Tropical Animal Health and Production

Synchronizing Ethiopian Highland Sheep for Artificial Insemination: Improvement of Conception Rate With a Double Injection of Prostaglandin at 11 days

--Manuscript Draft--

Manuscript Number:	
Full Title:	Synchronizing Ethiopian Highland Sheep for Artificial Insemination: Improvement of Conception Rate With a Double Injection of Prostaglandin at 11 days
Article Type:	Regular Articles
Keywords:	Menz sheep; Artificial Insemination; Synchronization, Prostaglandins; Conception rate
Corresponding Author:	Mariem Rouatbi Institut National Agronomique de Tunis Ariana, TUNISIA
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	Institut National Agronomique de Tunis
Corresponding Author's Secondary Institution:	
First Author:	Shanbel Besufkad
First Author Secondary Information:	
Order of Authors:	Shanbel Besufkad
	Simret Betsha
	Chekol Demis
	Tesfaye Zewude
	Mariem Rouatbi
	Tesfaye Getachew
	Aynalem Haile
	Barbara Rischkowsky
	Mourad Rekik
Order of Authors Secondary Information:	
Funding Information:	International Fund for Agricultural Development (IFAD) (2000000764-ICARDA) — Not applicable
	CGIAR Research Program on Livestock (Not applicable) — Not applicable
Suggested Reviewers:	Maria Dattena, Dr AGRIS Sardegna (Agricultural Research Agency of Sardinia) mdattena@agrisricerca.it
Opposed Reviewers:	

Synchronizing Ethiopian Highland Sheep for Artificial Insemination: Improvement of Conception Rate With a Double Injection of Prostaglandin at 11 days

Shanbel Besufkad[1], Simret Betsha[2], Chekol Demis[1], Tesfaye Zewude[1], Mariem Rouatbi[3], Tesfaye Getachew[4], Aynalem Haile[4], Barbara Rischkowsky[4], Mourad Rekik[5]

[1]Debre Birhan Agricultural Research Center, P.O. Box 112, Debre Birhan, Ethiopia

[2]Hawassa University, P.O. Box 05, Hawassa, Ethiopia

[3]Institut National Agronomique de Tunisie, 43 Avenue Charles Nicolle, Tunis, Tunisia

[4]International Centre for Agricultural Research in the Dry Areas (ICARDA), P.O. Box 5689, Addis Ababa, Ethiopia

[5]International Centre for Agricultural Research in the Dry Areas (ICARDA), P.O. Box 11195, Amman, Jordan

Correspondence: Mariem Rouatbi, Institut National Agronomique de Tunisie, 43 Avenue Charles Nicolle, Tunis, Tunisia E-mail: rouatbi.myriam@yahoo.fr, orcid: https://orcid.org/0000-0003-0117-0266

Abstract

The study investigated, for artificially-inseminated (AI) Menz ewes, the reproductive performance of prostaglandin-based treatments simultaneously to the standard "P_4+eCG" protocol using progestogens priming with intravaginal devices and gonadotropin. A total of 483 non-pregnant and non-suckling Menz ewes were assigned to either the "P_4+eCG" protocol, the "PGF_s" treatment where sheep were synchronized with a single injection of prostaglandin or "PGF_7" and "PGF_{11}" where the sheep had their estrus and ovulation synchronized with a double injection of prostaglandin 7 or 11 days apart, respectively. The ewes were artificially inseminated with fresh semen at 55 ± 1 h after the end of the hormonal treatment. Conception rate (60.87±4.2) was highest for PGF_{11} ewes in comparison to sheep in all other treatment groups (P<0.05). The lowest conception rate (34.07±4.1) was recorded for the PGF_s group. Location, body condition score and parity did not affect variation in conception rate (P>0.05). A higher proportion of ewes in the P_4+eCG group yielded multiple births (2 and 3) compared to the 3 prostaglandin-based protocols (P<0.01). Nevertheless, a higher (P=0.02) proportion (17.11±4.3) of ewes treated with PGF_{11} protocol yielded twins by comparison to their counterparts in the PGF_7 protocol (2.50±2.5). It is concluded that use of a prostaglandin-based protocol composed of 2 injections 11 days apart, preceded by a careful selection of non-pregnant ewes for cervical fixed-time AI with fresh semen, is a feasible reproductive management option to support sheep breeding programs in Ethiopia.

Keywords: Menz sheep, Artificial Insemination, Synchronization, Prostaglandins, Conception rate

Introduction

Artificial insemination (AI) represents the universal process through which the products of the primary breeding activities are out and up-scaled. Community based breeding programs (CBBPs) for sheep and goats have been operating in Ethiopia for some time and genetic progress in growth traits and reproduction has been observed together with other socio-economic benefits (Haile et al., 2019). AI has been identified as one of several strategies to further increase genetic progress and dissemination relying on a more intense use of sires (Mueller et al., 2019). Fixed-time AI using fresh, cooled semen is the most widely used method in sheep. Such a method requires synchronization of the estrus and ovulations in recipient females which are inseminated without prior estrous detection. Compared to other domestic species, AI in sheep has several technical and organizational limitations which are enumerated by Palacin et al. (2012) and were reviewed by Santolaria et al. (2011). One important factor which may condition the insemination outcome in sheep is the synchronization treatment type and its duration (Baril et al., 1996; Maurel et al., 2003). Most of the treatments require the application of intravaginal devices containing synthetic progestogens for 12–14 days coupled with an intra muscular injection of eCG at the end of treatment. In spite of the advantages of such treatments in inducing high estrus response in and out of the breeding season, there are many drawbacks like disturbance in the luteinizing hormone (LH) secretion patterns (Leyva et al., 1998; Viñoles et al., 2001), altered follicular dynamics (Berlinguer et al., 2007; Vilariño et al., 2011), production of eCG antibodies (Hervé et al., 2004) and impaired sperm motility inside the female reproductive tract (Manes et al., 2016). Additionally, Gatti and Ungerfeld (2012) reported that intravaginal devices decrease sexual attractiveness in ewes due to alterations in the vaginal flora, thus, limiting the effectiveness of a teaser ram in detecting ewes in estrus. Furthermore, intravaginal hormone-releasing devices have a high cost and are not available under extensive conditions of many countries (Flores-Najera et al., 2010). In inter-tropical and equatorial Africa, seasonality of reproduction in sheep is not pronounced and recently, (Mekuriaw et al., 2015; Rekik et al., 2016) demonstrated that cheaper, easy-to-apply protocols based on the use of prostaglandin and GnRH analogues were effective in inducing satisfactory levels of estrous synchrony and reproductive performance after natural mating. To our knowledge, there are no published results on the effectiveness of sheep AI in Ethiopia and current study aimed, in the Ethiopian Highlands, at comparing the reproductive performance of different prostaglandin-based protocols after fixed-time AI of Menz ewes participating in the breed CBBP.

Materials and methods

Study area

The study was conducted in different privately-owned flocks (n=8) belonging to two regions, Debre Birhan and Menz areas and members of the breed CBBP. The climate of the two locations has a bi-modal rainfall pattern, where the main rainy season is from June to September. Debre Birhan is a woreda (administrative sub-division) in central Ethiopia, located in the Semien Shewa Zone of the Amhara Region. It is located about 120 kilometers north east of Addis Ababa at an elevation of 2,840 m. Menz area is located in North Shewa administrative zone of Amhara regional state at north east of Addis Ababa. It is the main breeding tract for Menz sheep and found in the subalpine and cold highland agro-ecological zones of Ethiopia (Gizaw et al., 2007). Mixed crop-livestock dominated by sheep-barley is the principal production system of the study area. Menz sheep is small in body size (the smallest of the 14 Ethiopian breeds described by Gizaw et al., 2007), is characterized by a very low twinning rate of usually less than 3% and is well adapted to the cool Ethiopian highlands, and tolerant to drought, seasonal variation in feed availability, and endo-parasite infection (Haile et al., 2002; Gizaw et al., 2008). This breed is mainly reared for income generation from the sale of lambs at market age although they are also important as source of coarse wool, food, manure and socio-cultural benefits (Gizaw, 2008; Getachew et al., 2010).

Experimental animals

In this trial, a total of 483 non-pregnant and non-suckling Menz ewes were selected. Prior to synchronization, non-pregnancy was verified using CBBP records and ultrasonography (B-Ultrasonic Diagnostic 23500/1000 Minitub GmbH; Tiefenbach, Germany). Ewe's parity ranged from 1 to 4 and body condition score (BCS) was assessed using the method of Russel et al. (1969). The experimental animals were housed in the night and allowed to graze during the day on natural pastures daily for 6-7 h. Starting 20 days before artificial insemination (AI) and the subsequent 2 weeks, all experimental animals were fed quality hay ad libitum and received in addition a mixed commercial concentrate (200 g head/day). They had free access to fresh water twice a day. The experimental animals were drenched against internal parasites (Albendazole 300 mg at 1 bolus/30 kg body weight, Ashish life Science Pvt. Ltd., India), and were vaccinated against Ovine Pasteurellosis, Peste des Petits Ruminants (PPR) and Sheep and Goat Pox (National Veterinary Institute, Debre Zeit, Ethiopia).

Experimental procedures

Ewes were assigned randomly to four groups synchronized with different hormonal protocols. Ewes belonging to the same flock were randomly assigned to only 2 protocols. The first group (P_4+eCG, n=121) received the standard protocol using intravaginal polyurethane sponges impregnated with 30 mg fluorogestone (Syncro-part®; CEVA laboratories, Libourne, France) that were inserted and left in the vagina for 14 days. All ewes were checked twice daily (morning and evening) to ensure that sponges remained in place during the treatment period. At sponge withdrawal, each ewe received an i.m. injection of 300 I.U. of equine chorionic gonadotropin (eCG) (Syncro-part PMSG®; CEVA laboratories, Libourne, France). Ewes in the second group (PGF_S, n =135) received a singular i.m. of 5 mg of the $PGF_{2\alpha}$ analogue dinoprost (1 ml Enzaprost®; CEVA Laboratories, Libourne, France). Ewes in the two other groups (PGF_7 and PGF_{11}, n=89 and n=138, respectively) received each 2 i.m. of 5 mg of the $PGF_{2\alpha}$ analogue dinoprost (1 ml Enzaprost®; CEVA laboratories, Libourne, France) administered 7 and 11 days apart. The schedule of implementation of the 4 protocols was arranged so that fixed-time AI is carried out the same day for all sheep (Fig. 1).

Artificial insemination

Semen from 8 adult Menz rams (selected on the basis of their estimated breeding value) (4 per location), checked for normal breeding soundness, was collected using an artificial vagina. Only ejaculates presenting a minimum concentration of 2.5 x 10^9 sperm per ml, determined by spectrophotometry (Accucell®, Instruments de Médecine Vétérinaire, IMV Paris, France) and an individual motility of at least 3.5 (determined on a scale of 5) using a contrast-phase microscope were selected for use. Immediately after collection, the semen was diluted with a commercial sheep-specific diluent (OVIXcell®, Instruments de Médecine Vétérinaire, IMV Paris, France) and ewes were inseminated cervically with 0.2 ml of diluted semen containing in average 400 x 10^6 sperm. Cervical artificial insemination (AI) was performed by two well-trained technicians using a speculum equipped with a light source and an insemination gun (Instruments de Médecine Vétérinaire, IMV Paris, France) as described by Evans and Maxwell (1987). Ewes in all treatment groups were inseminated at 55 ± 1 h after the end of the hormonal treatment (Fierro et al., 2017).

Data collection and statistical analyses

Conception rate (CR) was attributed the value of 1 if lambing date ∈ [145, 155 days after AI] and 0 if the ewe did not lamb or the lambing date >155. When lambing occurred, the number of lambs born was also recorded. For statistical purposes, LS was considered as a binomial variable: 0 for single-born lambs and 1 for multiple-born lambs (2 and 3 in the particular case of this study). For each protocol, lambing rate (LR) was calculated according to the formula:

Lambing rate*=100 x [(number of lambs born alive)/(number of inseminated ewes)]*.

Since CR and LS follow a binomial distribution, an analysis of variance for categorical data were performed using the CR or LS as fixed effect (measured by 0 or 1). Therefore, a general linear model (GLM) procedures using the logit model of R-Studio® open source, with R version 3.4.3 (2017-11-30), was performed. The following model was used: $Fi_{jklm} = u + Loc_i + BCS_j + Prot_k + P_l + e_{ijklm}$,

where F_{ijklm} is the success of the insemination (CR or LS = 0/1); u = Overall mean; Loc_i is the location, i = Menz area or Debre Birhan; BCS_j is the body condition score, j = ≤2,]2, 3[or [3, 4]; $Prot_k$ is the used hormonal protocol of oestrus synchronization, k = P_4+ECG, PGF_S, PGF_7 or PGF_{11}; P_l is the parity of the ewe, l = from 1 to 4; e_{ijklm} is the residual error. For factors showing significant effect, a chi-square test was used to compare different classes (biostat TGV[1]). ANOVA (IBM SPSS Statistics® ver. 23.0) was conducted to compare the effect of different hormonal synchronization protocols on LR.

Results

Conception rate to AI

For the 483 ewes included in the experiment, 225 ewes conceived in response to AI giving an overall CR of 46.58±2.3. Only the protocol affected significantly the CR and the highest value was recorded for PGF_{11} (60.87±4.2). The two by two chi square comparison showed that ewes treated with PGF_{11} protocol had significant higher CR than animals synchronized with P_4+eCG, PGF_S and PGF_7 ($p<0.05$). CR tended to be higher for animals receiving the P_4+eCG treatment compared to those with only one injection of prostaglandin (P=0.06) (Table 1).

Litter size

For the 225 ewes which conceived, the data for litter size was only available for 217 ewes. For the remaining 8 ewes which conceived to AI, data on the number of lambs born was not recorded. The average number of lambs at lambing was 1.19±0.44 and the frequencies of ewes bearing single, double and triple LS were 82.95±2.6, 15.21±2.4 and 1.84±0.9, respectively. Statistical analysis showed that the proportion of ewes having multiple LS tended to be higher in Debre Birhan area (22.68±4.3 *vs* 12.50±3.0). The effect of the synchronization protocol on the proportion of animals bearing multiple litters just reached significance (p=0.05) (Table 1). The two by two chi square comparison showed that more ewes in P_4+eCG yielded multiple litters (36.36±6.5; $P<0.01$) when compared to the 3 other protocols. Percentages of animals having multiple LS was significantly higher when treated with PGF_{11} protocol (17.11±4.3) than PGF_7 ewes (P=0.02) and tended to be higher than for sheep in PGF_S treatment (P=0.09) (2.50±2.5 and 6.52±3.6, respectively).

Lambing rate

The overall LR following oestrous synchronization and artificial insemination was 53.42%. ANOVA showed that synchronization protocols had a significant effect on lambing rate and the highest percentages were recorded for P_4+eCG and PGF_{11} protocols (65.29 and 64.49, respectively) (Table 2).

[1] https://biostatgv.sentiweb.fr/?module=tests/chideux

Discussion

The longest interval between prostaglandin injections (11 days) improved conception rate at fixed-time AI compared to the 7-days interval or the single injection. The resulting conception rate is also higher than levels obtained with the standard method associating exogenous progestogens and eCG. It was not an aim of this study to test efficiency of the P_4+eCG protocol but it was included as a positive control in view of the universality of such protocol in sheep AI programs. In any case, the information obtained in the current study on the reproductive response of ewes supports previous reports on the drawbacks which may be associated to the use of progestogen-based protocols to control reproduction in sheep (Lopez-Sebastian et al., 2007; De Paula Vasconcelos et al., 2016). Using Menz ewes synchronized with P_4+eCG and despite a 100% oestrous response at the induced ovulation and a normal pattern of luteal function as assessed by progesterone secretion, Rekik et al. (2016) recorded a lambing rate after mating at the induced estrus slightly over 50%, significantly lower than for ewes synchronized with 2 injections of a prostaglandin-analogue.

Beyond the practical significance of this result in terms of validating a workable synchronization protocol for sheep AI in Ethiopia using prostaglandin analogues, some features of the reproductive performance in terms of both conception rate and litter size are important to discuss.

The estrous response is the physiological response to injections of the prostaglandin-analogue at different moments during the luteal phase in randomly cycling ewes. When longer intervals between prostaglandin injections are applied, this results in longer periods of higher progesterone levels prior to mating, higher oestradiol plasma levels around the onset of estrus (Fierro et al., 2016) and a higher fertilization rate (Fairnie et al., 1977). This has been strongly evidenced in the recent work of Fierro et al. (2017) who concluded that long-term prostaglandin-based protocols improve the reproductive performance after timed artificial insemination in sheep. They indeed demonstrated that 12, 14- or 16-days interval between prostaglandin injections enhances the pregnancy rate of ewes at cervical timed artificial insemination with fresh semen. When short intervals (7 days in this study) are used in artificial insemination, there is an altered progesterone profile during the luteal period preceding luteolysis and this affects the overall outcome negatively (Fierro et al., 2017). As anticipated, the least conception rate was obtained with the single injection of $PGF_{2\alpha}$. With only one single injection, ewes which are initiating their luteal phase (days 1-4) and those which have already initiated luteolysis are not responsive to the treatment and are not synchronized (Pope and Cárdenas, 2004). Our results are consistent with those reported by Mekuriaw et al. (2015) who obtained a lambing rate (ewes lambing/ewes treated) varying between 25 and 55% only when Menz ewes are naturally mated after being synchronized with only one single injection of different analogues and doses of prostaglandin. There is a very high variability in the response of ewes when synchronized with only one single injection of prostaglandin which may be a major limitation to the purpose for which AI is used as a delivery method of improved genetics to the largest possible proportion of females in the flocks.

The variation factors that were captured in this study, namely location, body condition score and parity did not affect the results of conception rate. The previous number of parturitions was found to be an important factor impacting fertility on AI in sheep (Palacin et al., 2012); likelihood of pregnancy decreased in ewes with more than five previous parturitions (by a factor of 0.87, 0.79 and 0.66 for the 6th, 7th and ≥8 parturitions, respectively). In the case of our study, the ewes had between 1 and 4 previous parturitions when fertility of the female sheep is at its peak. Although not statistically significant, conception rate was lower for ewes with a BCS less than 2. The relationship between BCS and the outcome of reproduction is very well documented and our results are very similar to those by (Bru, et al., 1995) for Rasa Aragonesa ewes who found that the lowest pregnancy rates (32.7 %) were obtained in sheep with a BCS<2, the average values (48.3%) with BCS between 3 and 2 and the higher values (58.8%) when BCS was >3.

Menz sheep is a non-prolific breed and average figures of litter size are very often inferior to 1.1. The proportion of ewes bearing multiple litters (2 and 3) in the P_4+eCG treatment group was expected because of the effect of eCG, even at lower doses, in increasing ovulation rate. Which was not expected is the increase of the proportion of ewes yielding twins in the PGF_{11} group (13/76) corresponding to an average litter size of 1.17. Such an increase was reflected in ewes bearing twins only and no triplet births were recorded. Although, Menz is a breed which thrives in a very harsh environment of the Ethiopian Highlands, the observed increase in litter size in the PGF_{11} group cannot be considered as an undesirable increase of prolificacy and the extra lambs born can be easily managed by farmers. Physiologically, the effect of prostaglandin analogues on ovulation rate and prolificacy is controversial, with reports showing no change, a decrease, and an increase in ovulation rate (Fierro et al., 2013). Combining litter size and the number of treated ewes under what we designated as lambing rate, puts the P_4+eCGand the PGF_{11} treatment groups in a similar rank significantly exceeding the reproductive outcome in both PGF_s and PGF_7 treatments.

In summary, the findings of this study indicate that the use of a prostaglandin-based protocol composed of 2 injections 11 days apart, preceded by a careful selection of non-pregnant ewes (BCS > 2) using ultrasonography for cervical fixed-time AI with fresh semen, is a feasible reproductive management option for the widespread of genetic gain in the framework of CBBPs in Ethiopia.

Acknowledgement

The authors acknowledge the contribution of the staff at Debre Birhan (Amhara Regional Agricultural Research Institute) for their technical support. They are also indebted to the engagement of the Menz CBBP farmers for contributing with their sheep and labor to the experimental work. This study was financially supported by the CGIAR Research Program on Livestock and the International Fund for Agricultural Development (IFAD)-funded project "Improving the Performance of Pro-Poor Sheep and Goat Value Chains for Enhanced Livelihoods, Food and Nutrition Security in Ethiopia (SmaRT-Ethiopia)" -IFAD Grant 2000000764-ICARDA

Compliance with ethical standards

The manuscript does not contain clinical studies or patient data. There are no ethical concerns to be reported and animals were handled in the presence of their owners by adhering to local animal practices and handling rules.

Conflict of interest

The authors declare no conflict of interest that would prejudice the impartiality of this scientific work.

References

Baril, G., Remy, B., Leboeuf, B., Beckers, J.F. and Saumande, J., 1996. Synchronization of estrus in goats: the relationship between eCG binding in plasma time of occurrence of estrus and fertility following artificial insemination, Theriogenology, 45, 1553–1559

Berlinguer, F., Gonzalez-Bulnes, A., Succu, S., Leoni, G., Mossa, F., Bebbere, D., Ariznavarreta, C., Treguerres, J.A.F., Veiga-Lopez, A. and Naitana, S. 2007. Effects of progestagens on follicular growth and oocyte developmental competence in FSH-treated ewes, Domestic Animal Endocrinology, 32, 303–314

Bru, C., Fantova, E., Sevilla, E., Quintin, F.J. and Alabart, J.L., 1995. Resultados de insemiancion artificial de las ovejas Rasa aragonea de las ganaderías de Carne Aragón, S.C.L. Influencia de la condición corporal, Proceedings of: XX Jornadas Científicas de la Sociedad Española de Ovinotecnia y Caprinotecnia., Madrid (España)

De Paula Vasconcelos, C.O., Zandonadi Brandao, F., Martins, G., Penna, B., Concalves de Souza-Fabjan, J.M. and Lilenbaum, W., 2016. Qualitative and quantitative analysis of bacteria from vaginitis associated with intravaginal implants in ewes following estrus synchronization, Ciência Rural, *46*, 632–636

Evans, G. and Maxwell, W.M.C., 1987. Insemination. In: Salamon's artificial insemination of sheep and goats. Editorial Butterworths :142e53.

Fairnie, I.J., Wales, R.G. and Gherardi, P.B., 1977. Time of ovulation, fertilisation rate, and blastocyst formation in ewes following treatment with a prostaglandin analogue (ICI 80996), Theriogenology, 8, 183

Fierro, S., Gil, J., Viñoles, C. and Olivera-Muzante, J., 2013. The use of prostaglandins in controlling estrous cycle of the ewe: a review, Theriogenology, 79, 399-408

Fierro, S., Viñoles, C. and Olivera-Muzante, J., 2016. Concentrations of steroid hormones, estrous, ovarian and reproductive responses in sheep estrous synchronized with different prostaglandin-based protocols, Animal Reproduction Science, 167, 74-82

Flores-Najera, M.J., Meza-Herrera, C.A., Echavarrıa, F.G., Villagomez, E., Iniguez, L., Salinas, H. and Gonzalez-Bulnes, A., 2010. Influence of nutritional and socio-sexual cues upon reproductive efficiency of goats exposed to the male effect under extensive conditions, Animal Production Science, 50, 897–901

Gatti, M. and Ungerfeld, R., 2012. Intravaginal sponges to synchronize estrus decrease sexual attractiveness in ewes, Theriogenology, 78, 1796–1799

Getachew, T., Haile, A., Tibbo, M., Sharma, A.K., Sölkner, J. and Wurzinger, M., 2010. Herd management and breeding practices of sheep owners in a mixed crop-ivestock and a pastoral system of Ethiopia, African Journal of Agricultural Research, 5, 685–691

Gizaw, S., Van Arendonk, J.A.M., Komen, H., Windig, J.J. and Hanotte, O., 2007. Population structure, genetic variation and morphological diversity in indigenous sheep of Ethiopia, Animal Genetics, 38, 621–8

Gizaw, S., Komen, H., Hanotte, O. and Van Arendonk, J.A.M., 2008. Indigenous sheep resources of Ethiopia: types, production systems, Animal Genetic Resources Information, 43, 25–39

Haile, A., Tembely, S., Anindo, D., Mukasa-Mugerwa, E., Rege, J.E., Yami, A. and Baker, R.L., 2002. Effects of breed and dietary protein supplementation on the responses to gastrointestinal nematode infections in Ethiopian sheep, Small Ruminant Research, 44, 247–61

Haile, A., Gizaw, S., Getachew, T., Mueller, J. P., Amer, P., Rekik, M. and Rischkowsky, B., 2019. Community-based breeding programmes are a viable solution for Ethiopian small ruminant genetic improvement but require public and private investments, Journal of Animal Breeding and Genetics, 136, 319-328

Hervé, V., Roy, F., Bertin, J., Guillou, F. and Maurel, M.C., 2004. Anti-equine chorionic gonadotropin (eCG) antibodies generated in goats treated with eCG for the induction of ovulation modulate the luteinizing hormone and follicle-stimulating hormone bioactivities of eCG differently, Endocrinology, 145, 294–303

Leyva, V., Buckrell, B.C. and Walton, J.S., 1998. Regulation of follicular activity and ovulation in ewes by exogenous progestogen, Theriogenology, 58, 395–416

Lopez-Sebastian, A., Gonzalez-Bulnes, A., Carrizosa, J.A., Urrutia, B., Diaz-Delfa, C., Santiago-Moreno, J. and Gomez-Brunet, A., 2007. New estrus synchronization and artificial insemination protocol for goats based on male exposure, progesterone and cloprostenol during the non-breeding season Theriogenology, *68*, 1081–1087

Manes, J., Rios, G., Fiorentino, M.A. and Ungerfel, R., 2016. Vaginal mucus from ewes treated with progestogen sponges affects quality of ram spermatozoa, Theriogenology, 85, 856–861

Maurel, M.C., Roy, F., Hervé, V., Bertin, J., Vaiman, D., Cribiu, E., Manfredi, E., Bouvier, F., Lantier, I., Boue, P. and Guillou, F., 2003. Réponse immunitaire à la eCG utilisée dans le traitement de l'induction d'ovulation chez la chèvre et la brebis, Gynécologie Obstétrique & Fertilité, 31, 766–769

Mekuriaw, Z., Assefa, H., Tegegne, A. and Muluneh, D., 2015. Estrus response and fertility of Menz and crossbred ewes to single prostaglandin injection protocol, Tropical Animal Health and Production, 48, 53-7

Mueller, J.P., Haile, A., Getachew, T., Rekik, M. and Rischkowsky, B., 2019. Genetic progress and economic benefit of community-based breeding programs for sheep out- and upscaling options in Ethiopia, Small Ruminant Research, 177, 124-132

Palacín, I., Yániz, J.L., Fantova, E., Blasco, M.E., Quintín-Casorrán, F.J., Sevilla-Mur, E. and Santolaria, P., 2012. Factors affecting fertility after cervical insemination with cooled semen in meat sheep, Anim Reprod Sci, 132, 139-44

Pope, W.F. and Cárdenas, H., 2004. Sensitivity of sheep to exogenous prostaglandin F2α early in the estrous cycle, Small Ruminant Research, 55, 245–248

Rekik, M., Haile, A., Abebe, A., Muluneh, D., Goshme, S., Ben Salem, I., Hilali, M.E., Lassoued, N., Chanyalew, Y. and Rischkowsky, B., 2016. GnRH and prostaglandin-based synchronization protocols as alternatives to progestogen-based treatments in sheep, Reproduction in Domestic Animals, 51, 924-929

Russel, A.J.F., Doney, J.M. and Jun, R.G. 1969. Subjective assessment of body fat in live sheep, The Journal of Agricultural Science, 72, 451-454

Santolaria, P., Palacin, I. and Yaniz, J., 2011. Management Factors Affecting Fertility in Sheep, Artificial Insemination in Farm Animals, Dr. Milad Manafi (Ed.), ISBN: 978-953-307-312-5, InTech, Available from: http://www.intechopen.com/books/artificial-insemination-in-farm-animals/management-factors-affectingfertility-in-sheep

Vilariño, M., Rubianes, E. and Menchaca, A., 2011. Re-use of intravaginal progesterone devices associated with the short-term protocol for timed artificial insemination in goats, Theriogenology, 75, 1195–1200

Viñoles, C., Forsberg, M., Banchero, G. and Rubianes, E., 2011. Effect of long-term and short-term progestagen treatment on follicular development and pregnancy rate in cyclic ewes, Theriogenology, 55, 993-1004

Table 1 Differences in conception rate (CR) and litter size (LS) according to the different parameters

		Conception rate		***Litter size***	
Parameter		*Ewes conceiving/Total (%±SD)*	*P value*	*Number of animals having multiple LS/Ewes conceiving (%±SD)*	*P value*
Location	Menz	125/260 (48.08±3.1)	0.511	15/120 (12.50±3.0)	**0.096**^{t}
	Debre Birhan	100/223 (44.84±3.3)		22/97 (22.68±4.3)	
BCS	≤2	22/69 (31.88±5.6)	0.487	5/22 (22.73±8.9)	0.515
	]2, 3[	116/227 (51.10±3.3)		20/111 (18.02±3.6)	
	[3, 4]	87/187 (46.52±3.6)		12/84 (14.29±3.8)	
Protocol	P_4+eCG	55/121 (45.45±4.5)^{a}	**0.004****	20/55 (36.36±6.5)^{a}	**0.050***
	PGF_S	46/135 (34.07±4.1)^{a}		3/46 (6.52±3.6)^{cb}	
	PGF_7	40/89 (44.94±5.3)^{a}		1/40 (2.50±2.5)^{b}	
	PGF_{11}	84/138 (60.87±4.2)^{b}		13/76 (17.11±4.3)^{dc}	
Parity	1	99/207 (47.83±3.5)	0.621	14/92 (15.22±3.7)	0.819
	2	48/108 (44.44±4.8)		12/47 (25.53±6.4)	
	3	42/89 (47.19±5.3)		7/42 (16.67±5.8)	
	4	35/76 (46.05±5.7)		3/35 (8.57±4.7)	
Overall		225/483 (46.58±2.3)		37/217 (17.05±2.6)	

CR: Conception rate
LS: Litter Size
SD: Standard Deviation
P: probability
*: Significant ($0.001 \le p \le 0.05$)
**: Highly significant ($p<0.001$)
^{t}: Tendency
Values with different superscripts (a, b, c and d) in the same column are significantly different ($P < 0.05$).

Table 2 Effect of synchronization protocols on lambing rate in Menz sheep

Parameter		***LR (%)***	***P value***
Protocol	P_4+eCG	79/121 (65.29)^{a}	**<0.001****
	PGF_S	49/135 (36.30)^{b}	
	PGF_7	41/89 (46.07)^{b}	
	PGF_{11}	89/138 (64.49)^{a}	
Overall		258/483 (53.42)	

LR: Lambing Rate

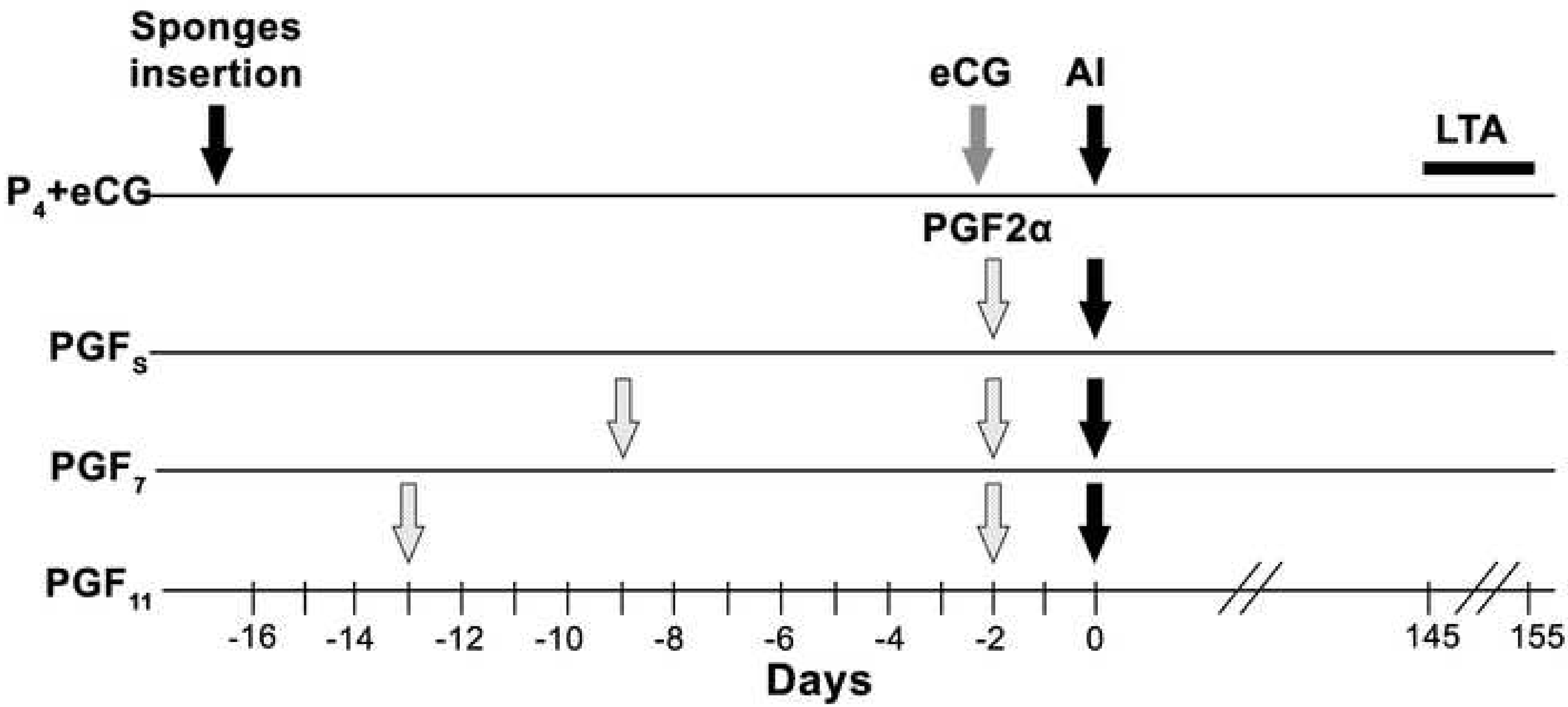
P: probability

**: Highly significant (p<0.001)

Values with different superscripts (a and b) in the same column are significantly different ($P < 0.05$).

Figure caption

Fig. 1 Schematic representation of the experimental design. P_4+ECG group: ewes synchronized with the standard protocol using intravaginal polyurethane sponges and 1 injection of ECG. PGF_S group: ewes synchronized with 1 injection of. PGF_7 and PGF_{11} groups: ewes synchronized with two injections of dinoprost given 7 and 11 days apart respectively, starting 13 days before the timed artificial insemination (Day 0). AI: day of artificial insemination. LTA: Lambing to AI between 145 and 155 days.


Sponges insertion
eCG
AI
LTA
P_4+eCG
PGF2α
PGF_S
PGF_7
PGF_{11}
-16
-14
-12
-10
-8
-6
-4
-2
0
145
155
Days