Applicability of Day 0 superovulation protocol in Boer goats

C.R. Mogase[a], K.C. Lehloenya[b,*] and M. Dattena[c]

[a]Department of Agriculture Forestry and Fisheries, Animal Production Division, South Africa
[b]Department of Animal & Wildlife Sciences, University of Pretoria, South Africa
[c] AGRIS-Sardegna (DIRPA), Olmendo, Italy

*∗ Corresponding author. E-mail address: khoboso.lehloenya@up.ac.za (K.C. Lehloenya).

Highlights

• Day 0 protocol can induce superovulation in Boer goats although studies are needed to ascertain its beneficial effects.
• Follicular size and population at the onset of a superovulation treatment can determine the superovulation response.
• The size and number of follicles at the beginning of a superovulation can determine embryo quality.

Abstract

This study evaluated the applicability of the Day 0 superovulation protocol in Boer goats by comparing it to a traditional pFSH protocol. Twenty Boer goat does were allocated into two groups comprising of 10 animals per group. For the Day 0 protocol, does oestrous cycles were synchronized for 9 days and superovulated with pFSH starting 84 h after the termination of progesterone treatment. For the traditional pFSH protocol, the oestrous cycle of does was synchronized for 9 days, followed by superovulation with pFSH initiated 48 h before CIDR withdrawal. For both groups does had two timed cervical inseminations with fresh undiluted semen. Embryos from both groups were flushed on day 6 following AI. The response to superovulation did not differ significantly between treatments but a tendency ($P = 0.06$) was found for both fertilization and number of unfertilized ova in favour of the Day 0 protocol. The number of follicles 2–3 mm, 4–5 mm and total number of follicles at the beginning of a superovulation treatment was positively correlated to the total number of structures and embryos recovered. It is therefore concluded that the Day 0 protocol can be used for superovulation in Boer goat does however, more studies with large number of animals are recommended to ascertain its benefits. The correlation results suggest that the response to superovulation and quality of embryos recovered could be more determine by the size and number of follicles at the beginning of a superovulation treatment.

Keywords: Goat embryos; Follicular size; Superovulation protocols

1. Introduction

The South African Boer goat has outstanding meat characteristics which makes this breed the most popular for goat meat and enjoys high demand across the globe (Christopher, 2002). Multiple ovulation and embryo transfer programme has been widely used to disseminate this breed across the globe as in most countries; the entry of live animals is prohibited because of health risks. Therefore, import or export of cryopreserved semen and embryos under strict regulatory conditions are the most viable options (Steele and Smith, 1996) for dissemination of genetic materials across the globe. To date, superovulation, the most important step for production of multiple embryos, continues to lack consistency and as a result there is always variability in terms of quantity and quality of embryos obtained in small ruminants (Cognie, 1999 and Gonzalez-Bulnes et al., 2004a).

Variation in response to superovulation can be ascribed to several factors including oestrous synchronization treatment, the quality of gonadotrophin and the superovulation protocol used (Gonzalez-Bulnes et al., 2004a and Lehloenya and Greyling, 2010a). Currently, superovulation protocols are designed to reduce excessive handling of animals to minimise stress and avoid starting a superovulation treatment in the presence of the dominant follicle (Menchaca et al., 2002, Menchaca et al., 2007, Menchaca et al., 2010, Gibbons et al., 2007, Simonetti et al., 2008 and Lehloenya, 2013). In recent years, a Day 0 protocol first described by Menchaca et al. (2002) in which a superovulation treatment is applied coinciding with emergence of follicular wave, has been used. Although there is limited research concerning this protocol, promising results have been reported (Menchaca et al., 2007, Menchaca et al., 2009, Martemucci et al., 2008 and Tasdemir et al., 2011). Despite few publications of the beneficial effects of the Day 0 protocol in some aspects of the ovarian response, there is still not enough data to inspire its use not only in South Africa but across the globe. Like other countries, in South Africa the most commonly used superovulation protocol in goats is still the traditional FSH. Therefore, this study evaluated the efficiency of using the Day 0 protocol in Boer goats through comparison to the traditional FSH protocol.

2. Materials and methods

2.1. Experimental area

The experimental procedures for this study were approved by the Agricultural Research Council (ARC, APIEC 10/14) and Tshwane University of Technology (AREC 2010/07/003) ethics committees. The study was conducted during the spring season at Agricultural research council (ARC), Irene. The area is situated at 25° 53′ 59.6″ south latitude and 28° 12′ 51.6 east longitudes with altitude of 1525 m above sea level. The weather conditions range from hot days in summer (17.5 °C to 32 °C) to moderate winter days with very cold nights (1 °C to 17 °C).

2.2. Animals

All the experimental does grazed on natural pastures during the day and were supplemented with lucerne hay when kraaled. Water was provided *ad libitum* throughout the experimental period. A total of 20 Boer goats does used in this study were divided into two groups comprising of 10 animals per treatment group. The age of animals were estimated based on the number of permanent incisors and it ranged from 1 to 6 years. The body weight of does also varied from 35 to 62 kg. Therefore, the animals were allocated into two treatment groups

balanced according to age (2.20 ± 0.4 and 2.12 ± 0.5) parity (2.11 ± 0.4 and 1.98 ± 0.3) and body weight (43.20 ± 3.0 kg and 43.78 ± 3.3 kg) for Day 0 and traditional protocols, respectively.

2.3. Treatments

For synchronization of the emergence of follicular wave from the Day 0 protocol, controlled internal drug release dispensers (CIDR) containing 0.3 g progesterone (Pfizer,™ New Zealand Ltd) were inserted intravaginally for nine days. At CIDR insertion, does were injected with 160 µg/doe prostaglandin (PGF2α) analogue (Estrumate, Cloprostenol Sodium, Intervet Schering-Plough Animal Health, South Africa). The does were also injected with 300 IU of equine chorionic gonadotrophin (eCG) (Intervet Schering-Plough Animal Health, South Africa) at CIDR withdrawal. For superovulation the does were injected with 200 mg pFSH (Folltropin®-Vetrepharm, Bioniche, Canada) initiated 84 h following CIDR withdrawal. The pFSH treatment was administered in seven decreasing dosages, administered twice daily (the first dose being 50 mg and the rest being 25 mg) at 12 h intervals. Two doses of PGF2α were administered concurrently with the fifth and sixth pFSH treatment. At 24 h after the first PGF2α administration, does were injected with 8.4 µg per doe of GnRH (Receptal®VET, Buserelin acetate, MSD Animal Health, India). For the traditional FSH protocol, does oestrous cycles were synchronized with the aid of CIDR inserted intravaginally for a period of nine days. Then, the does were superovulated with pFSH initiated 48 h before CIDR withdrawal. The superovulation dosage was similar to the Day 0 protocol. All the injected hormones were administered intramuscularly.

2.4. Oestrous detection

Oestrous detection was performed at 12 h interval, using intact bucks wearing aprons. From the Day 0 protocol, the detection of oestrus was performed from CIDR withdrawal for a period of 96 h and thereafter from the second PGF2α injection. For the traditional FSH protocol, oestrous detection was initiated at CIDR withdrawal. From both protocols the oestrous detection following superovulation continued for a period of three days (72 h).

2.5. Artificial insemination (AI)

Fixed-time cervical inseminations with 0.1 mL ($200 - 300 \times 10^6$ sperm/mL) fresh undiluted Boer goat semen were performed at 24 and 36 h after the second PGF2α administration for the Day 0 protocol and 24 and 36 h after CIDR withdrawal for the traditional multiple FSH protocol. Semen used for AI was collected with an electro ejaculator from two Boer goat bucks of proven fertility. Ejaculates were assessed for wave motility under the microscope (×10 magnification) by examining a drop (5 µL) of semen on a warmed (35 °C) glass slide. Each sample was scored using a scale ranging from 0 (no wave movement) to 5 (extreme wave movement) (Evans and Maxwell, 1987 and Avdi et al., 2004). Only ejaculates with wave motility (≥ 3) were used for AI.

2.6. Ultrasonography evaluation

Transrectal ultrasonographic examinations of the ovaries were performed with the aid of an ultrasound scanner (Aloka 210, Tokyo, Japan), using a rectal probe with 7.5 MHz linear array transducer. Ultrasonographic measurements were taken at the beginning of the superovulation treatment for both groups. The diameter and number of follicles were

recorded from both ovaries. Follicles were classified as small (2–3 mm), medium (4–5 mm) or large (≥6 mm) (Gonzalez-Bulnes et al., 2004c).

2.7. Ovulatory evaluation and embryo yield

On day 5 after AI, all does were deprived of feed and water for 24 h. The embryos were surgically flushed on day 6 following the second AI, as described by Lehloenya et al. (2008). Before embryo flushing, ovaries were laparoscopically examined and the total number and quality of corpus lutea (CL) were recorded. The flush media recovered were scrutinized microscopically and evaluated under a stereomicroscope to identify and classify the structures (unfertilized ova and embryos) collected. The flushed structures were classified using the International Embryo Transfer Society criteria recommended by International Embryo Transfer Society (IETS) (1990) as unfertilized ova (no cleavage), degenerated and transferable embryos (grade 1, 2 and 3).

2.8. Statistical analyses

Data for the onset of oestrus, duration of the induced oestrous period, the total number of ovulations, total number of CL, total number of structures, unfertilized ova, embryos and transferable embryos recovered were analysed using the analysis of variance (ANOVA) procedure of (SAS, 2003). Categorical data pertaining to the oestrous response, fertilization rate and embryo viability was analysed using the chi-square test.

3. Results

The time from CIDR withdrawal to the onset of oestrus recorded for the Day 0 before superovulation treatment was 34.80 ± 4.2 h and the oestrous period lasted for 32.00 ± 2.8 h. One Boer goat from the traditional multiple FSH protocol was removed from the trial due to illness. The oestrous response following superovulation for the two protocols was 100%. The time to onset of oestrus (27.60 ± 4.2 h vs 33.11 ± 5.0 h) and duration of the induced oestrous period (26.67 ± 3.3 h vs 37.80 ± 4.5 h) did not differ significantly ($p > 0.05$) for the Day 0 and traditional protocols, respectively.

Table 1. Ovarian response and embryo yield of Boer goats following Day 0 or traditional superovulation protocols.

Parameters	Day 0 protocol (n = 10)	Traditional FSH protocol (n = 9)	p-value
No of corpora lutea/doe)	9.00±2.3	12.89±2.9	0.305
Total no of structures recovereda	6.50±2.1	8.00±2.2	0.634
Total no of embryo recovered/doe	5.70±2.2	5.5.44±2.3	0.937
Total no of unfertilized ova/doe	0.80±0.5	2.56±1.2	0.066
Fertilization rateb	78.67±12.0	51.94±15.2	0.055
Total no of degenerated embryos/doe	2.10±0.6	1.78±0.9	0.756
Total no of transferable embryos/doe	3.60±2.0	3.67±2.2	0.417
Viabilityc	44.10±13.6	38.44±16.7	0.793

[a]Total number of embryos and unfertilized ova.
[b]Total number of Embryos/total number of structures.
[c]Total number of transferable embryos/total number of structures.

The response to superovulation following the two superovulatory protocols is set out in Table 1. The mean total number of corpus lutea (CL), structures recovered, embryos, degenerated and transferable embryos, did not differ significantly ($p > 0.05$) between treatment groups. The number of unfertilized ova was numerically lower and the fertilization rate also tended to be higher in the Day 0 protocol than the traditional protocol, without significant difference. For these two parameters, the variation within treatment was greater in the traditional protocol.

The number and size of follicles at the initiation of a superovulation treatment did not differ significantly between treatment groups ($p > 0.05$). The values were 6.56 ± 2.4 vs 2.43 ± 2.4 mm, 0.8 ± 0.5 vs 1.89 ± 0.7 mm and 0.4 ± 0.2 vs 1.63 ± 0.7 mm for small, medium and large follicular sizes, respectively.

As there were no significance differences observed between the two treatment groups in relation to follicular activity, data was then pulled and correlated with the superovulation response parameters. The number of corpora lutea was positively ($p < 0.05$; $r = 0.7527$) correlated to the number of small follicles (2–3 mm) at the onset of the superovulation treatment. There was also a positive correlation between the total number of structures recovered with small follicles (2–3 mm) ($p < 0.05$; $r = 0.7523$), and the total number of follicles ($p < 0.05$; $r = 0.9524$) at the initiation of the superovulation treatment. There was also a positive correlation between medium follicles (4–5 mm) $p < 0.05$; $r = 0.5866$ and the total number of embryos recovered. Furthermore, the number of large follicles (≥6 mm) was positively ($p < 0.05$; $r = 0.9007$) correlated to the number of degenerated embryos at the initiation of a superovulation treatment.

4. Discussion

The short (9 days) progestagen treatment from the Day 0 protocol was efficient for synchronizing oestrus, as indicated by the 100% oestrous response. Besides being consistent, the short progestagen treatment is also reported to increase fertility in several goat breeds (Romano, 2004 and Fonseca et al., 2005a). The time interval from CIDR removal to the onset of oestrus recorded in the Day 0 protocol proceeding superovulation treatment, was closer to 40.2 ± 10.5 h reported by Romano (2004) after a 13-days progesterone priming treatment and 37.2 ± 0.7 h where the Day 0 protocol was used (Lehloenya and Greyling, 2010a). The time to onset of oestrus value that is more comparable to the current Day 0 protocol was obtained in Alpine goats following a nine days progesterone treatment (Fonseca et al., 2005b). The results emphasize the reliability of using CIDR for short period synchronization treatment. Furthermore, the duration of the induced oestrous period for this Day 0 protocol was also in line with 36.4 ± 0.5 h reported by Lehloenya and Greyling (2010a) following the Day 0 protocol and 39.2 ± 10.9 h reported by Romano (2004), where progesterone treatment was carried for 12 days.

The 100% oestrous response recorded from both protocols following superovulation was not a surprise as 85–100% oestrous response has been previously reported in other goat breeds using the traditional and Day 0 protocols (Menchaca et al., 2007, Tasdemir et al., 2011 and Sanchez-Davila et al., 2014). The time interval from CIDR withdrawal to the onset of oestrus and the duration of the induced oestrus for both protocols was in line with previous research in Alpine and Boer goats after using similar or long progestagen synchronization protocols (Baril and Vallet, 1990, Greyling et al., 2002 and Lehloenya and Greyling, 2010a). However, does in group 1 (Day 0 protocol) had a tendency to respond earlier to

synchronization with shorter duration of oestrus compared to does in the traditional FSH protocol, although not significantly different. This observation might be attributed to the ability of more follicles to produce oestrogen. Unfortunately, in this study the oestrogen concentration was not measured but the number of follicles were numerically (not significant) more for the Day 0, compared to the traditional FSH protocol at the beginning of the superovulation treatment. Therefore, more oestrogen concentration would be expected from many follicles.

Both protocols did not differ regarding the ovarian activity, embryo yield and quality, probably due to limited number of animals used. However, the number of unfertilized ova and fertilization rate tended to favour the Day 0 protocol. These results suggest that, the Day 0 protocol provides an environment for production of competent oocytes, which are more likely to be fertilised. In other studies, the Day 0 protocol produced more quality embryos (grade 1 & 2) compared to the traditional protocol, which emphasizes competency of oocytes (Menchaca et al., 2007). Nevertheless, there was poor superovulation response in terms of total CL, ova, embryos and transferable embryos recovered regardless of a superovulation protocol used, compared to previous studies in Boer goats (Lehloenya et al., 2008, Lehloenya and Greyling, 2009 and Lehloenya and Greyling, 2010a). Much of this observation may have been largely attributed to age effect, as in this study, more than halve of the experimental animals aged 1–2 years. In our previous studies it was observed that the superovulation response is lower in young does compared to the adult does (Lehloenya and Greyling, 2010b).

There were no significant differences between the two protocols. However the correlation results suggest that the response to superovulation treatment can be predicted based on the follicular status at the initiation of the superovulation treatment. Therefore, if the follicular status is not taken into consideration, the variability in response to superovulation will continue regardless of tremendous developments in superovulation protocols achieved. The number of corpora lutea improved with increase in the number of small follicles at the initiation of a superovulation treatment. Similar observation was reported in sheep (Gonzalez-Bulnes et al., 2002). This confirms the hypothesis that high number of small follicles coincides with emergence of follicular wave and absence of a dominant follicle. The latter is said to supress the response of other follicles to supplemented FSH therefore, leading to follicular atresia and reducing the number of follicles destined for ovulation hence reduced corpora lutea number (Menchaca et al., 2002, Menchaca et al., 2007, Menchaca et al., 2010 and Gonzalez-Bulnes et al., 2004a). The total number of embryos recovered also increased with the total number of medium follicles (4–5 mm). The association of medium follicles and embryo recovery is relevant, as these follicles have attained full competence and there are likely to be fertilised compared to the small follicles (Gonzalez-Bulnes et al., 2004b). It has been indicate in previous studies that the presence of a large follicle before superovulation leads to abnormality in preovulatory LH secretion and hence altering the final stages of oocyte maturation eventually, affecting the quality of embryos recovered (Lussier and Carruthers, 1989 and Greve et al., 1984). This was confirmed in the present study, where the higher the number of large follicles (≥6 mm) at initiation of superovulation was associated with the increase in the number of degenerated embryos.

There were no significant differences between the two protocols. However the correlation results suggest that the response to superovulation treatment can be predicted based on the follicular status at the initiation of the superovulation treatment. Therefore, if the follicular status is not taken into consideration, the variability in response to superovulation will

continue regardless of tremendous developments in superovulation protocols achieved. The number of corpora lutea improved with increase in the number of small follicles at the initiation of a superovulation treatment. Similar observation was reported in sheep (Gonzalez-Bulnes et al., 2002). This confirms the hypothesis that high number of small follicles coincides with emergence of follicular wave and absence of a dominant follicle. The latter is said to supress the response of other follicles to supplemented FSH therefore, leading to follicular atresia and reducing the number of follicles destined for ovulation hence reduced corpora lutea number (Menchaca et al., 2002, Menchaca et al., 2007, Menchaca et al., 2010 and Gonzalez-Bulnes et al., 2004a). The total number of embryos recovered also increased with the total number of medium follicles (4–5 mm). The association of medium follicles and embryo recovery is relevant, as these follicles have attained full competence and there are likely to be fertilised compared to the small follicles (Gonzalez-Bulnes et al., 2004b). It has been indicate in previous studies that the presence of a large follicle before superovulation leads to abnormality in preovulatory LH secretion and hence altering the final stages of oocyte maturation eventually, affecting the quality of embryos recovered (Lussier and Carruthers, 1989 and Greve et al., 1984). This was confirmed in the present study, where the higher the number of large follicles (≥6 mm) at initiation of superovulation was associated with the increase in the number of degenerated embryos.

5. Conclusion

The response to superovulation did not differ between the two protocols. However, the number of unfertilized ova and fertilization rate tended to favour the Day 0 protocol. It is therefore concluded that the Day 0 protocol could be used in Boer goats however, more studies with large number of animals are warranted to establish its benefits. The correlation results on the other hand, suggest that the size and number of follicles at the beginning of a superovulation treatment may play a major role in determining the ovarian response and quality of embryos recovered.

Conflict of interest

None.

Acknowledgements

Authors wish to thank the National Research Foundation and the Tshwane University of Technology for financial support of this research.

References

Avdi, M., Leboeuf, B., Terquic, M., 2004. Advanced breeding and “buck effect” in indigenous Greek goats. Livest. Prod. Sci 87, 251–257.

Baril, G., Vallet, J.C., 1990. Time of ovulations in dairy goats induced to superovulate with porcine follicle stimulating hormone during and out of the breeding season. Theriogenology 34, 303–311.

Christopher, D.L., 2002. Boer Goat Production: Progress and Perspective. Office of Vice Chancellor for Academic Affairs, University of Hawai’i, Hilo 96720. USA.

Cognie, Y., 1999. State of art in sheep-goat embryo transfer. Theriogenology 51, 105–116.

Evans, G., Maxwell, W.M.C., 1987. Salamon's Artificial Insemination of Sheep and Goats. Butterworths, Sydney.

Fonseca, J.F., Bruschi, J.H., Santos, I.C.C., Viana, J.H.M., Magalhaes, A.C.M., 2005a. Induction of estrus in non-lactating dairy goats with different estrous synchrony protocols. Anim. Reprod. Sci. 85, 117–124.

Fonseca, J.F., Torres, C.A.A., Costa, E.P., Maffili, V.V., Carvalho, G.R., Alve, N.G., Rubert, M.A., 2005b. Progesterone and reproductive performance of estrous-induced Alpine goats given hCG five days after breeding. Anim. Reprod. 2 (1), 54–59.

Gibbons, A., Bonnet, F.P., Cueto, M.I., Salamone, D.F., Gonzalez-Bulnes, A., 2007. Procedure for maximising oocyte harvest for in vitro embryo production in small ruminants. Reprod. Domest. Anim. 42, 423–426.

Gonzalez-Bulnes, A., Santiago-Moreno J.Cocero, M.J., Souza, C.J.H., Groome, N.P., Garcia-Garcia, R.M., Lopez-Sebastian, A., Baird, D.T., 2002. Measurement of inhibin A and follicular status predict the response to superovulatory FSH treatments. Theriogenology 57, 1263–1272.

Gonzalez-Bulnes, A., Baird, D.T., Campell, B.K., Corero, M.J., Garcia-Garcia, R.M., Santiago-Moreno, J., 2004a. Multiple factors affecting the efficiency of multiple ovulation and embryo transfer in sheep and goats. J. Anim. Sci. 16, 421–435.

Gonzalez-Bulnes, A., Garcia-Garcia, R.M., Carrizosa, J.A., Urrutia, B., Souza, C.J.H., Cocero, M.J., Lopez-Sebastian, A., McNeilly, A.S., 2004b. Plasma inhibin A determination at start superovulation FSH treatments is predictive for embryo outcome in goats. Domest. Anim. Endocrinol. 26, 259–266.

Gonzalez-Bulnes, A., Santiago-Moreno, J., Garcia-Garcia, R.M., Souza, C.J.H., Lopez-Sebastian, A., McNeilly, A.S., 2004c. Effect of GnRH antagonists treatment on gonadotropin secretion, follicular development and inhibin A secretion in goats. Theriogenology 61, 977–985.

Greve, T., Callesen, H., Hyttel, P., 1984. Plasma progesterone profiles and embryo quality in superovulated dairy cows. Theriogenology 21, 238 ([abstract].).

Greyling, J.P.C., van Der Nest, M., Schwalbach, L.M.J., Muller, T., 2002. Superovulation and embryo transfer in South African Boer and indigenous feral goats., Small Rumin. Res. 43, 45–51.

International Embryo Transfer Society (IETS), 1990. Manual of the International Embryo Transfer Society. In: Stringfellow, D.A., Seidel, S.M. (Eds.), 2nd ed. IETS, Champaign, Illinoins, USA.

Lehloenya, K.C., Greyling, J.P.C., 2009. Effect of route of superovulatory gonadotrophin administration on embryo recovery rate of Boer goat does. Small Rumin. Res. 87, 39–44.

Lehloenya, K.C., Greyling, J.P.C., 2010a. The ovarian response and embryo recovery rate in Boer goat does following different superovulation protocols, during the breeding season. Small Rumin. Res. 88, 38–48.

Lehloenya, K.C., Greyling, J.P.C., 2010b. The effect of embryo donor age and parity on the superovulatory response in Boer goat does. SA J. Anim. Sci. 40 (1), 65–69.

Lehloenya, K.C., Greyling, J.P.C., Grobler, S., 2008. Effect of season on the superovulatory response in Boer goats. Small Rumin. Res. 78, 74–79.

Lehloenya, K.C., 2013. Prelimenary results evaluating a simplified superovulation protocol in Boer goats. Small Rumin. Res. 113, 171–174.

Lussier, J.G., Carruthers, T.D., 1989. Endocrine and superovulatory response in heifers pretreated with FSH or bovine follicular fluid. Theriogenology 26, 209–219.

Martemucci, G., D'Alessandro, A.G.D., Paradiso, N., Terzano, M., Rufrano, D., Casamassima, D., 2008. Oestrus and ovarian response following superovulatory treatment soon after ovulation in dairy goats. In: Torres-Acosta, F. (Ed.), Proceedings of the 9th International Conference on Goats, Queretaro, Mexico. International Goat Association: Queretaro (Abstract).

Menchaca, A., Pinzak, A., Rubianes, E., 2002. Follicular recruitment and ovulatory response to FSH treatment initiated on Day 0 or Day 3 postovulation in goats. Theriogenology 58, 1713–1721.

Menchaca, A., Vilarino, M., Crispo, M., Pinzak, A., Rubianes, E., 2007. Day 0 protocol: superstimulatory treatment initiated in the absence of a large follicle improves ovarian response and embryo yield in goats. Theriogenology 68, 1111–1117.

Menchaca, A., Vilarino, M., Crispo, M., Kmaid, S., Saldana, J.M., 2009. Progesterone treatment, FSH plus eCG, GnRH administration, and Day 0 Protocol for MOET programs in sheep. Theriogenology 72, 477–483.

Menchaca, A., Vilarino, M., Crispo, M., de Casto, T., Rubianes, E., 2010. New approaches to superovulation and embryo transfer in small ruminants. Reprod. Fertil. Dev. 22, 113–118.

Romano, J.E., 2004. Synchronization of oestrus using CIDR: FGA or MAP intravaginal pessaries during the breeding season in Nubian goats. Small Rumin. Res. 55, 15–19.

SAS, 2003. SAS Institute Inc. Carry, NC 27513, USA.

Sanchez-Davila, F., Ledezma-Torres, R.A., Padill-Rivas, G., del Bosque-Gonzalez, A.S., Bernal-Barragan, H., 2014. Effect of three pFSH doses on superovulation and embryo quality in goats during two breeding seasons in North-eastern Mexico. Reprod. Domest. Anim. 49, e40–e43.

Simonetti, L., Forcada, F., Rivera, O.E., Carou, N., Alberio, R.H., Alberio, J.A., Palacin, I., 2008. Simplified superovulation treatments in carriedale ewes. Anim. Reprod. Sci. 104, 237–240.

Steele, M., Smith, J., 1996. The Tropical Agriculturalists: Goats. Macmillan Education, Chester, UK.

Tasdemir, U., Agaoglu, A.R., Kaymaz, M., Karakas, K., 2011. Ovarian response and embryo yield of Angora and Kilis goats given the Day 0 protocol for superovulation in the non-breeding season. Trop. Anim. Health Prod. 43, 1035–1038.