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Can repeated superovulation and embryo recovery in Boer goats limit donor participation in a MOET programme?

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Abstract

This study evaluated the effect of repeated superovulation and embryo recovery in 15 Boer goat does. Does were synchronised for oestrus using CIDR's for 17 days and superovulated with pFSH during the natural breeding season (autumn). 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. Does superovulated for the first time recorded a shorter mean (± s.e.) induced duration of oestrus (20.8 ± 1.0 h), when compared to those repeatedly superovulated (30.4 ± 6.7 h). The mean (±s.e.) number of structures and embryos recovered were significantly lower in does treated repeatedly (6.0 ± 1.7 and 3.8 ± 1.7) than does superovulated for the first time (12.9 ± 0.5 and 11.7 ± 0.5), respectively. The mean (±s.e.) number of unfertilised ova per donor was significantly higher in repeatedly superovulated does (5.5 ± 1.6), compared to does superovulated for the first time (0.1 ± 0.1). The fertilisation rate and the number of transferable embryos were significantly lower in does treated repeatedly, compared to does superovulated for the first time. These results indicate that the number of times that a Boer goat doe can be utilised as the embryo donor may be limited to three times.

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Introduction

The potential of MOET to accelerate genetic progress in goats can be accomplished through repeated superovulation and the recovery of embryos from superior donors. Successive superovulation and the collection of embryos in less seasonal breeds such as the Boer goat may lead to faster progress, above that achieved in a natural breeding management system. Several findings concerning the effect of repeated superovulation and embryo recovery have been reported in goat breeds. However, limited information is available for indigenous South African goat breeds, like the Boer goat. Repeated superovulation with porcine follicle stimulating hormone (pFSH) in goats has been reported to reduce the number of ovulations and embryos recovered, as well as the number of transferable embryos (Nuti *et al*., 1987; Baril *et al*., 1989; Beckers *et al*., 1990). Similar observations have been reported in other species (Al-Kamali *et al*., 1985; Bavister *et al*., 1986). In addition to reduced ovulation rates in sheep, the oestrous response and the number of ewes ovulating were also reduced (Al-Kamali *et al*., 1985; Fuki *et al*., 1985).

Several factors have been suggested to attribute to the reduction in superovulation response e.g. the refractoriness of the ovaries if superovulation is repeated within an interval of 2 to 6 months and the formation of gonadotrophin antibodies following successive superovulation (Nuti *et al*., 1987; Brebion *et al*., 1992; Holtz, 2005). The method of embryo flushing used in goats is seen as a major limitation, as it is generally surgical (Nowshari *et al*., 1995; Baril *et al*., 1996), while embryos are transferred with the aid of a laparoscope (Besenfelder *et al*., 1994). This method does not only impose surgical trauma and formation of post-operative adhesions, but mostly limits the number of times surgical flushings may be performed on the same animal (Pereira *et al*., 1998; Suyadi *et al*., 2000). The alternative method of transcervical embryo collection in goats has been limited in the past because of the difficulty of passing the catheter through the cervix (Ishwar & Memon, 1996; Suyadi *et al*., 2000). Even after the discovery that by injecting animals with hormones such as oxytocin, oestrogen and prostaglandin, could ripen the cervix, this procedure is still limited by the number of flushings needed, as well as the time taken to flush one donor (Holtz *et al*., 2000;

Suyadi *et at*., 2000; Holtz, 2005). Surgical embryo collection still remains the most commonly utilised method in goat MOET programmes and therefore research on improving its efficiency is warranted. This study evaluated the effect of repeated superovulation on the ovarian response and embryo recovery rates of South African Boer goat does.

Materials and Methods

Materials and methods used in this trial were approved by the ethical committee of the University of the Free State. This study was conducted during the natural breeding season (autumn 2007) at the University of the Free State's experimental farm. Does were kept in open pens and fed a maintenance diet of pellets and lucerne hay. Water was provided *ad libitum* throughout the experiment. The does utilised had 2 to 8 permanent incisors (1 - 4 years of age) and this classification was used as an indicator of the age of the experimental animals. The does utilised thus included maiden does (also superovulated for the first time) and does which had previously been superovulated three times. The body weight of the does ranged between 28.7 and 68.6 kg.

Oestrous synchronisation of all the does was performed using CIDR devices (Pharmacia & Upjohn, Auckland, New Zealand), inserted for a 17 d period, while does were superovulated with a total of 200 mg pFSH/doe (Folltropin®-Vetrepharm). The superovulation treatment (pFSH) was administered intramuscularly in seven dosages, at 12 h intervals, starting 48 h prior to CIDR removal (the first dose being 50 mg and all others being 25 mg). Oestrous detection was performed three times daily at 8 h intervals following CIDR withdrawal, with the aid of teaser bucks. Fixed-time cervical inseminations (0.01 mL fresh undiluted semen – density 3000 x 10^6 sperm/mL) were performed 36 h and 48 h following CIDR withdrawal. The semen used for AI was collected from bucks with the aid of an artificial vagina and only semen samples with a 3+ motility score was utilised for AI. On day 6 following the second AI, embryos were recovered surgically under general anaesthesia. Does were deprived of feed and water 24 h before embryo recovery. A mid-ventral incision (laparotomy) was made cranial to the udder to exteriorise the reproductive tract. The ovaries were visually examined and the numbers of corpora lutea (CL's) on the ovaries were recorded. A two-way Folleys catheter was inserted at the base of the uterine horn and the cuff was inflated with a flushing media. An intravenous 18G catheter was inserted at the utero-tubal junction. The embryos were flushed using Emcare™ flushing media and transferred into Emcare™ holding media (Donnison *et al*., 1996). The flushing media that was recovered was scrutinized and evaluated under a stereomicroscope to identify and classify the structures (unfertilised ova and embryos) collected.

Data regarding the onset and duration of the induced oestrous period, total corpora lutea (CL's), total structures flushed, unfertilised ova and embryos collected were analysed using an ANOVA. Fertilisation rates were analysed using the Chi-square test (SAS, 2003).

Results and Discussions

The effect of repeated superovulation treatment on ovarian response is set out in Table 1. The time response from CIDR removal to the onset of oestrus for does superovulated for the first time (32.8 ± 0.7 h) and for the 4th time (27.2 ± 0.9 h) is in line with comparable times of 32.0 ± 3.5 h and 27.6 ± 3.5 h previously reported in other goat breeds following superovulation (Baril & Vallet, 1990; Pendleton *et al*., 1992). The significantly ($P < 0.05$) shorter duration of the induced oestrous period for does superovulated for the first time compared to does treated repeatedly, may be due to an age factor in this trial, as the majority of does superovulated for the first time were maiden does. In previous trials, the duration of the induced oestrus in younger does tended to be shorter by approximately 5.5 h, compared to adult does (Lehloenya *et al*., 2008a).

The number of CL's recorded was not affected by the repeated superovulation treatment. This observation contradicts previous findings in goats where repeated superovulation has been recorded to reduce the number of CL's obtained (Nuti *et al*., 1987; Baril *et al*., 1989) - especially following the 4th and 5th superovulation treatment, as was the case in this study. The lack of differences in the number of CL's induced in this trial may mainly be attributed to an age effect. There were more young does available in this trial which was shown to lead to a lower superovulatory response. The reduced number of CL's from repeatedly-treated does was also obscured by the lower response in the young does. From the previous studies utilising multiparous Boer goat does aged between 3 and 4 years, the average number of CL's for the

1st and 3rd superovulatory treatment ranged from 18.0 ± 5.8 to 21.3 ± 5.9 during and outside the breeding season (Lehloenya *et al*., 2006; Lehloenya *et al*., 2008b). These mean numbers of CL's were higher compared to the present study – indicating a possible reduction in response from repeatedly treated does by approximately two CL's per doe

The number of structures recovered (recovery rate), embryos and transferable embryos collected following repeated superovulation treatment were also significantly (P <0.05) lower in those does that were superovulated repeatedly. This was the case in spite of the latter group being older on average than those does superovulated for the first time. The lower response to superovulation treatment following repeated treatment may be attributed to reduced activity of the exogenous gonadotrophin applied, due to it being neutralised by antibodies generated from previous gonadotrophin treatments. The higher occurrence of antibodies following repeated superovulation treatment with FSH in goats is generally observed after the 3rd treatment. These FSH antibodies following repeated superovulation treatment in goats have been highly correlated with a reduced superovulatory response (Beckers et al., 1990; Remy *et al*., 1991). The reduction in superovulatory response can also be ascribed to the formation of post operative adhesions following repeated surgical collection of the embryos. This factor has also been shown to reduce ovulation rate and the number of embryos recovered (Al-Kamali *et al*., 1985; Cognie, 1999).

Table 1 The mean **(± s.e.)** effect of repeated superovulation treatment on the ovarian activity of Boer goat does

Parameters	First time superovulation	Repeated superovulation
No. of does	10	5
Time to onset of oestrus (h)	32.8 ± 0.7	27.2 ± 0.9
Duration of oestrus (h)	20.8 [a] ± 1.0	30.4 [b] ± 1.4
No. of ovulations (total CL's/donor)	14.8 ± 0.5	16.8 ± 1.4
Total number of structures recovered per doe flushed (unfertilised ova & embryos)	12.9 [a] ± 0.5	6.0 [b] ± 1.7
Total number of embryos recovered /donor	11.7 [a] ± 0.5	3.8 [b] ± 1.7
Total number of unfertilised ova/donor	0.1 [a] ± 0.1	5.5 [b] ± 1.6
Fertilisation rate (%)	99.4 [a] ± 0.1	50.0 [b] ± 14.1
Total number of degenerate embryos/donor	2.2 ± 0.3	0 ± 0
Total number of transferable embryos/donor	10.7 [a] ± 0.4	3.8 [b] ± 1.7

[ab] Values within the same row with different superscripts differ significantly (P <0.05).

The increased number of unfertilised ova obtained from repeatedly treated does has been emphasized by a poor fertilisation rate. This phenomenon may also be attributed to the formation of FSH antibodies following repeated superovulation treatment. It has been reported that gonadotrophin antibodies are usually formed following successive superovulation (Holtz, 2005). This antibody formation can influence the hormonal balance and hence affect the developmental competence of the oocytes. Thus, if the oocytes have improper developmental competence, the end result would either be reduced fertilisation capacity, or improper development of the embryos following fertilisation (Kumar *et al*., 1990; Kumar *et al*., 1991).

Conclusion

The number of times that a donor can be utilised in a goat MOET programme is limited, as embryos are routinely flushed surgically at present. This protocol is associated with the formation of adhesions. The

adverse effect of repeated superovulation treatment on ovulation rate was not observed and was probably obscured by an age effect in the present study. However the 4th superovulatory treatment in the does resulted in a lower number of structures and embryos being recovered. Moreover, the number of unfertilised ova increased substantially following repeated superovulation, hence reducing the fertilisation rate and eventually the number of transferable embryos being recovered. It would seem as if the number of times that Boer goat does can be utilised as donors in a MOET programme is limited to three flushings.

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