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Short communication

Applicability of Day 0 superovulation protocol in Boer goats

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ABSTRACT

This study evaluated the applicability of the Day 0 superovulation protocol in Boer goats by comparing it to a traditional pFSH protocol. Twenty Boer goat does were allocated into two groups comprising of 10 animals per group. For the Day 0 protocol, does oestrous cycles were synchronized for 9 days and superovulated with pFSH starting 84 h after the termination of progesterone treatment. For the traditional pFSH protocol, the oestrous cycle of does was synchronized for 9 days, followed by superovulation with pFSH initiated 48 h before CIDR withdrawal. For both groups does had two timed cervical inseminations with fresh undiluted semen. Embryos from both groups were flushed on day 6 following AI. The response to superovulation did not differ significantly between treatments but a tendency ($P = 0.06$) was found for both fertilization and number of unfertilized ova in favour of the Day 0 protocol. The number of follicles 2–3 mm, 4–5 mm and total number of follicles at the beginning of a superovulation treatment was positively correlated to the total number of structures and embryos recovered. It is therefore concluded that the Day 0 protocol can be used for superovulation in Boer goat does however, more studies with large number of animals are recommended to ascertain its benefits. The correlation results suggest that the response to superovulation and quality of embryos recovered could be more determine by the size and number of follicles at the beginning of a superovulation treatment.

1. Introduction

The South African Boer goat has outstanding meat characteristics which makes this breed the most popular for goat meat and enjoys high demand across the globe (Christopher, 2002). Multiple ovulation and embryo transfer programme has been widely used to disseminate this breed across the globe as in most countries; the entry of live animals is prohibited because of health risks. Therefore, import or export of cryopreserved semen and embryos under strict regulatory conditions are the most viable options (Steele and Smith, 1996) for dissemination of genetic materials across the globe. To date, superovulation, the most important step for production of multiple embryos, continues to lack consistency and as a result there is always variability in terms of quantity and quality of embryos obtained in small ruminants (Cognie, 1999; Gonzalez-Bulnes et al., 2004a).

Variation in response to superovulation can be ascribed to several factors including oestrous synchronization treatment, the quality of gonadotrophin and the superovulation protocol used (Gonzalez-Bulnes et al., 2004a; Lehloenya and Greyling, 2010a). Currently, superovulation protocols are designed to reduce excessive handling of animals to minimise stress and avoid starting a superovulation treatment in the presence of the dominant follicle (Menchaca et al., 2002, 2007, 2010; Gibbons et al., 2007; Simonetti et al., 2008; Lehloenya, 2013). In recent years, a Day 0 protocol first described by Menchaca et al. (2002) in which a superovulation treatment is applied coinciding with emergence of follicular wave, has been used. Although there is limited research concerning this protocol, promising results have been reported (Menchaca et al., 2007, 2009; Martemucci et al., 2008; Tasdemir et al., 2011). Despite few publications of the beneficial effects of the Day 0 protocol in some aspects of the ovarian response, there is still not enough data to inspire its use not only in South Africa but across the globe. Like other countries, in South Africa the most commonly used superovulation protocol in goats is still the traditional FSH. Therefore, this study evaluated the efficiency of using the Day 0 protocol in Boer goats through comparison to the traditional FSH protocol.

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2. Materials and methods

2.1. Experimental area

The experimental procedures for this study were approved by the Agricultural Research Council (ARC, APIEC 10/14) and Tshwane University of Technology (AREC 2010/07/003) ethics committees. The study was conducted during the spring season at Agricultural research council (ARC), Irene. The area is situated at 25° 53′ 59.6″ south latitude and 28° 12′ 51.6 east longitudes with altitude of 1525 m above sea level. The weather conditions range from hot days in summer (17.5 °C to 32 °C) to moderate winter days with very cold nights (1 °C to 17 °C).

2.2. Animals

All the experimental does grazed on natural pastures during the day and were supplemented with lucerne hay when kraaled. Water was provided *ad libitum* throughout the experimental period. A total of 20 Boer goats does used in this study were divided into two groups comprising of 10 animals per treatment group. The age of animals were estimated based on the number of permanent incisors and it ranged from 1 to 6 years. The body weight of does also varied from 35 to 62 kg. Therefore, the animals were allocated into two treatment groups balanced according to age (2.20 ± 0.4 and 2.12 ± 0.5) parity (2.11 ± 0.4 and 1.98 ± 0.3) and body weight (43.20 ± 3.0 kg and 43.78 ± 3.3 kg) for Day 0 and traditional protocols, respectively.

2.3. Treatments

For synchronization of the emergence of follicular wave from the Day 0 protocol, controlled internal drug release dispensers (CIDR) containing 0.3 g progesterone (Pfizer,™ New Zealand Ltd) were inserted intravaginally for nine days. At CIDR insertion, does were injected with 160 μg/doe prostaglandin (PGF2α) analogue (Estrumate, Cloprostenol Sodium, Intervet Schering-Plough Animal Health, South Africa). The does were also injected with 300 IU of equine chorionic gonadotrophin (eCG) (Intervet Schering-Plough Animal Health, South Africa) at CIDR withdrawal. For superovulation the does were injected with 200 mg pFSH (Folltropin®-Vetrepharm, Bioniche, Canada) initiated 84 h following CIDR withdrawal. The pFSH treatment was administered in seven decreasing dosages, administered twice daily (the first dose being 50 mg and the rest being 25 mg) at 12 h intervals. Two doses of PGF2α were administered concurrently with the fifth and sixth pFSH treatment. At 24 h after the first PGF2α administration, does were injected with 8.4 μg per doe of GnRH (Receptal®VET, Buserelin acetate, MSD Animal Health, India). For the traditional FSH protocol, does oestrous cycles were synchronized with the aid of CIDR inserted intravaginally for a period of nine days. Then, the does were superovulated with pFSH initiated 48 h before CIDR withdrawal. The superovulation dosage was similar to the Day 0 protocol. All the injected hormones were administered intramuscularly.

2.4. Oestrous detection

Oestrous detection was performed at 12 h interval, using intact bucks wearing aprons. From the Day 0 protocol, the detection of oestrus was performed from CIDR withdrawal for a period of 96 h and thereafter from the second PGF2α injection. For the traditional FSH protocol, oestrous detection was initiated at CIDR withdrawal. From both protocols the oestrous detection following superovulation continued for a period of three days (72 h).

2.5. Artificial insemination (AI)

Fixed-time cervical inseminations with 0.1 mL ($200 - 300 \times 10^6$ sperm/mL) fresh undiluted Boer goat semen were performed at 24 and 36 h after the second PGF2α administration for the Day 0 protocol and 24 and 36 h after CIDR withdrawal for the traditional multiple FSH protocol. Semen used for AI was collected with an electro ejaculator from two Boer goat bucks of proven fertility. Ejaculates were assessed for wave motility under the microscope (×10 magnification) by examining a drop (5 μL) of semen on a warmed (35 °C) glass slide. Each sample was scored using a scale ranging from 0 (no wave movement) to 5 (extreme wave movement) (Evans and Maxwell, 1987; Avdi et al., 2004). Only ejaculates with wave motility (≥ 3) were used for AI.

2.6. Ultrasonography evaluation

Transrectal ultrasonographic examinations of the ovaries were performed with the aid of an ultrasound scanner (Aloka 210, Tokyo, Japan), using a rectal probe with 7.5 MHz linear array transducer. Ultrasonographic measurements were taken at the beginning of the superovulation treatment for both groups. The diameter and number of follicles were recorded from both ovaries. Follicles were classified as small (2–3 mm), medium (4–5 mm) or large (≥6 mm) (Gonzalez-Bulnes et al., 2004c).

2.7. Ovulatory evaluation and embryo yield

On day 5 after AI, all does were deprived of feed and water for 24 h. The embryos were surgically flushed on day 6 following the second AI, as described by Lehloenya et al. (2008). Before embryo flushing, ovaries were laparoscopically examined and the total number and quality of corpus lutea (CL) were recorded. The flush media recovered were scrutinized microscopically and evaluated under a stereomicroscope to identify and classify the structures (unfertilized ova and embryos) collected. The flushed structures were classified using the International Embryo Transfer Society criteria recommended by International Embryo Transfer Society (IETS) (1990) as unfertilized ova (no cleavage), degenerated and transferable embryos (grade 1, 2 and 3).

2.8. Statistical analyses

Data for the onset of oestrus, duration of the induced oestrous period, the total number of ovulations, total number of CL, total number of structures, unfertilized ova, embryos and transferable embryos recovered were analysed using the analysis of variance (ANOVA) procedure of (SAS, 2003). Categorical data pertaining to the oestrous response, fertilization rate and embryo viability was analysed using the chi-square test.

3. Results

The time from CIDR withdrawal to the onset of oestrus recorded for the Day 0 before superovulation treatment was 34.80 ± 4.2 h and the oestrous period lasted for 32.00 ± 2.8 h. One Boer goat from the traditional multiple FSH protocol was removed from the trial due to illness. The oestrous response following superovulation for the two protocols was 100%. The time to onset of oestrus (27.60 ± 4.2 h vs 33.11 ± 5.0 h) and duration of the induced oestrous period (26.67 ± 3.3 h vs 37.80 ± 4.5 h) did not differ significantly ($p \square 0.05$) for the Day 0 and traditional protocols, respectively.

The response to superovulation following the two superovulatory protocols is set out in Table 1. The mean total number of corpus lutea (CL), structures recovered, embryos, degenerated and transferable embryos, did not differ significantly ($p \square 0.05$) between