The effect of embryo donor age and parity on the superovulatory response in Boer goat does

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Abstract

This study was conducted to evaluate the effect of the age and parity of the embryo donor on the superovulatory response and embryo recovery rate in Boer goat does. The oestrous cycles of seven maiden does (young, 1 - 2 years) and nine multiparous does (adult, 3 - 4 years) were synchronised using controlled internal drug release dispensers (CIDR's) for a period of 17 days, and superovulated with pFSH during the natural breeding season. The superovulation treatment was administered as a total dose of 200 mg pFSH/doe given i.m. in seven dosages (at 12 h intervals) - starting 48 h prior to CIDR removal. Does were observed for oestrous behaviour three times daily, at 8 h intervals following CIDR withdrawal. Cervical inseminations (0.01 mL fresh undiluted semen) were performed 36 h and 48 h following CIDR removal and the embryos surgically flushed six days following the second artificial insemination. All does showed overt signs of oestrus. The time interval from CIDR removal to the onset of oestrus in the adult multiparous does (24.0 ± 4.0 h**)** was significantly shorter than that recorded in the younger does (32.0 ± 4.6 h). The age and parity of the embryo donors did not have any effect on the duration of the induced oestrous period. The mean number of CL's, structures and embryos recovered were significantly higher in the adult multiparous does (19.8 ± 4.8, 21.3 ± 3.9 and 20.9 ± 4.5, respectively), compared to the young maiden does (13.7 ± 3.8, 11.7 ± 5.0 and 11.7 ± 5.0, respectively). The fertilisation rate, the mean number of unfertilised ova and degenerate embryos recorded did not differ between the young and adult multiparous does. The mean number of transferable embryos in the adult does (15.8 ± 6.4) was, however, significantly higher than in the young does (9.5 ± 3.7). The longer response time taken to the onset of oestrus had no influence on the fertilisation rate in young does. Although the young does recorded an acceptable fertilisation rate, the number of transferable embryos was, however, lower due to the lower total number of embryos produced by the young donor does and this may favour the use of older multiparous does as embryo donors in a multiple ovulation and embryo transfer programme.

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Introduction

Multiple ovulation and embryo transfer (MOET) is utilised to accelerate the genetic improvement in sheep and goat breeding. This potential of accelerating genetic progress has, however, not yet realised. The efficiency of this technique could have more impact if the generation interval could be shortened by utilising young animals in the programme. Several studies have been conducted in sheep and cattle to evaluate the effect of age of the donors on the ovarian response to superovulation. However, there is limited information and investigations comparing young and adult females; most of the studies have been comparing pre-pubertal to adult animals. There is a general agreement that pre-pubertal and young (age, 1 – 2 years) animals can be superovulated and these follicles are also sensitive to gonadotrophin stimulation (Donaldson, 1984; Driancourt *et al*., 1990; Rangel-Santos *et al*., 1991; Hasler, 1992; Kuhholzer & Brem, 1999). Although the ovaries of young females can respond to external gonadotrophins administered, there have been incidences of a poorer ovarian response to superovulation (in terms of ovulation and fertilisation rates), as well as lower embryo recovery and survival rates recorded, compared to multiparous or adult females (Quirke & Hanrahan, 1977; Mahmood *et al*., 1991; Rangel-Santos *et al*., 1991; Driancourt & Avdi, 1993).

More trials evaluating the effect of donor age on the ovarian response to superovulation have been conducted in sheep and cattle for example than in goats. Contradictory results have been reported regarding

the response to superovulation regarding different parameters. Ova and embryos recovered from young ewes (pre-pubertal and ewe aged 9 - 12 months) have been reported to have a lower potential for development, when compared to the ova produced by adult ewes (Quirke & Hanrahan, 1977; Wright *et al*., 1981; McMillan & McDonald, 1985). It was also reported that adult ewes yield higher ovulation rates compared to young ewe (aged, 1 - 2 years) (Baril *et al*., 2000; Lopes *et al*., 2006). In cattle, however, the effect of donor age on the superovulatory ovarian response is not always taken into account, as there is either too small a difference in the superovulatory response due to age, or no effect of donor age observed regarding the total number of embryos recovered (Donaldson, 1984; Hasler, 1992). The limited information regarding the effect of donor age on response to superovulation in goats and the contradictory results reported in other ruminants warrant further research to evaluate the effect of age and parity of an embryo donor on the ovarian response to superovulation in goats.

Materials and Methods

The study was conducted in autumn (the natural breeding season) at the University of the Free State. The University is located at 28.57º south longitude and 25.89º east latitude, at an altitude of 1304 m above sea level. The highest mean maximum ambient temperature recorded in winter is 22.8 °C, and 37.9 °C in summer, with the mean minimum temperature being -5.5 °C and 7.7 °C in the winter and summer, respectively. The average annual rainfall in this area is 559 mm, and falls predominately during the summer months (December to February), with the mean daylight length ranging from 9.8 h in winter to 13.2 h in summer. Materials and methods used in this study were approved by the ethics committee of the University of the Free State.

Seven maiden does (young; 1 to 2 years of age) and nine multiparous does (adult; 3 to 4 years of age) were used in this trial. Does were maintained in open pens and fed a maintenance diet of pellets and lucerne hay *ad libitum*. In all does, oestrus was synchronised with the aid of controlled internal drug release dispensers (CIDR; Phamacica & Upjohn, Auckland, New Zealand), inserted intravaginally for a period of 17 d. All does were superovulated with pFSH (Folltropin, Vetrepharm). The superovulatory treatment consisted of a total dose of 200 mg FSH/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 being 25 mg).

Starting at CIDR withdrawal, all does were tested for overt signs of oestrus three times per day (at 8 h intervals) for a period of three days (72 h) to determine the oestrous response; the onset and duration of the induced oestrous period. 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 artificial insemination (AI) was collected with the aid of an artificial vagina and only semen samples with a motility score 3+ (out of 5) were utilised for AI. On day 5 following AI all does were deprived of feed and water for a 24 h period and the following day (day 6 after AI), a laparoscopic evaluation of the ovaries was performed to determine the presence, absence and quality of the corpora lutea (CL's) prior to laparotomy and embryo flushing.

Prior to surgery, does were pre-treated with atropine, anaesthetised and maintained in this state with halothane combined with nitrous oxide. Once anaesthetised, a mid-ventral surgical incision was made cranial to the udder to exteriorise the reproductive tract and the number of CL's recorded. Thereafter, a two-way Foley's catheter was inserted at the base of the uterine horn and the cuff inflated with flushing media, while an intravenous 18G catheter was inserted at the utero-tubal junction. The embryos were then flushed using Emcare™ flushing media and transferred to Emcare™ holding media. The total number of recovered structures (unfertilised ova and embryos) was evaluated microscopically for the stage of development and quality, using morphological criteria. The embryos were classified as unfertilised ova (no cleavage), degenerate embryos (embryos at 8-cell stage and earlier stage) or as transferable Grade 1, 2 and 3 embryos. Grade 1 embryos were morphologically intact with an even granulation and cell distribution; Grade 2 embryos had small deviations, like a few excluded blastomeres; while Grade 3 embryos had an uneven cell organisation, loose structures, and numerous free blastomeres (Lindner & Wright, 1983; Nuti *et al*., 1987).

Data regarding the onset of oestrus following the cessation of treatment and the duration of the induced oestrous period, the total number of ovulations, the total number of structures recovered, unfertilised ova and embryo yield and recovery rates were analysed using the general linear model (GLM) while, data pertaining to the oestrous response were analysed with the aid of the Chi-square test (SAS, 2003).

Results

The oestrous and ovarian response of the maiden young and adult multiparous Boer goat does following superovulation are set out in Table 1. The time interval from CIDR removal to the onset of oestrus in the adult or older does was significantly ($P < 0.01$) shorter than that recorded in the young does. Age and parity, however, did not have any evident effect on the duration of the induced oestrous period. The mean duration of oestrus being 19.4 ± 11.2 h and 24.9 ± 4.8 h for young and adult does, respectively. The variation between animals with respect to the duration of the induced oestrous period was also higher in the young maiden does. The mean number of CL's, structures and embryos recovered were significantly ($P < 0.01$) higher in the adult does compared to the young does. No recovery of unfertilised ova was recorded in the young does and the fertilisation rate and mean number of unfertilised ova did not differ between the two groups. Age and parity as such had no effect on the mean number of degenerate embryos per donor recorded - with a mean of 2.1 ± 3.2 and 5.1 ± 4.3 for the young and adult does, respectively. The mean number of transferable embryos in the adult does (15.8 ± 6.4) was, however, significantly ($P < 0.01$) higher, than in the young does (9.5 ± 3.7).

Table 1 The mean (± s.d.) effect of age and parity on the response of Boer goat does to superovulation treatment

Parameters	Young	Adult
Number of does	7	9
Time to onset of oestrus (h)	32.0 [a] ± 4.6	24.0 [b] ± 4.0
Duration of oestrus (h)	19.4 ± 11.2	24.9 ± 4.8
No. of ovulations (total CL's/donor)	13.7 [a] ± 3.8	19.8 [b] ± 4.8
Total number of structures recovered per doe flushed (unfertilised ova & embryos)	11.7 [a] ± 5.0	21.3 [b] ± 3.9
Total number of embryos recovered/donor	11.7 [a] ± 5.0	20.9 [b] ± 4.5
Fertilisation rate (%)	100.0 ± 0	97.4 ± 5.2
Total number of unfertilised ova/donor	0	0.4 ± 0.8
Total number of degenerate embryos/donor	2.1 ± 2.3	5.1 ± 4.3
Total number of transferable embryos/donor	9.6 [a] ± 3.7	15.7 [b] ± 6.4

[ab] Means within the same row with different superscripts differ significantly ($P < 0.01$).

Discussion

The effect of donor age on the ovarian response to superovulation is an aspect almost always ignored in most farm animal species. However, age could contribute greatly to the variation in ovarian response to superovulation between animals within the same group - as animals of different ages have been shown to have different physiological needs and respond differently (Jainudeen *et al*., 2000). In goats there is limited information relating to the effect of age on the ovarian response to superovulation, compared to other ruminants. When evaluating the effect of age on oestrous response following superovulation, younger does recorded a longer time interval from CIDR removal to the onset of oestrus. Similar results have been reported in cattle and goats (Bari *et al*., 2000; Drion *et al*., 2001). For the young does it was the first time to be treated with exogenous hormones – thus, these does may have responded slower and were less sensitive to the exogenous hormones due to high sensitivity to the negative effects of steroids (Bari *et al*., 2000). It could imply that the hormonal threshold level is higher and a greater stimulus is needed to elicit an ovarian response. The duration of the induced oestrous period also tended to be 5.5 h shorter in the young does. However, due to the high variation recorded between animals within groups, these differences were not significant. It would thus be pure speculation to comment on this phenomenon. The results regarding the

oestrous response in this trial are important, especially where fixed-time AI is performed as part of a MOET programme.

The response to superovulation as reflected by ovulation rate, total number of structures and embryos collected was lower in the maiden young does. In both sheep and goats poorer superovulatory responses have been recorded in younger females (less than three years of age) (Torres *et al*., 1987, Mahmood *et al*., 1991; Dingwall *et al*., 1993; Lopes *et al*., 2006). Several factors may contribute to this tendency. Firstly, the ovaries of young does may not be mature enough and developing, to be sensitized by exogenous gonadotrophin treatment, while on the other hand, the lower response to superovulation may indicate a poor recruitment of the follicles to the ovulatory stage on the ovary of the young does. The poor recruitment or stimulation of small follicles could also be attributed to the suppressive effect of a dominant follicle, which is known to secrete steroids (together with inhibin), which eventually suppresses the total follicular recruitment (Driancourt, 2001; Senger, 2003). Thus, eventually maiden does may be more sensitive to this inhibitory effect of the steroids and inhibin, compared to adult does.

The longer reaction time taken to the onset of oestrus did no influence the fertilisation rate in the young does and a fertilisation rate of 100% was recorded. The fertilisation rate thus obtained was not affected by age, which is in contrast to observations in pre-pubertal sheep, where pre-pubertal ewes recorded a lower fertilisation rate compared to adult ewes (Rangel-Santos *et al*., 1991). Although young does in this trial recorded a higher fertilisation rate and harvested good quality embryos (based on the degeneration rate of the embryos), the number of transferable embryos was lower, when compared to the older does. The lower number of acceptable quality embryos could again be ascribed to a lower number of embryos recovered from the young does.

Conclusion

Age and parity of the donor Boer goat doe had an effect on the ovarian activity following superovulation. Young does generally took longer to exhibit overt signs of oestrus following the CIDR's withdrawal, compared to the older, multiparous does. Mature does also produced a higher number of CL's, structures and embryos recovered, compared to the younger does. Although the young does recorded an acceptable fertilisation rate, the number of transferable embryos was lower. Thus, if a large number of embryos (the maximum) are to be expected in a MOET programme, it would seem more appropriate to superovulate multiparous, older goats. This may ultimately lead to the production of more transferable embryos. However, young does can still be utilised in a MOET programme with acceptable success.

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