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Superovulatory response in Boer goats pre-treated with a GnRH agonist outside the natural breeding season

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Abstract

The aim of this study was to evaluate the ovarian response and embryo recovery rates in Boer goat does superovulated with pFSH following a pre-treatment with a GnRH agonist (GnRHa) outside the natural breeding season. Oestrus was synchronised in 22 does with CIDR's for 17 days, and these were superovulated with a total dose of 200 mg pFSH/doe administered i.m. in seven dosages, at 12 h intervals, starting 48 h prior to CIDR removal (the first dose being 50 mg and all others 30 mg each). Half of the does (n = 11; treatment group) received GnRHa (Lucrin®) for seven days, starting on day 7 of CIDR insertion, while the other half (n = 11; control group) received no GnRHa. Cervical inseminations with fresh undiluted semen were performed 36 h and 48 h following CIDR removal and the embryos surgically flushed six days after the second AI. The oestrous response, onset- and duration of the induced oestrous period did not differ significantly between groups. There were also no significant differences between pFSH (21.3 ± 5.9) and pFSH/GnRHa (16.1 ± 7.0) treatments, with respect to the mean ovulation rate per donor. However, the mean total number of structures recovered (unfertilised ova and embryos) per doe flushed and the fertilisation rates (%) were significantly lower in the pFSH/GnRHa treated does (12.7 ± 6.0; 11.5 ± 5.3; 81.6 ± 32.2%, respectively), compared to the control group (17.5 ± 4.5; 16.5 ± 6.1; 92.6 ± 19.6%, respectively). In addition, the mean number and percentage of transferable embryos were also significantly lower in the pFSH/GnRHa treated does (4.3 ± 4.0 and 32.7 ± 36.9%), compared to the control does (13.1 ± 5.3 and 75.2 ± 26.8%, respectively). However, no significant differences were recorded in the mean total number of unfertilised ova/doe between groups. The pFSH/GnRHa treatment resulted in a higher number of degenerated embryos per donor (6.9 ± 4.5) compared to the control (3.2 ± 4.2). The pre-treatment with a GnRHa to the pFSH superovulation protocol outside their natural breeding season seemed to be detrimental to embryo production and quality in Boer goats, and is not warranted.

Keywords: Boer goats, superovulation, oestrous synchronisation, pFSH, GnRH agonist, embryo, MOET
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Introduction

Superovulation is an important part of a multiple ovulation and embryo transfer (MOET) programme and has the potential to increase the reproductive performance of selected donors and breeds in high demand. In order to realise the commercial application of MOET programmes, a continuous supply of embryos throughout the year is crucial. However, in most countries, MOET in small ruminants is limited to the natural breeding season because of the natural seasonal cyclic activity of the small ruminants (Chagas e Silva *et al*., 2003). In South Africa, it has been reported that Boer goat does show peak cyclic activity in autumn and lowest activity in spring (Greyling & Van Niekerk, 1987). In some goat breeds the highest ovulation rate and embryo yields are obtained during their natural breeding season and the lowest output is recorded in the anoestrus period (Gonzalez-Bulnes *et al.*, 2003). Although superovulation is an important part of MOET programmes, it continues to produce variable responses in small ruminants (Cognie, 1999; Gonzalez-Bulnes *et al.*, 2004). The major factor leading to the variable ovulation rate and embryo output currently seems to be the follicular status of the donor at the onset of superovulatory treatment (Gonzalez-Bulnes *et al.*, 2004). So for example, the presence of a dominant follicle at the onset of superovulation has been reported to decrease the ovarian response in small ruminants (Rubianes *et al.*, 1995; Lopez-Sebastian *et al.*, 1999; Rubianes & Menchaca, 2003). It is assumed that by lowering the LH concentration, the establishment of a dominant follicle could be avoided, and therefore more follicles will have the opportunity to develop and ovulate under

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the exogenous FSH influence (Gonzalez-Bulnes *et al.*, 2004). Low LH concentration can be achieved by administrating an exogenous gonadotrophin-releasing hormone antagonist (GnRHa) or agonist prior to the superovulatory treatment (Gonzalez-Bulnes *et al.*, 2004). The aim of this study was to evaluate the effect of a GnRHa (Lucrin®) prior to the superovulation protocol using pFSH in Boer goat does outside the natural breeding season.

Materials and Methods

Twenty-two mature multiparous Boer goat does were used in this trial (conducted during spring, i.e. outside their natural breeding season), maintained in open pens and fed milled lucerne hay. All does were synchronised for oestrus with controlled internal drug release dispensers (CIDR; Pharmacia & Upjohn, Auckland, NZ) for 17 days and superovulated with 200 mg/doe porcine FSH (pFSH; Folltropin® - Vetrepharm). The superovulatory treatment (pFSH) was administered i.m. in seven dosages, at 12 h intervals, starting 48 h prior to CIDR removal (the first dose being 50 mg and all others 30 mg each). Half the does (n = 11; control group - pFSH), received only this superovulatory treatment, while the other half (n = 11; treatment group – pFSH/GnRHa) additionally received a gonadotrophin releasing hormone agonist (GnRHa). A daily dose of 40 µg/doe of Leuprolide (Lucrin®, NL) was administered as two injections of 20 µg each, 12 hours apart for seven days, starting on day 7 of CIDR insertion. Following CIDR withdrawal, oestrous detection was performed three times daily at 8 h intervals with the aid of teaser bucks to determine the onset and duration of the induced oestrous period. Fixed time cervical inseminations with fresh undiluted semen were performed 36 h and 48 h following CIDR withdrawal.

On day 6 following the second AI, embryos were recovered surgically under general anaesthesia. A mid-ventral incision (laparotomy) was made cranial to the udder to exteriorise the reproductive tract. The ovaries were visually examined and the number of *corpora lutea* (CL) on the right and left ovaries recorded. A two-way Folley's catheter was inserted at the base of the uterine horn and the cuff inflated with flushing media. An intravenous 18G catheter was inserted at the utero-tubal junction. The embryos were flushed using Emcare™ flushing media and transferred to Emcare™ holding media (Donnison *et al*., 1996).

The total number of structures recovered per doe flushed (unfertilized ova and embryos) was evaluated and classified using a stereomicroscope according to their morphological appearance (Lindner & Wright, 1983; Nuti *et al*., 1987). Embryos were further classified as degenerated and transferable embryos (grades 1, 2 and 3).

Data regarding the onset and duration of the induced oestrous period, total CL, total structures flushed, unfertilised ova and embryos collected per donor were analysed using ANOVA procedures, while the oestrous response, embryo recovery rates and fertilisation rates were analysed using the Chi-square test (SAS, 1999).

Results and Discussions

Results regarding the oestrous response, time to onset of oestrus and its duration are set out in Table 1. All does responded to oestrous synchronisation. Similar results have been reported in different goat breeds (Selvaraju *et al*., 2003, Espinosa-Marquez *et al*., 2004).

Table 1 Mean (± s.d.) oestrous response, onset and duration of the induced oestrous period in Boer goat does superovulated with pFSH (control) or pFSH/GnRHa protocols

Treatment	N	Oestrous response (%)	Time to oestrus (h)	Duration of oestrus (h)
pFSH (control)	11	100	30.6 ± 9.1	18.2 ± 3.7
pFSH/GnRHa	11	100	31.1 ± 8.8	18.9 ± 4.0

On average oestrus was demonstrated 30.8 ± 8.9 h following CIDR removal, and lasted for 18.6 ± 3.9 h, with no significant differences between the two treatment groups. The onset of oestrus was in line with the

32.0 ± 3.5 h and 33.4 ± 4.7 h reported previously in other goat breeds (Pendletone *et al.*, 1992; Selvaraju *et al*., 2003). The mean duration of the induced oestrous period was shorter when compared to the 36.6 ± 3.5 h reported by Selvaraju *et al*. (2003), however, comparable with the 22.7 ± 1.3 h recorded by Espinosa-Marquez *et al*. (2004).

The mean number of CL's (ovulation rate) and ova/embryo recovery rates are set out in Table 2. It was expected that pre-treatment with GnRHa would increase the ovulation rate, as reported in sheep (Naqvi & Gulyani, 1998) and in mice (Kanter *et al*., 2004). However, in the present study the pre-treatment with GnRHa did not improve the ovulation rate. These results thus contradict the findings of Cognie (1999) in sheep, where FSH/GnRHa treated ewes recorded a significant higher ovulation rate (19.2 ± 4.1) than those treated with FSH alone (13.2 ± 5.5). The mean ovulation rate (18.8 ± 6.5) recorded in this study is in line with the averages of 18 in Boer goats and 15 in feral goats reported by Greyling *et al.* (2002), as well as the mean number of 17.6 ± 5.5 reported by Armstrong *et al.* (2003) in feral goats. Nevertheless, the mean ovulation rate recorded in this study was higher than the 8.3 ± 1.8 ovulations reported by Selvaraju *et al.* (2003) in mixed goat breeds as well as the 10.2 ± 3.1 ovulations recorded in Tellicherry goats treated with the same pFSH as that used in the present study (Senthil Kumar *et al.*, 2003).

Table 2 Mean (±SD) ovulation rate, number of ova and embryos (total, transferable and degenerated) in Boer goat does superovulated with pFSH (control) or pFSH/GnRHa protocols

Parameters	pFSH	pFSH/GnRHa
No. of does flushed	11	11
No. of ovulations (total CL's/donor)	21.3 [a] ± 5.9	16.1 [a] ± 7.0
Total number of structures recovered per doe flushed [*]	17.5 [a] ± 4.9	12.6 [b] ± 6.0
Total number of embryos recovered /donor	16.5 [a] ± 6.1	11.5 [b] ± 5.3
Fertilisation rate (%)	92.6 [a] ± 19.5	81.6 [b] ± 3.2
Total number of unfertilised ova/donor	0.9 ± 2.4	1.2 ± 2.5
Total number of transferable embryos/donor	13.1 ± 5.3	4.3 [d] ± 4.0
Transferable embryos rate (%)	75.2 [c] ± 26.8	32.7 [d] ± 4.9
Total number of degenerated embryos/donor	3.2 [a] ± 4.2	6.9 [b] ± 4.5

[a,b] Values with different superscripts within the same row differ significantly ($P < 0.05$)
[c,d] Values with different superscripts within the same row differ significantly ($P < 0.01$)
[*] Unfertilised ova & embryos (degenerated and transferable: grades 1, 2 & 3)

The mean total number of structures (unfertilised ova & embryos) flushed, embryos recovered and fertilisation rates were significantly higher in the control group (pFSH), compared to the treatment (pFSH/GnRHa) group. These results clearly indicate that pre-treatment with a GnRHa has a detrimental effect on the total recovery rate (ova/embryo recovered) and fertilisation rate in Boer goat does. Similar trends have been reported in goats treated with a GnRH antagonist, where a low fertilisation rate (28.5%) in FSH/GnRH antagonist treated goats was recorded, compared to FSH treated does (93.6%; Cognie *et al.*, 2003). The number of unfertilised ova per donor did not differ between treatments. However, the pFSH/GnRHa treatment resulted in a higher ($P < 0.05$) number of degenerated embryos than in the control group. This led to a significantly lower mean number of transferable embryos in the pFSH/GnRHa treated does compared to the control group. Similar tendencies have been reported in does treated with a GnRH antagonist (Cognie *et al.*, 2003; Gonzalez-Bulnes *et al.*, 2004).

Conclusions

The oestrous response, onset and duration of the induced oestrous period in Boer goat does are not affected by the administration of a GnRH agonist prior to superovulation with pFSH. However, this pre-treatment reduces the mean number of transferable embryos recovered in Boer goat does and therefore its use is not warranted.

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