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Effect of breed and follicular status on response to superovulation in South African goats

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ABSTRACT

This study evaluated the effect of breed and follicular status of Boer and indigenous goats on response to superovulation and embryo yield. The oestrous cycles were synchronized with progestagen for nine days and superovulated with porcine follicle-stimulating hormone. Does were cervically inseminated and embryos were surgically flushed on day six following artificial insemination. The oestrous activity, ovarian response, embryo yield and quality, did not differ significantly between breeds. The number and size of follicles at the onset of superovulation treatment and during oestrus did not differ significantly between breeds. The follicles 2–3 mm, 4–5 mm and total number of follicles at the onset of superovulation treatment were positively correlated with the number of structures, embryos and transferrable embryos recovered. The number of medium follicles (4–5 mm) at the beginning of superovulation treatment increased the number of transferable embryos. The total number of follicles >6 mm at the onset of superovulation was positively correlated to the number of unfertilized ova. Although limited number of animals was used, the results suggest that breed has limited effect on superovulation response. Instead, the quality and yield of embryos are dependent on number and size of follicles present at the beginning of a superovulation treatment.

1. Introduction

South Africa has a vast variety of goat breeds, such as the Boer goat, Savanna, Kalahari Red and unimproved indigenous goats. Among these breeds, the Boer goats are renowned worldwide for meat production and having good body conformation, rapid growth and good carcass quality (Lu 2002). The other breed which had little research attention previously is the South African indigenous goat. The goats are mostly kept by the rural communities and are used for both meat and milk. According to the Agricultural Research Council ARC (2006) all indigenous goats are remarkably hardy; survive on harsh environment such as high temperature and humidity as well as in dry conditions. They can also survive on the poor pastures and are known for being non-selective grazers. They are more resistant to diseases and their reproductive performance under normal conditions is acceptable (Lehloenya et al. 2005; ARC 2006). These goats are mostly recognized for being able to survive and reproduce under extremely unfavourable conditions (Campbell 2003). Due to climatic changes which has led to increased temperature and humidity resulting in prevalence of numerous diseases and unfortunately the animals are increasingly becoming resistant to antibiotics, animals that are hardy and resistant to disease will be important. Therefore, because of the valuable characteristics possessed by the South African indigenous goats, it is postulated that they have a potential to be included in the breeding programmes in order to transfer their genetic material in relation to hardiness

and disease resistance. These will be accompanied by an increase in their demand, necessitating the increase in numbers and the ways to disseminate their genetic material to be further researched.

Reproductive technologies such as oestrous synchronization, artificial insemination, and multiple ovulation and embryo transfer (MOET) can be used to increase numbers and disseminate the valuable characteristics of South African goats worldwide. In Africa and across the globe, movement of live animals is highly restricted due to contagious diseases such as anthrax and therefore the only quarantined option will be through semen and embryos. MOET is the fastest way of speeding up the genetic progress of any herd and the most effective reproductive technology for disseminating the genetic materials between countries. In South Africa, most of the reproductive technologies mentioned have been thoroughly researched using the Boer goats with acceptable results; however, there is limited information regarding utilization of these technologies on South African indigenous goats (Greyling et al. 2002; Lehloenya et al. 2008; Lehloenya & Greyling 2009, 2010). In goats and other species like cattle, breed is implicated to affect ovarian response and embryo quality. In general, prolific breeds have better superovulation response (Breuel et al. 1991; Gonzalez-Bulnes et al. 2004a).

On the other hand, the size of the follicle does not only determine ovulation rate but also is an important characteristic that indicates competence of the oocyte and eventually embryo development. In goats it has been observed that the

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number of recovered and viable embryos following superovulation is associated with follicles of 4–6 mm size (Gonzalez-Bulnes et al. 2003). From our recent study comparing different superovulation protocols, the size and number of follicle were more predictive of embryo recovery rate and quality than the superovulation protocol (Mogase et al. 2016). In sheep, the total number of embryos and their viability were positively correlated to 3 mm follicle size while the embryo degeneration rate was associated with small follicles (2 mm), following superovulation (Veiga-Lopez et al. 2005). The observation is to a large extent associated with the contents of medium-large follicles that support embryo development. One of the medium-large follicular contents is inhibin. High concentration of inhibin has been positively correlated to the number of follicles with 4–6 mm in size and high number of viable embryos following superovulation (Gonzalez-Bulnes et al. 2004b). The effect of follicular size on oocyte competence and embryo development has been extensively studied *in vitro*. In cattle, for example, high developmental competence of oocytes from follicles ≥6 mm compared to oocytes from smaller follicles has been reported (Lequarre et al. 2005). *In vitro* studies in goats also emphasize the importance of follicular size on embryo development and provide more information on the relationship between follicular size and oocyte competency. It has been observed that the diameter of a follicle is associated with increase in oocyte growth and number of oocytes with cumulus cell layers. Hence, oocytes with larger diameter had higher maturation rate (indicated by oocytes reaching metaphase II stage) and yielded higher proportion of hatched blastocyst (Martino et al. 1994; Crozet et al. 1995). Clearly *in vitro* and *in vivo* studies emphasize the importance of follicular size on oocyte competency and embryo development, and therefore it is imperative to evaluate the effects of follicular growth on response to superovulation. This study evaluated the response to superovulation of South African indigenous goats using the Boer goats as the control and the effect of follicular status on response to superovulation.

2. Materials and methods

2.1. Animals and samplings

The trial was conducted at the Small Stock Section of the Agricultural Research Council (Irene) in Pretoria, South Africa. The area is situated in the Highveld area with an altitude of 1525 m above sea level. A total of 20 female goats (10 Boer goats and 10 Indigenous goats) were used in this trial. The age of does ranged from 2 to 4 years and the average body weight was 26.0 ± 2.7 kg and 26.1 ± 2.8 kg for indigenous and Boer goats, respectively. Does were allocated in such a way that age, parity and body weight were balanced for both groups. All does were maintained in open pens and were allowed to graze during the day and fed lucerne as a supplement when kraaled throughout the experiment and water was provided *ad libitum*.

The onset of oestrus was synchronized using controlled internal drug release dispensers (CIDRs) containing 0.3 g progesterone (Pfizer,[TM] New Zealand Ltd) inserted intravaginally for nine days. Superovulation was performed with porcine

follicle-stimulating hormone (pFSH) (Folltropin, Vetrepharm). In all does regardless of breed, the superovulatory treatment consisted of a total dose of 200 mg FSH/doe, administered intramuscularly in seven dosages, at 12 h intervals, starting from 48 h prior to CIDR removal. The first dosage consisted of 50 mg pFSH and the remaining dosages of 25 mg. Does were teased with the aid of intact bucks fastened with aprons. Oestrous behaviour was observed twice daily at 12 h intervals, starting from CIDR removal for 72 h to determine the oestrous response, onset and duration of the induced oestrous period. Cervical inseminations with 0.1 mL (density 200×10^6 sperm/mL) fresh undiluted semen (Boer goat and unimproved indigenous buck) were performed 24 and 48 h following CIDR withdrawal. The semen used for artificial insemination (AI) was collected using an electro-ejaculator and each animal was collected once per session. Only semen samples with 3+ motility score (out of 5) were used.

Ovarian activity was evaluated with an ultrasonographic scanner (Aloka 500, Tokyo, Japan) using a 7.5 MHz linear array transrectal probe to obtain an ovarian image, as described by Menchaca et al. (2002). The first ultrasound was performed at the beginning of the superovulation treatment and the second at 48 h after CIDR withdrawal. Goats were restrained in the lying-down position. The probe was lubricated with an ultrasound gel (BAC Laboratories KYA sand, Gauteng RSA) before insertion in the rectum. The urinary bladder, cranial vagina and the cervix were viewed in the longitudinal planes while the probe was inserted. Thereafter, the probe was rotated 45–90° clockwise to locate the ovaries. Both ovaries were examined and the number of follicles as well as the diameter of all follicles were recorded and classified as small (2–3 mm), medium (4–5 mm) or large (≥6 mm).

A laparoscopy was performed just before embryo flushing according to the technique described by Oldham and Lindsay (1980) to determine the ovulation rate. Goats with no active or only abnormal corpora lutea (CL) were not flushed. Embryo flushing was carried out as described by Lehloenya et al. (2008). On day 5 after the second AI, all does were deprived of feed and water and on day 6, embryos were flushed surgically. The total number of recovered structures (unfertilized ova and embryos) was evaluated microscopically for stage of development and quality, using morphological criteria. The embryos were classified as unfertilized ova (if there was no cleavage), degenerated embryos (embryo at 8-cell stage and earlier) and transferable grades 1, 2 and 3 embryos, according to Lindner and Wright (1983) and Stringfellow and Seidel (1990).

2.2. Statistical analyses

Data collected were analysed using ANOVA through the Stata Software Version 11. A *t*-test was adopted to compare the effect of breed on the onset and duration of oestrus, CL, total structures (unfertilized ova and embryo), degenerated and transferable embryos, size and number of follicles. The results were expressed as mean ± SE. and differences and correlations were accepted as statistically significant at $p \leq .05$. The response to oestrous data were analysed using the chi-square test.
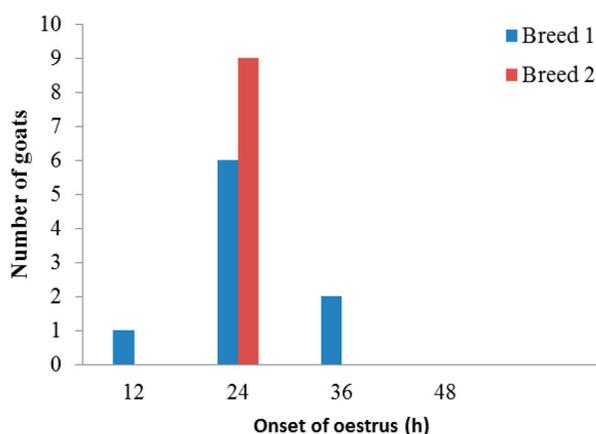
Table 1. Effect of breed on response to oestrous synchronization following superovulation in Boer and Indigenous goats (mean ± SE).

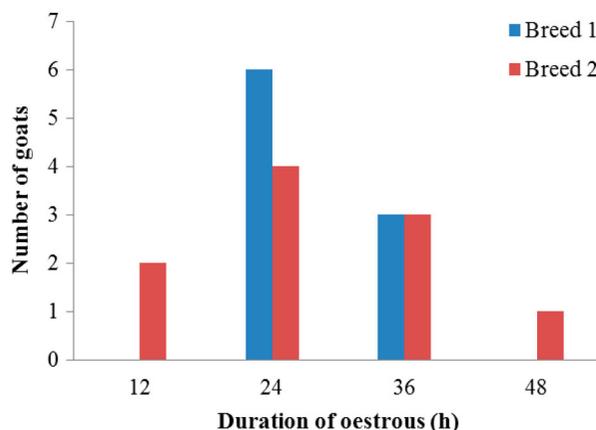| Parameters	Boer goats	Indigenous goats
Oestrous response (%)	100 ± 0.1	90 ± 0.0
Onset of oestrus (h)	25.20 ± 2.1	24.00 ± 0.0
Duration of oestrous (h)	27.60 ± 3.6	28.00 ± 2.0

3. Results and discussion

One indigenous goat was removed from the experiment due to sickness. Table 1 presents the oestrous response of the two breeds following oestrous synchronization and superovulation. The response to oestrous synchronization, the time to the onset of oestrous from CIDR removal and duration of induced oestrous period did not differ significantly ($p > .05$) between breeds. The onset of oestrous for indigenous goats was 24.00 ± 0.0 h while for the Boer goats it ranged from 12 to 36 h (Figure 1). The duration of oestrous ranged 24–36 and 12–48 h for indigenous and Boer goats, respectively (Figure 2).

One doe from the indigenous and five does from the Boer goat breed did not have active CL and therefore were not flushed. The total CL, structures (unfertilized ova and embryos), degenerated and transferable embryos following superovulation are given in Table 2. The mean numbers of

Figure 1. Interval from CIDR removal to onset of oestrus for two South African goat breeds.

Figure 2. Oestrous duration of two South African goat breeds following superovulation.

Table 2. Effect of breed on response to superovulatory treatment with pFSH (mean ± SE).

Parameters	Boer goats	Indigenous goats
Number of does flushed	5	8
Total CL	12.78 ± 3.0	14.37 ± 2.1
Total structure[a]	9.00 ± 3.2	10.62 ± 1.1
Unfertilized ova	0.60 ± 0.4	2.50 ± 1.8
Total embryos	8.40 ± 3.4	8.12 ± 1.6
Degenerated embryos	1.80 ± 0.9	2.12 ± 0.9
Transferable embryos	6.60 ± 3.4	6.00 ± 1.8

[a]Structures (unfertilized ova and embryos).

Table 3. Number and sizes of follicles in Boer and indigenous goats (mean ± SE).

Parameters	Boer goats	Indigenous goats
At onset of pFSH treatment		
Follicles 2–3 mm	3.40 ± 1.5	2.00 ± 0.7
Follicles 4–5 mm	2.60 ± 0.9	2.25 ± 0.4
Follicles >6 mm	0.60 ± 0.2	0.75 ± 0.2
Total no of follicles	6.60 ± 1.6	5.00 ± 0.6
48 h after CIDR withdrawal		
Follicles 2–3 mm	0.22 ± 0.2	0.89 ± 0.3
Follicles 4–5 mm	4.55 ± 1.5	4.22 ± 0.9
Follicles >6mm	5.22 ± 1.3	5.22 ± 1.1
Total no. of follicles	10.4 ± 0.7	10.8 ± 0.3

CLs were (12.78 ± 3.0) and (14.37 ± 2.1) for Boer and indigenous goats respectively, and these values did not differ significantly. Breed also did not have any significant effect $p > .05$ on the total number of structures (unfertilized ova and embryos), degenerated embryos and transferable embryos recovered.

The number and size of follicles at the beginning of superovulation and 48 h after CIDR removal are given in Table 3. The number and size of follicles did not differ between the two breeds. The number of smaller and medium follicles (2–3 and 4–5 mm) was numerically higher than the large follicles (>6 mm) at the onset of superovulation, higher. At 48 h following CIDR removal there were more number of medium and large follicles (4–5 and > 6 mm) for both breeds.

Since the number and size of follicles did not differ between the two breeds, the follicular data were pooled and correlated with the superovulation parameters. The correlations between follicle size and superovulation parameters are given in Table 4. The number of follicles 2–3 mm, 4–5 mm and the total number of follicles at the onset of pFSH treatment were positively correlated with total number of embryos recovered. The total number of follicles at the onset of superovulation treatment was also positively correlated with the number of

Table 4. Correlations between superovulation parameters and follicle size at the onset of a superovulation treatment in South African goats.

	No follicles 2–3 mm	No follicles 4–5 mm	No follicles >6 mm	Total no of follicles
Total corpora lutea	0.316	−0.163	0.313	0.281
Total structures[a]	0.413	0.281	0.249	0.623*
Unfertilized ova	−0.296	−0.441	0.656*	−0.398
Total embryos	0.565*	0.557*	−0.252	0.819*
Degenerated embryos	0.374	−0.083	−0.266	0.249
Transferrable embryos	0.399	0.555*	−0.157	0.681*

[a]Structures (unfertilized ova and embryos).
*Values with are statistically significant $p < .05$.

structures recovered. The number of medium follicles (4–5 mm) and the total number of follicles at the onset of pFSH treatment were positively correlated with the total number of transferrable embryos. The total number of follicles with a diameter of >6 mm was also positively correlated with the number of unfertilized ova.

In the present study, the oestrous response, onset of oestrous, and duration of induced oestrous following oestrous synchronization and superovulation did not differ significantly between breeds (Boer and indigenous goats). This lack of differences may be due to similar genetic origin as Boer, Kalahari red and Savanna goats are believed to originate from the indigenous goats found in South Africa (Ramsey et al. 2000) or due to adaptation of animals to similar geographical origin (Tasdemir et al. 2011). The was more precise onset of oestrous for indigenous goats at 24 h while for Boer goats it was spread between 12 and 36 h. Although, there was no significant difference, the results suggest that the onset of oestrous is more synchronized in indigenous goats, compared to Boer goats where the onset of oestrous was distributed over a period of time. The effect on the onset of oestrous was emphasized by Nuti et al. (1987), who reported that more Nubian does came into oestrous earlier than the Alpine goats following superovulation.

One doe from the indigenous and five does from the Boer goat breed were not flushed due to the absence of active CL/ only having early CL regression. As the embryo collection in sheep and goats is surgical, it is always advisable not to flush ewes/does with evidence of early/premature CL regression. In goats superovulated with FSH, approximately 33% will have premature CL regression. In sheep and goats, premature CL regression leads to lower embryo recovery rate coupled with recovery of unfertilized ova and degenerated embryos (Armstrong et al. 1982; Armstrong et al. 1983; Baldassarre & Karatzaz 2004). Luteal dysfunction in these species following superovulation is generally characterized by a transient rise and fall in circulating progesterone concentrations within 4 days after ovulation (Armstrong et al. 1982; Stubbing et al. 1986; Southee et al. 1988). Due to the lack of progesterone support in goats with premature luteal regression, there is loss of most embryos before collection is attempted on day 6 or 7 after the onset of oestrous (Armstrong et al. 1982, 1983, 1987). Premature CL regression may also be attributed to persistence of large follicles after superovulation (Cognie et al. 2003). At the beginning of the superovulation treatment there were some medium and large follicles in this study that might have contributed to high occurrence of premature CL regression.

The mean number of CLs, structures recovered, unfertilized ova, total number of embryos, degenerated and transferable embryos for Boer and indigenous goats did not differ between breeds. These results are in agreement with the observations by Nuti et al. (1987), who reported that breed had no effect on response to superovulation in Nubian and Alpine goats. The results might be due to adaptation of animals to similar environments and likelihood of relationship between the two breeds as the Boer goats origin, although scanty, is indicated to be from indigenous African goats (Campbell 2003; Tasdemir et al. 2011). To emphasize the relationship between the two breeds, Osterhoff et al. (1987) found no differences in gene frequencies of polymorphisms between the South African indigenous goat population and the Boer goats. Tasdemir et al. (2011) also reported similar observation in Angora and Kilis goats except that in their study the transferable embryo yields differed. There was no effect of breed on number and size of follicles. There is scarce information on effect of breed on number and size of follicles in sheep and goats. However, differences on the number and size of follicles have been observed in different cattle breeds ascribed to environment or climate (Sergerson et al. 1984; Dominguez 1995), in contrast to the present results where breed had no effect on number and size of follicles. The numerically more number of small and medium-sized follicles at the beginning of a superovulation treatment and more number of medium and large-sized follicles 48 h following CIDR removal were as expected. The total number of follicles at the beginning of a superovulation treatment was associated with the number of structures and embryos recovered. In the current study, more number of small and medium-sized follicles was observed at the beginning of superovulation than large follicles. The presence of large number of small follicles in the ovaries during the absence of a dominant follicle (secreting inhibin) at the beginning of superovulation can significantly improve ovulation rate (Cognie et al. 2003; Gonzalez-Bulnes et al. 2004a).

The number of follicles 4–5 mm was positively correlated with total number of the transferrable embryos. This observation supports the findings of Gonzales-Bulnes et al. (2003) who emphasized that the number of recovered and transferable embryos could be associated with the number of follicles 4–6 mm in size. Furthermore, de Smedt et al. (1994) reported that the oocytes of goats are fully competent to mature in follicles larger than 3 mm therefore, more quality embryos from these oocytes are expected. In the current study there was also a positive correlation between the number of follicles > 6 mm and the unfertilized ova at the beginning of superovulation treatment. These results are in agreement with the previous studies that reported the negative effects of large follicles on response to superovulation (Grasso et al. 1989; Gonzales-Bulnes et al. 2003). Large follicles are associated with secretion of steroids and inhibin, which suppresses growth and competency of recruited follicles following superovulation (Driancourt 2001; Senger 2003), therefore leading to a high number of undersized ova.

4. Conclusion

In conclusion, breed had no effect on the response to oestrous and superovulation parameters evaluated. It should be noted that the indigenous goats had a more predictable onset of oestrous, while the Boer goats demonstrated a range concerning the onset of oestrous. The number of large follicles at the beginning of the superovulation treatment was associated with the total number of unfertilized ova. On the other hand, the number of medium follicles (4–5 mm) at the beginning of superovulation treatment increased the number of transferable embryos. As breed had no effect to response to superovulation, these results although limited number of animals was used suggest that the focus should be more diverted to follicular development at the onset of the superovulation treatment.

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