (April–May, 26° N).

the largest interval to estrus onset in those ewes exposed to GC and GG rams, while the shortest estrus onset was observed in those ewes exposed to the GT and GGT rams, with no differences among GG, GT, and GGT ( $P > 0.05$ ) (Figures 4 and 5). The largest pregnancy rate ( $P < 0.05$ ) occurred in the ewes exposed to the GGT rams, followed by the GGT, GT, GC, and GG groups.

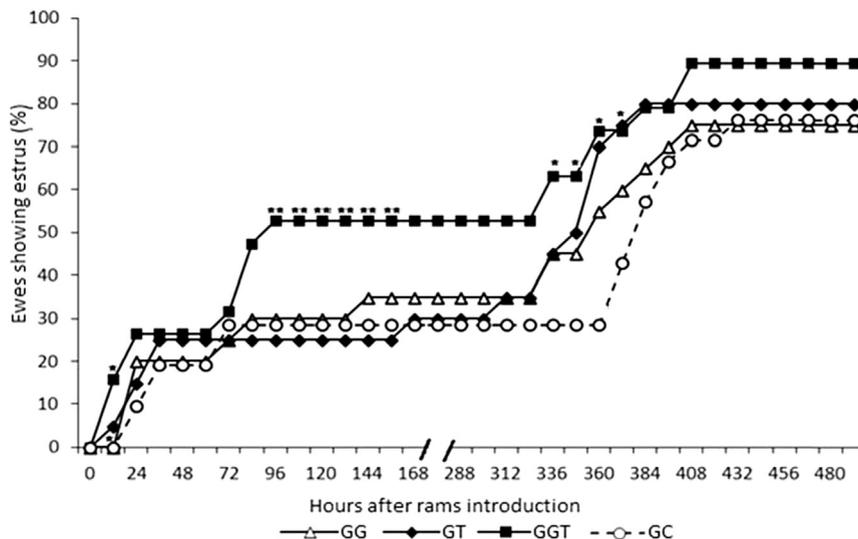
The cumulative percentage of sheep presenting estrus during the sampling period (every 12 hours during the 20 days) is presented in Figure 3. The expression of the estrus response in the ewes of the GGT group was constantly higher than the groups GG, GT, and GC; however, only significant differences were found between the GGT and the GC group ( $P < 0.05$ ).

**Discussion**

Based on our working hypothesis, the central inquiries we sought to answer in this study were 1) Will the iv administration of glutamate, testosterone, or both induce both appetitive and consummatory sexual behaviors of young Dorper rams during the natural sexual resting season?, 2) Would glutamate and testosterone act in a synergic fashion to enhance such sexual behaviors?

3) Would these treated young rams be able to induce, throughout the “male effect”, the reactivation of ovarian function of anestrus-acyclic Dorper ewes and fertilize them? In regard to the first question, our results demonstrate that frequent treatments with glutamate + testosterone in young Dorper rams increased both the appetitive and consummatory sexual frequencies. Because such increased sexual behavior was notably observed in the GGT group, this upshot denotes a possible synergistic action of glutamate + testosterone, providing elements to answer the second query. With respect to the third inquire, all group males were able to induce reactivation of ovarian function throughout the stimulus of the “male effect”, yet, the shortest onset to estrus and the largest pregnancy rate were observed in those ewes exposed to the GGT-treated pubertal rams.

In the present study, the sexual behavior of the GGT rams was not only 17% superior to the control group but also 13% greater when adding the CSB and CSC sexual frequency behaviors regarding to the control group. Simultaneously, those Dorper ewes exposed to these GGT rams had a reduced interval to estrus (359 hours vs. 385 hours) as well as an increased pregnancy rate (89 % vs. 61 %) with respect to those ewes exposed to the control rams. These



**Figure 5.** Cumulative percentage of ewes that exhibited estrus behavior when exposed during an experimental breeding (20 days) to Dorper rams treated with glutamate (GG), testosterone (GT), glutamate + testosterone (GGT), and saline solution (GC) under the natural anestrus season (April–May, 25° North).\*, Statistical differences among GGT versus GC ( $P < 0.05$ ); \*\*, statistical differences among GGT versus GC ( $P < 0.07$ ).

encouraging sexual responses of those females exposed to GGT rams can be attributed to the enhanced sexual behavior performed by these rams. In fact, previous studies state that the number of females responding to the male effect is strongly influenced by the intensity of sexual behavior and the quality of the stimulatory signals provided by males such as pheromones, in addition to a combination of visual and tactile signals (Martínez-Alfaro et al., 2014; Orihuela, 2014; Perkins and Fitzgerald, 1994; Pellicer-Rubio et al., 2013). Interestingly, more than 50% of ewes exposed to GGT rams exhibited standing estrus during the first 96 hours compared to GG, GT, and GC (Figure 3). This behavior could be because of the socio-sexual interaction between rams and ewes potentiated by the administration of glutamate + testosterone, a scenario that may have diminished the negative feedback of steroids responsible for the state of anestrus, promoting in turn, a positive stimulus on the GnRH secretion and reactivating the reproductive cycle in a sooner fashion (Fabre-Nys et al., 2015). Therefore, the observed results, backing to the GGT group, suggest a synergistic action of glutamate + testosterone and not necessarily to the individual action of either glutamate or testosterone alone.

The obtained results in the present work provide evidence that glutamate potentiates its effect on sexual behavior in the pubertal treated males when exogenous testosterone is added. The above can be explained by the existence of the hormone-glutamate interaction that may reflect bidirectional influences; in that some hormones regulate glutamate activity and glutamate also regulates endocrine function by stimulating sexual behavior (Dominguez, 2009; Estienne, 2000). Certainly, because glutamate receptors are located in a myriad of hypothalamic nuclei (Durand et al., 2008; Meza-Herrera et al., 2014), this excitatory amino acid may have acted on the GnRH neurons (Iremonger et al., 2010), which are responsible to initiate the hormonal cascade that leads to the hormone synthesis by the testes (Hull & Dominguez, 2006; Will et al., 2014).

Besides, the administration of glutamate may have acted also in an indirect fashion, by promoting the activation of the androgen receptors, and in turn, stimulate copulatory behaviors (Will et al., 2014). In rodents, glutamate has shown to elicit sexual behaviors such as erection and ultrasonic vocalizations that occur during mating and facilitates socio-sexual contact (Brudzynski & Pniak, 2002; Fu & Brudzynski, 1994). In addition, glutamate has been involved in the expression of copulation behaviors (Dominguez et al., 2006; Ebling et al., 1998; Li et al., 2013; Melis et al., 2004). Furthermore, in many species, the activation of male copulatory behaviors seems to depend on the central aromatization of testosterone. Certainly, it has previously shown that the aromatase activity is present in some brain areas that control the male sexual behavior, whereas the enzymatic activity along the steroidogenic pathway seems to be regulated by testosterone (Balthazart & Foidart, 1993).

According to other studies, glutamate indirectly influences the expression of sexual behavior, specifically at brain level in the medial preoptic area, by modulating the male's sexual behavior through its effect on dopamine action; indeed, testosterone is required to maintain basal dopamine levels to increase copulatory ability (Dominguez & Hull, 2005; Mahesh & Brann, 2005; Will et al., 2014). On the other hand, rams treated only with testosterone had 42% more indicators of sexual rest. A possible response to such endocrine scenario is that when applying exogenous testosterone, the negative feedback system diminished the production of this hormone and, as a result, a reduction in the libido expression was motivated, decreasing its ability to express sexual behavior (Bustos et al., 2012; Henley et al., 2010). Finally, although the ewes were treated with 20 mg intramuscularly of progesterone to reduce the presence of short cycles once exposed to males, silent ovulations

were not completely abolished, suggesting that with the aim of observing an evident standing estrus, a greater level of progesterone should be applied to block the synthesis of prostaglandins, thus affecting luteolysis (Chemineau et al., 2006). The observed socio-sexual response in our study, besides to be quite encouraging, they can also be an important reproductive alternative to induce reactivation of the ovarian response of acyclic ewes not only under out-of-season scenarios but also in a shorter period of time. Whereas the GGT treatment generated promising sexual and reproductive outcomes, our main outcomes are also thought-provoking from a comparative sexual behavior perspective, while they may embrace potential translational applications.

## Conclusions

Our study seems to be the first report that demonstrates that sexual behavior of young Dorper rams can be improved throughout intravenous administration of glutamate + testosterone (GGT) because we are not aware of any previous study scrutinizing such possibility. Subsequently, such GGT-treated males were able to promote encouraging reproductive outcomes from those previously anestrus-acyclic Dorper ewes throughout the male effect. Therefore, our study unveils a possible synergistic stimulus provided by the administration of glutamate + testosterone on both the sexual behavior and performance of the anestrus ewes once exposed to these treated rams.

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