Preliminary results evaluating a simplified superovulation protocol in Boer goats

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Abstract

This study was conducted to evaluate the use of one shot follicle stimulating hormone (FSH) plus equine chorionic gonadotrophin (eCG) (simplified) superovulation protocol in mature Boer goat does. Eighteen does used were allocated into two groups, during the natural breeding season (autumn). The two group were Group 1: the control (n = 9); multiple FSH protocol and Group 2: treatment group (n = 9); one shot FSH plus eCG protocol. All does were synchronised for oestrus, using controlled internal drug release dispensers (CIDR's) for 9 days. Does in the multiple FSH protocol group were superovulated with 200 µg pFSH/doe, administered i.m. in 7 dosages at 12 h intervals, starting 48 h prior to CIDR removal. In the treatment group (one shot FSH plus eCG protocol), the superovulation treatment was administered once as 200 µg FSH plus 350 IU eCG, 48 h prior to CIDR removal. All does were then observed for oestrous behaviour twice daily, at 12 h intervals following CIDR removal for a period of 72 h. Cervical inseminations (with 0.1 ml fresh undiluted Boer goat semen) were performed 36 h and 48 h following CIDR removal. Embryos were then surgically flushed on day 6 following the second artificial insemination. The time interval from CIDR removal to onset of oestrus was not affected by treatment. The duration of the oestrous period however was significantly longer in the one shot group, compared to the multiple FSH protocol. Does from the one shot FSH plus eCG protocol recorded a significantly ($p < 0.05$) lower mean number of CL's, structures (unfertilised ova plus embryos) and ($p < 0.01$) embryos recovered. A significantly ($p < 0.01$) higher number of unfertilised ova were observed from the one shot FSH plus eCG group (4.06 ± 0.9), compared to the multiple FSH group (0.24 ± 0.1). However, the number of degenerated embryos was not affected by treatment. The total number of transferable embryos was significantly lower in the one shot FSH plus eCG protocol (0.22 ± 0.2), compared to the multiple FSH protocol (9.56 ± 1.9). The size of follicles was not affected by treatment, however the total number of follicles at CIDR removal was significantly lower in the one shot FSH plus eCG group (11.67 ± 0.7), compared to the multiple FSH group (14.83 ± 0.7). AI might have been performed at the wrong time, because of the duration of the oestrous period being longer in Group 2. The multiple FSH superovulation protocol overall seemed to be more efficient in the Boer goat. More research is needed on the doses of gonadotrophins administered and the time of AI, in order for the simplified protocol (Group 2) to be effective.

Keywords : Follicular size; Goat; Embryo; eCG; FSH; Superovulation

1. Introduction

Multiple ovulation and embryo transfer (MOET) technologies remain the fastest way for genetic improvement in small ruminants. However, the response to superovulation, one of the most important steps in MOET, is usually unpredictable (Cognie et al., 2003 and Gonzalez-Bulnes et al., 2004). Superovulation in goats can generally be performed utilising FSH or eCG gonadotrophins. The FSH protocol most commonly used involves oestrous synchronisation with the aid of progestagens for 9–17, days followed by the administration of FSH for 6–8 dosages, beginning approximately 48 h before progestagen removal (Gonzalez-Bulnes et al., 2003, Goel and Agrawal, 2005, Lehloenya et al., 2008, Lehloenya et al., 2009 and Lehloenya and Greyling, 2010). On the other hand, eCG is usually administered as a single injection, due to its long half-life, approximately 48 h before progestagen removal (Espinosa-Marquez et al., 2004 and Holtz, 2005). When comparing the efficiency of FSH and eCG as superovulation agents in goats, it has been reported that FSH produced higher ovulation and embryo recovery rates, as well as more transferable embryos, although more variable than eCG (Mahmood et al., 1991 and Goel and Agrawal, 2005). Although the utilisation of FSH in the superovulation programme of goats has demonstrated the ability to produce better results, this regime is more labour intensive and imposes more stress to the animals, due to excessive handling – as it must be administered twice daily over a 4 day period. Therefore, the simplicity of administering eCG (one injection), the variation in response to superovulation and labour intensity when utilising FSH, warrant attempts to simplify the current FSH protocol used in goats. One of the attempts could be to combine FSH and eCG as one protocol. The main advantage of eCG is then that it can be administered in one dose, which is simple and more practical than when 6–8 injections of FSH are used. Studies in using a simplified superovulation protocol have been extensively conducted in sheep, compared to goats, with contradictory results being reported. The use of one shot FSH and eCG treatment for superovulation has been reported to lead to a higher ovulation rate, increased number of large follicles and the number of oocytes aspirated for in vitro embryo production. Not only was the oocyte recovery rate improved, but also a higher number of good quality embryos (Gibbons et al., 2007 and Simonetti et al., 2008). To the contrary, Cueto et al. (2011) reported that one shot protocol reduced the number of corpora lutea (CL's) and embryo recovery rate regardless of the season in sheep. In goats where a multiple regime and one shot FSH and eCG protocols were compared, a similar superovulation response was reported, based on the number of follicles visualised and aspirated, as well as the number of oocytes collected (Baldassarre et al., 2002 and Freitas and Melo, 2010). Although one shot protocol resulted in an increased number of large follicles in goats, the oocyte recovery rate was not improved, as was the case in sheep (Gibbons et al., 2007). Due to these contradictory results obtained in sheep and the limited research performed on goats concerning the next stage of embryo yield and quality – this study was conducted to evaluate the effect of the one shot FSH plus eCG superovulation protocol on ovarian response and embryo production, compared to the traditional multiple FSH protocol in Boer goats.

2. Materials and methods

The study procedures were approved by Agricultural Research Council ethics committee (Ref.: APIC2010/14).

2.1. Study area

The study was conducted during the natural breeding season (autumn) at the Agricultural Research Council, Irene at the small stock section in the Germplasm Conservation and Reproductive Biotechnologies laboratory. The area is located at 25° 55 south latitude and 28° 12 east longitude in Pretoria, South Africa. The altitude is 1525 m above sea level. The weather conditions range from hot days and cool nights in summer (17.5 °C–32 °C), to moderate winter days with very cold nights (1 °C–17 °C) (Webb et al., 2004).

2.2. Animals

The 18 Boer goat used in this study, were maintained in open pens, allowed to graze on natural pastures during the day and supplemented with lucerne at night when kraaled. Water was provided ad libitum throughout the experiment. The does used were 2–4 years of age, while the average body weight ranged between 27 and 40 kg. Allocation of animals into the respective treatment groups was stratified, based on age and body weight, so that each group consisted of an equal number of young and adult does, as well as having a similar average body weight.

2.3. Treatment

The oestrous cycles of the does were synchronised with the aid of controlled internal drug release dispensers (CIDR; Phamacica & Upjohn, Auckland, New Zealand), inserted intravaginally for 9 days. Thereafter does were allocated to two treatment groups ($n = 9$ per group). In Group 1 (multiple FSH protocol) does were superovulated with 200 mg pFSH (Folltropin, Vetrepharm, Canada) per doe, administered in 7 decreasing dosages at 12 h intervals – starting 48 h before and finishing 24 h after CIDR withdrawal. In Group 2 (one shot FSH plus eCG) does were superovulated with a one dose of 200 mg pFSH (Folltropin, Vetrepharm, Canada) plus a single dose of 350 IU eCG (Fostim; Upjohn, South Africa) 48 h before CIDR withdrawal. Oestrous detection was performed with the aid of vasectomised bucks, at 12 h intervals for a period of 3 days (72 h) – starting at CIDR withdrawal. All does were cervically inseminated with 0.1 ml fresh undiluted semen (approximately 350×10^6 sperm/insemination), performed at a fixed time (36 h and 48 h), following CIDR withdrawal. The semen used for AI was collected with the aid of an artificial vagina from certified fertile bucks and was microscopically evaluated for progressive motility and percentage live spermatozoa. Only semen samples with motility score of 3+ (out of 5) was utilised for AI.

2.4. Ovarian assessment

From all does, the number and size of the ovarian follicles were evaluated, with an ultrasonographic scanner (Aloka SSD-500, Tokyo, Japan), using a 7.5 MHz linear array transrectal probe. The sonographic evaluations were performed at the onset of the superovulation treatment, CIDR withdrawal and at 48 h following CIDR removal (when all the does were in oestrus).

2.5. Ovulation assessment and embryo collection

Embryos were surgically flushed under general anaesthesia on day 6 following the second AI as described by Lehloenya et al. (2008). Before embryo flushing, the number of CL's was recorded. The embryos were then flushed using Emcare™ flushing media and transferred into Emcare™ holding media (ICPbio Reproduction, New Zealand). The recovered structures (unfertilised ova and embryos) were evaluated microscopically for the stage of development and quality, using morphological criteria. The embryos were classified as unfertilised ova (no cleavage), degenerated embryos (embryos at 8-cell stage and earlier) or as transferable embryos (Grade 1, 2 or 3) (Lindner and Wright, 1983 and Nuti et al., 1987). Data were analysed using the general linear model programme of SAS (2003) and the means between the two groups were compared using the *T*-test.

3. Results

The effect of the superovulation protocols on ovarian response and embryo production in Boer goat does is set out in Table 1. Treatment had no effect on the time from CIDR removal to the onset of oestrus. However, does which received the one shot FSH plus eCG protocol recorded a significantly ($p < 0.05$) longer duration of the oestrous period, compared to the does which received the multiple FSH protocol. The total number of corpora lutea ($p < 0.05$) measured, the structures ($p < 0.05$) and total embryos ($p < 0.01$) and transferable embryos ($p < 0.01$) recovered were significantly lower in Group 2 (one shot FSH plus eCG treatment), compared to Group 1 (multiple FSH treatment). The number of unfertilised ova was significantly ($p < 0.01$) higher in Group 2, in comparison to Group 1. On the other hand, the number of degenerated embryos was similar for the two protocols.

Table 1. Mean (± s.e.) ovarian response and embryo production in Boer goat does following superovulation using a multiple FSH or one shot FSH plus eCG protocol.

Parameter	FSH	FSH + eCG
Number of does flushed	9	9
Onset of oestrus (h)	21.33 ± 2.4^{a}	18.66 ± 2.6^{a}
Duration of oestrus (h)	33.88 ± 2.6^{a}	44.44 ± 3.2^{b}
No. of CL	12.00 ± 1.1^{a}	9.67 ± 2.1^{b}
No. of structures	11.24 ± 1.8^{a}	5.11 ± 1.4^{b}
No. of unfertilised ova	0.24 ± 0.1^{c}	4.06 ± 0.9^{d}
No. of embryos	11.00 ± 1.9^{c}	1.33 ± 0.6^{d}
No. of degenerated embryos	1.44 ± 0.4^{a}	1.11 ± 0.5^{a}
No. of transferable embryos	9.56 ± 1.9^{c}	0.22 ± 0.2^{d}

Values with different superscripts within the same row are significantly different ($^{a,b}p < 0.05$ and $^{c,d}p < 0.01$); FSH = follicle stimulating hormone; eCG = equine chorionic gonadotrophin.

The size and number of follicles following superovulation with multiple FSH or one shot FSH plus eCG are presented in Table 2. The administration of both superovulation protocols increased the number of large follicles at CIDR removal and 48 h following CIDR removal (Table 2). The

size and total number of follicles were similar for both superovulation protocols at the onset of the superovulation treatment and when all does were in oestrus. However, at CIDR removal the number of follicles ≥3 mm ($p < 0.05$) and the total number of follicles ($p < 0.01$) were significantly lower in the one shot FSH plus eCG group than in Group1. For both protocols there were no or few small follicles (≤2 mm) 48 h following CIDR removal.

Table 2. Mean (± s.e.) follicular number and size in Boer does superovulated with multiple the FSH or one shot FSH plus eCG protocol.

Parameters	Treatments	
	FSH	**FSH + eCG**
CIDR removal		
2–3 mm	1.33 ± 1.0^{a}	1.83 ± 1.1^{a}
≥3 mm	13.5 ± 0.5^{a}	9.83 ± 1.4^{b}
4–5 mm	8.83 ± 1.4^{a}	7.33 ± 1.6^{a}
≥6 mm	4.67 ± 1.3^{a}	2.50 ± 1.0^{a}
Total follicles	14.83 ± 0.7^{c}	11.67 ± 0.7^{d}
48 h after CIDR removal		
2–3 mm	0^{a}	0.11 ± 0.1^{a}
≥3 mm	11.11 ± 0.7^{a}	9.78 ± 0.9^{a}
4–5 mm	3.89 ± 1.4^{a}	2.11 ± 1.0^{a}
≥6 mm	7.22 ± 1.6^{a}	8.11 ± 1.4^{a}
Total follicles	11.11 ± 0.7^{a}	10.33 ± 0.6^{a}

Values with different superscripts within the same row are significantly different ($^{a,b}p < 0.05$ and $^{c,d}p < 0.01$); FSH = follicle stimulating hormone; eCG = equine chorionic gonadotrophin.

4. Discussion

The time interval from CIDR removal to the onset of oestrus was not affected by treatment in all Boer goat does. Similar results were obtained in goats superovulated with FSH or eCG (Goel and Agrawal, 2005). However, this observation came as a surprise as goats superovulated with eCG usually exhibit oestradiol-17β peak concentration earlier, which could lead to the early onset of oestrus (Riesenberg et al., 2001a). Contradictory results in sheep regarding the onset of oestrus, when comparing the traditional multiple FSH regime and the simplified protocol (single dose of FSH plus eCG) have been reported. So for example, Simonetti et al. (2008) reported that ewes superovulated using the simplified protocol came into oestrus earlier, compared to those superovulated with a traditional multiple FSH protocol. However, Cueto et al. (2010) reported ewes treated with the one shot FSH plus eCG protocol (simplified) to record a longer time interval from CIDR removal to the onset of oestrus, compared to ewes treated with multiple FSH treatment. The one shot FSH plus eCG protocol on the other hand, led to longer duration of the oestrous period. This observation could be a result of a high blood oestrogen concentrations produced by the continuous ovarian stimulation of eCG due to its long half-life. It has been observed that elevated oestradiol concentration continued even to the day of embryo collection in does superovulated with eCG (Senthil Kumar et al., 2003).

The number of ovulations measured and structures recovered were reduced when the one shot FSH plus eCG protocol was used. Similar results have been reported in sheep (Cueto et al., 2011). However in goats the ovulation and oocyte recovery rate were reported to be similar between the multiple FSH protocol and the simplified protocol (Baldassarre et al., 2002 and Freitas and Melo, 2010). In the current study, the lower number of ovulations measured may be due to ovulations not being visible because of the premature luteal regression or obscured by unovulatory follicles – which are commonly reported following superovulation with eCG (Riesenberg et al., 2001b). However in goats where eCG or FSH are combined with hCG, the ovulation and embryo recovery rate were similar (Goel and Agrawal, 2005). In the present study not only was the superovulation response reduced when the simplified protocol was used, but also the number of embryos recovered while the number of unfertilised ova recovered increased. The increased number of unfertilised ova may have been attributed to either fertilisation failure due to incompetent ova produced or an untimed AI – as the duration of the induced oestrous period was longer in one shot FSH plus eCG protocol (Group 2). Usually incompetent ova are associated with high blood oestrogen levels after ovulation from follicles which did not ovulate when eCG is used as a superovulation agent (Armstrong et al., 1983, Mahmood et al., 1991 and Saharrea et al., 1998). In sheep, one shot protocol (simplified) tended to lead to the occurrence of large follicles observed during embryo flushing (Simonetti et al., 2008). Furthermore, unfertilised ova may also be attributed to the abnormal maturation of oocytes, due to premature activation of the initial stages of meiotic oocyte maturation. This abnormality usually occurs as a result of increased blood LH concentrations following eCG superovulation treatment – as this hormone exhibits both LH and FSH bioactivity (Cameron et al., 1988 and Kumar et al., 1991).

In both protocols the number and size of the follicles were effectively increased. Similar observations have been reported in sheep and goats (Pierson et al., 2004 and Gibbons et al., 2007). However, the number of follicles ≥3 mm and the total number of follicles at CIDR withdrawal were reduced when one shot FSH plus eCG protocol was used, compared to the multiple FSH regime. This observation then contradicts reports that a similar number of large and visualised aspirated follicles were obtained when multiple FSH and one shot protocols were compared (Baldassarre et al., 2002 and Freitas and Melo, 2010). The reduced number of follicles ≥3 mm and the total number of follicles at CIDR removal in one shot FSH plus eCG group may have contributed to a lower embryo production – as these follicles are positively correlated with the number of recovered and transferable embryos (Gonzalez-Bulnes et al., 2003).

5. Conclusion

In conclusion, the longer oestrus duration in the simplified protocol (one shot FSH plus eCG) may have affected the timing of AI – as increased numbers of unfertilised ova were collected from does which received the one shot FSH plus eCG protocol. Furthermore, the number of follicles ≥3 mm and total number of follicles at CIDR removal, as well as embryo yield decreased in does which received the simplified protocol. Therefore, it can be said that the multiple FSH superovulation regime seemed to be more efficient for superovulation in Boer goats. However, bearing in mind the simplicity of a one shot protocol, more research is needed on the doses of gonadotrophins and the time of AI during this simplified protocol.

Acknowledgements

This study was funded by National Research Foundation (Thuthuka).

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