Animal Reproduction Science 149 (2014) 194–198

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Equine chorionic gonadotrophin administration to rams improves their effectiveness to stimulate anoestrous ewes (the "ram effect")

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ARTICLE INFO

Article history:
Received 7 May 2014
Received in revised form 1 June 2014
Accepted 4 July 2014
Available online 11 July 2014

Keywords:
Gonadotropin
Pheromones
Reproductive seasonality
Socio-sexual signals
Testosterone

ABSTRACT

Ewes' response to ram effect is related to the strength of androgen-dependent ram signals. Experiment 1 aimed to determine if the administration of a single dose of 1000 IU of eCG to rams three days before joining them with ewes enhance their ability to stimulate females. Based on the results of Experiment 1, in a second experiment rams received two doses seven and three days before their introduction to females. In Experiment 1, rams treated or not with eCG were joined with ewes, and estrous was recorded until Day 5 (Day 0 = rams and ewes were joined), and from Day 15 to Day 23. In addition, serum testosterone concentration was measured in all rams in the first recorded period. Testosterone values were greater in eCG-E1 than in Con-E1 rams on Days 0 and 2. The percentage of ewes in estrus was similar in both groups. In Experiment 2, rams were treated with two doses of eCG on Days −7 and −3 or remained as untreated controls. Estrous was recorded until Day 5, and pregnancy rate on Day 46; testosterone was measured in samples collected from all rams. Testosterone concentration was greater in eCG-E2 than Con-E2 rams from Day −5 to Day 1, and tended to do so on Day 2. More eCG-E2 than Con-E2 ewes came into estrus and became pregnant. It was concluded that treatment of rams with two high doses of eCG before joining them with anestrous ewes, enhanced their ability to induce ewes' cyclic activity (the "ram effect").

1. Introduction

The "ram effect" (RE) is an inexpensive technique, used for estrus induction during the non-breeding season in extensive sheep management systems. The sudden introduction of rams into an anovulatory group of ewes induces a synchronized ovulation that occurs 2–3 days later (RE) (see reviews: Martin et al., 1986; Ungerfeld et al., 2004). This ovulation is not associated with heat (Martin et al., 1986). To obtain heat in connection with the first ovulation and prevent the occurrence of short luteal phases, progestogen primings should be applied before rams are introduced (Hunter et al., 1971; Ungerfeld et al., 2003).

The response of a ewe to the RE depends on her responsiveness to the stimulus and the strength of the stimulus provoked by the rams. There are ewes that will not respond, regardless of the strength of the stimulus (e.g., breeds with a strong seasonal pattern). On the other hand, some ewes will respond to a very light stimulus (e.g., breeds with a light/shallow anoestrus close to the onset of the breeding season). The strength of the stimulus is related to the characteristics or the management of the males used (see

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http://dx.doi.org/10.1016/j.anireprosci.2014.07.004
0378-4320/

review: Ungerfeld et al., 2004). The ability of the rams is related with their sexual behavior (Perkins and Fitzgerald, 1994) as well as chemical signals released from the skin glands (Knight and Lynch, 1980; Ungerfeld et al., 2008). As these signals are androgen-dependent (Croker et al., 1982; Fulkerson et al., 1981; Signoret et al., 1982), managements that increase the testosterone concentration in rams may be effective in increasing the number of ewes that respond to the stimulation.

During the non-breeding season, rams from temperate climates and high latitudes undergo a decrease in their reproductive pattern, including their sexual performance (libido), testosterone concentration, testicular weight and volume, and semen characteristics (Avdi et al., 2004; Dufour et al., 1984; Gündogan, 2007). For example, when Corriedale rams are used to induce the RE, their reproductive status is just beginning to recover from their nadir (Ungerfeld, 2012). Males' reproductive status may be stimulated by treatment with melatonin (Rosa et al., 2000), or applying artificial lighting regimes (Delgadillo et al., 2004). However, due to practical management, these alternatives are not useful in extensive grazing systems.

Another alternative may be to directly stimulate the testicular activity. In this sense, equine chorionic gonadotrophin (eCG or PMSG) is a hormone with an FSH/LH effect, that has been widely used to induce ovulation in females, but has been scarcely used in males. It has been demonstrated that eCG administration can stimulate testosterone secretion in rams (Hochereau-De Reviers et al., 1990; Price et al., 1991) and even spermatogenic activity in hypophysectomized rams (Courot et al., 1979), with the advantage of a sustained effect, as in ruminants it is still detected 10 days after administration (Price et al., 1991).

Therefore, the objective of this paper was to determine if the administration of a single dose of 1000 IU of eCG to rams 3 days before joining them with ewes enhances their ability to stimulate anestrous females. As in that experiment we administered a single dose, obtaining an increase in testosterone concentration but not in the number of ewes that responded to those rams, we hypothesized that the short time during which testosterone remained elevated before the rams were joined with anestrous ewes limited their effectiveness. Thus, we designed a second experiment administering two doses seven and three days before their introduction to females.

2. Materials and methods

2.1. General management

Two studies were performed on a private farm in Colonia, Uruguay (35°S), during the non-breeding season (October–November; natural light (L)/dark (D) ratio = 14L:10D) with 18 rams and 259 multiparous Corriedale ewes.

Ewes were isolated from rams so that they could not see, hear, or smell them (minimum distance 1000 m), on Day −40 (Day 0 = introduction of the rams). During the experimental period, ewes grazed on native pastures.

In both experiments, intravaginal sponges impregnated with 60 mg of medroxyprogesterone acetate (Syntex, Buenos Aires, Argentina) were inserted to all ewes on Day −7. At sponge withdrawal (Day 0), each group of ewes was joined with sexually experienced Corriedale rams fitted with crayon markers.

2.2. Experiment 1

2.2.1. Animal management

One hundred and sixty six ewes with a body condition score of 2.47 ± 0.03 (scale 1 to 5, where 1 = extremely emaciated, and 5 = excessively fat) (mean ± SEM) were used. Ewes were allocated to two experimental groups homogeneous according to body condition, and ewes were joined with five rams treated with eCG (group eCG-E1; $n = 84$) or five untreated rams (group Con-E1; $n = 82$).

Rams joined with eCG-E1 ewes received 1000 IU of eCG im (Novormon, Syntex, Buenos Aires, Argentina) on Day −3. Marked ewes were detected every 24 h from Day 0 to Day 5, and from Day 15 to Day 23.

2.2.2. Blood samples

Blood samples were collected from all rams by jugular puncture on Days −4, −3 (2 h before eCG administration), 0, 2 and 4, always at 09:30. Samples were allowed to clot for 1 h at room temperature before being centrifuged for 10–20 min, and stored at −20 °C until testosterone measurement. Serum testosterone concentration was measured with a Count-A-Count (TKPG, Diagnostic Products Corporation) solid-phase kit. The detection limit of the assay was 0.14 nmol/L and the intra-assay coefficient of variation was 3.4%.

2.3. Experiment 2

2.3.1. Animal management

Ninety three ewes with a body condition of 2.87 ± 0.05 were used. Ewes were assigned to two experimental groups homogeneous according to body condition, and were joined with four rams treated with eCG (group eCG-E2; $n = 46$) or four untreated rams (group Con-E2; $n = 47$).

Rams joined with eCG-E2 ewes received two eCG im doses of 1000 IU, one on Day −7, and the second on Day −3. Marked ewes were detected every 24 h from Day 0 to Day 5.

Transrectal ultrasound (Aloka 500 with a 7.5 MHz transducer, ALOKA, Tokyo, Japan) was performed on Day 46 on all ewes that showed estrus on Days 0 to 5, and pregnancy from those matings was determined based on fetus size.

2.3.2. Blood samples

Blood samples were collected from the jugular vein of all rams on Days −7, −5, −3, 0, 1, 2 and 3, early at the morning. Samples from Days −7 and −3 were collected before the administration of eCG. Samples were allowed to clot for 1 h at room temperature before being centrifuged for 10 to 20 min, and the serum was stored at −20 °C until assayed for testosterone. Serum testosterone concentration was measured with a Count-A-Count (TKPG, Diagnostic Products Corporation) solid-phase kit. The detection limit of