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USE OF CIDR AND ESTRADIOL CYPIONATE IN SYNCHRONIZATION PROTOCOLS ON ESTRUS PRESENTATION, PREGNANCY AND BIRTH RATE IN CREOLE SHEEP UNDER HIGH ALTITUDE CONDITIONS

Uso del CIDR y cipionato de estradiol en protocolos de sincronización sobre la presentación de celo, tasa de preñez y natalidad en borregas criollas bajo condiciones de altura

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ABSTRACT

The objective of this research was to evaluate the use of the intravaginal device (CIDR) and estradiol cypionate in synchronization protocols, on oestrus presentation, pregnancy and birth rate in Creole sheep under high altitude conditions. The study was carried out in a non-reproductive season, in the peruvian highlands, using 57 ewes. Four experimental groups were formed: group T1 (n=14) and T3 (n=14), CIDR progesterone device for 7 days and 12 days, respectively; group T2 (n=15) and T4 (n=14), CIDR progesterone device for 7 days and 12 days plus 1 mg of estradiol cypionate, 24 hours after removal of the device in both groups, respectively. Estrous presentation was observed from CIDR removal to 48 hours using vasectomized rams and IATF was performed with fresh semen 48 hours after device removal. The pregnancy rate was determined by ultrasonography at 46 and 90 days after FTAI and the birth rate was recorded. The chi-square test was used for statistical analysis. There was no difference (P>0.05) between groups, for estrous presentation, but there was difference (P<0.05) for pregnancy rate at 46 days between groups with: T1 (42.9%), T3 (38.5%), T4 (21.4%), with respect to T2 (0%) (The difference in results are shown in Table 2). There was a difference (P<0.05) for the pregnancy rate at 90 days: T1 (35.7%) and T3 (30.8%), with respect to T4 (7.1%) and T2 (0 %). The birth rate showed differences (P<0.05) for T1 (28.6%) and T3 (28.6%), with respect to T2 (0%) and T4 (0%). In conclusion, a high pregnancy rate was obtained with CIDR for 7 and 12 days, compared to the use of CIDR plus estradiol cypionate. However, no births were obtained with progesterone plus estradiol cypionate treatment.

Keywords: Ewe lambs, birth rate, pregnancy, fixed-time insemination

RESUMEN

El objetivo de esta investigación fue evaluar el uso del dispositivo intravaginal (CIDR) y cipionato de estradiol en protocolos de sincronización, sobre la presentación de celo, tasa de preñez y natalidad en borregas criollas bajo condiciones de altura. Se realizó en estación no reproductiva, en el altiplano peruano, se utilizaron 57 borregas. Se formaron cuatro grupos experimentales: grupo T1 (n=14) y T3 (n=14), dispositivo de progesterona CIDR por 7 días y 12 días, respectivamente; grupo T2 (n=15) y T4 (n=14), dispositivo de progesterona CIDR por 7 días y 12 días más 1 mg de cipionato de estradiol, 24 horas después del retiro del dispositivo en ambos grupos, respectivamente. Se observó la presentación de celo, desde el retiro del CIDR hasta las 48 horas mediante carneros vasectomizados y se realizó IATF con semen fresco 48 horas después del retiro del dispositivo. Se determinó la tasa de preñez mediante ultrasonografía a los 46 y 90 días después de IATF y se registró la tasa de natalidad. Para el análisis estadístico se utilizó la prueba de chi cuadrada. No existió diferencias (P>0.05) entre grupos, para la presentación de celo, pero hubo diferencia (P<0.05) para la tasa de preñez a los 46 días entre grupos con: T1 (42.9%), T3 (38.5%), T4 (21.4%), con respecto a T2 (0%) (La diferencia de resultados se muestran en el cuadro 2). Existió diferencia (P<0.05) para la tasa de preñez a los 90 días: T1 (35.7%) y T3 (30.8%), con respecto a T4 (7.1%) y T2 (0 %). La tasa de natalidad mostró diferencias (P<0.05) para T1 (28.6%) y T3 (28.6%), con respecto a T2 (0%) y T4 (0%). En conclusión, se obtuvo una alta tasa de preñez con CIDR por 7 y 12 días, comparado al uso de CIDR más cipionato de estradiol. Sin embargo, no se obtuvo ningún nacimiento con el tratamiento de progesterona más cipionato de estradiol.

Palabras clave: borregas, tasa de nacimiento, preñez, inseminación a tiempo fijo.

INTRODUCTION

Sheep production is a traditional activity in southern Peru and gives rise to four main products: meat, wool/hair, milk and skins, meat is the main product and the importance of its production is increasing, although the number of sheep is decreasing generating its scarcity (Morris, 2009; Ahmad et al., 2020). On the other hand, sheep have seasonal reproduction (Smith, 2012; Weems et al., 2015) at times of the year when it is advantageous for offspring survival and growth (Weems et al., 2015; Abecia et al., 2019), with annual periods of reproductive inactivity in response to the increased photoperiod in late winter through spring (Smith, 2012).

Sheep are seasonal polyestrous and in the non-reproductive season (anestrus), pulsatile GnRH secretion from the brain is reduced, due to increased estrogen negative feedback activity (Smith, 2012; Ahmad et al., 2020) photoreceptors in the eye are involved in photoperiod perception and nocturnal secretion of melatonin, which provides an endocrine signal (Yoshimura, 2013) and at the end of anestrus, the main mechanism responsible for seasonal reproduction is the increased responsiveness of neurons to gonadotropin-releasing hormone (Weems et al., 2015; Ando et al., 2018). However, there are breeds such as Santa Inés and native sheep from southeastern Peru and highlands of Colombia, which have generally low seasonality (Bravo, 1986; De Carvalho Menezes de Almeida et al., 2018; Lozano et al., 2020).

Creole bighorn sheep in southern Peru reach puberty earlier and with lower weight (Bravo, 1986), also as in other hair breeds, a high proportion of ewes are able to ovulate throughout the year (Bravo, 1986; Arroyo et al., 2016) and with lower requirement to the use of eCG, a hormone that has a significant cost for sheep breeders.

To manage the estrous cycle of the ewe, synchronization protocols based on intravaginal progesterone or progestogen release devices combined with cloprostenol and equine chorionic gonadotropin (eCG) are used (Carlson et al., 1989; Abecia et al., 2012, Wildeus, 2000). And in Peru, estrus synchronization protocols with progestogens and eCG are used, both in reproductive season (Caballa, 2019) and in anestrus (Verdoljak et al., 2017). In addition, it is possible to use this protocol in ewes with temporary weaning and fixed-time artificial insemination (Molina et al., 2020). However, because eCG, is a heavily glycoprotein hormone glycosylated, its administration leads to humoral immune responses, resulting in lower fertility rates, as observed in goats inseminated at a fixed time, after repeated treatment (Hervé et al., 2004).

On the other hand, progesterone-impregnated controlled internal drug release devices (CIDR) are approved to synchronize and induce fertile estrus in ewes (Knights and Singh-Knights, 2016), the duration of the device is 12 to 14 days (Evans et al., 2001; De et al., 2015; Hasani et al., 2018) or for 5, 6 or 7 days (Viñoles et al., 2001; Letelier et al., 2009; Menchaca et al., 2018; Martinez-Ros et al., 2018). The CIDR device, can be associated with the use of estradiol salts such as estradiol cypionate to induce estrus in goats (Maffili et al., 2005a) and sheep (Gómez et al., 2021). In addition, the combination of the CIDR device for five days and 100 ug of estradiol benzoate at withdrawal, allow obtaining an ovulatory follicle with the possibility of supplanting traditional eCG and implementing an FTAI (Menchaca and Rubianes, 2007; Verano, 2020), with a lower cost (Verano, 2020).
Therefore, our objective was to evaluate reproductive efficiency, using different intravaginal dwell times of the internal controlled release device (CIDR) with or without the administration of estradiol cypionate, in Creole sheepherds under high altitude conditions.

MATERIAL AND METHODS

Place of study

The study was carried out at the Chuquibambilla Experimental Center, Faculty of Veterinary Medicine and Animal Husbandry, belonging to the National University of the Altiplano, located in the district of Umachiri, province of Melgar, region of Puno, located at 13°47'37" south latitude and 70°47'50" west longitude and at an altitude of 3974 meters above sea level. It is characterized by a cold temperate climate, the area has a maximum temperature of 20.4°C in December and a minimum temperature of -18.4°C in June, with an annual average of 8°C, the average annual relative humidity is 53% (maximum 81%, minimum 18%); presenting an average annual rainfall of 659 mm.

Animals and experimental design

From a herd of 600 animals, 57 Criollo ewe lambs of three years of age were selected, with an average weight of 38.5 ± 3.5 kg and a body condition of 3.1 ± 0.2. The ewe lambs were identified with earrings and painted and were exposed to the same management, health and feeding conditions in natural pastures.

Two Creole breed rams with proven fertility were used, which were fed on oat hay and natural pastures. This work was carried out outside the reproductive season (November to March), at the beginning of the rainy season and was completed at the beginning of the dry season. To synchronize the estrus of the ewe lambs, intravaginal devices containing 0.3 g of progesterone (CIDR®) (Controlled Internal Drug Release, EAZI-BREED, Pfizer Animal Health) and 1 mg of Estradiol Cypionate (ECP, Pfizer Animal Health) were used, using one of the following treatments:

In group T1(n=14), the progesterone-based intravaginal device was placed for a period of 7 days; in group T2 (n=15), the CIDR intravaginal device was placed for 7 days and an injection of 1 mg estradiol cypionate (ECP) was administered 24 hours after device removal. In the T3 group (n=14), the CIDR intravaginal device was placed for 12 days. In the T4 group (n=14), the intravaginal CIDR device was placed for 12 days and a 1 mg estradiol cypionate injection was administered 24 hours after removal (Figure 1).

Estrous detection and artificial insemination.

- Estrous detection

For estrous detection, 5 vasectomized rams were used immediately after removal of the CIDR intravaginal device, from 0 to 48 hours, every 4 hours. Estrous detection was based on the observation of the behavior and sexual receptivity of the ewes in front of the vasectomized ram.

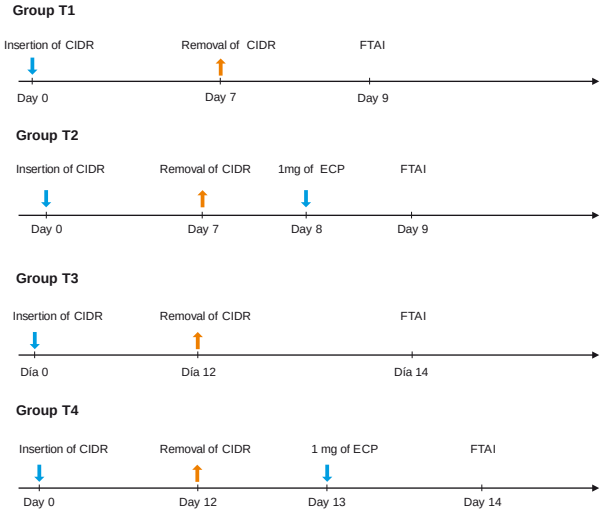

Figure 1. Estrous synchronization protocols of the ewe lambs subjected to the experimental treatments.

- Diluent preparation

For the preparation of the diluent, 11 grams of powdered skim milk diluted in 100 ml of distilled water, one Creole hen egg yolk and 5 ul of gentamicin were used.

- Obtaining the semen

To obtain the semen, two male breeders of good reproductive aptitude were used, which were routinely used in the artificial insemination campaigns prior to this research. The artificial vagina was used at a temperature of 41°C and in the seminal evaluation, the semen volume was determined in a graduated tube, the sperm concentration in the Neubauer chamber and the motility in a light microscope with a 200X thermal stage. Samples with a minimum volume of 1 ml, a motility of 4 to 5 (on a scale of 1 to 5) and a minimum sperm concentration of 3500 x 106 spermatozoa/ml were considered adequate.

- Artificial insemination

Artificial insemination was performed 48 hours after removal of the progesterone device. Two males were used and the distribution of the ewes for FTAI was random. To obtain the insemination dose with fresh semen, a 1:2 dilution (semen: diluent) was performed with a concentration per dose per ewe of 100 million spermatozoa/0.1 ml, which was placed at the entrance of the cervix, using a vaginoscope and an insemination gun tempered at 37°C. Pregnancy rate was determined using a Chison Ecovet 6 ultrasound scanner (CHISON 6 VET Ultrasound Doppler), at 46 and 90 days post insemination. The birth rate was recorded at the end of the gestation period of the pregnant ewes.

Statistical analysis

The presentations of estrus, pregnancy rate and birth rate were analyzed by Chi-square test, using the SPSS 20 program (SPSS Inc., Chicago, IL, USA).

RESULTS

Estrous presentation

In Table 1, no significant differences (P>0.05) were observed for estrous presentation between groups.

Table 1. Estrous presentation in Creole ewes, after different times of CIDR withdrawal with or without estradiol cypionate treatment.

Trat	n	(%) Percentage of ewes in estrous		
		36 h	48h	TOTAL
Group T1	14	57.1(8/14)	42.9(6/14)	100.0(14/14) [a]
Group T2	15	60.0(9/15)	33.3 (5/15)	93.3(14/15) [a]
Group T3	14	64.3 (9/14)	21.4 (3/14)	85.7(12/14) [a]
Group T4	14	64.3(9/14)	35.7(5/14)	100.0(14/14) [a]

Group T1= CIDR for 7 days = CIDR for 7 days + application of estradiol cypionate 24 hours later, group T3 = CIDR for 12 days, group T4 = CIDR for 12 days + application of estradiol cypionate 24 hours later. Different letters indicate significant difference (P<0.05).

Table 2. Effect of CIDR and estradiol cypionate on estrous presentation, pregnancy rate and birth rate in Creole ewes inseminated in non-breeding season.

Variable/Treatments	Group T1 n=14	Group T2 n= 15	GroupT3 n=14	Group T4 n=14
Estrous presentation (%)	100	93.3	85.7	100
Pregnacy rate (46 días) (%)	42.9[a]	0[c]	38.5[a]	21.4[b]
Pregnacy rate (90 días) (%)	35.7[a]	0[c]	30.8[a]	7.1[b]
Birth rate (%)	28.6[a]	0[c]	30.8[a]	0[c]

Group T1= CIDR for 7 days = CIDR for 7 days + application of estradiol cypionate 24 hours later, group T3 = CIDR FOR 12 days, group T4 = CIDR for 12 days + application of estradiol cypionate 24 hours later. Different letters in each row indicate significant difference (P<0.05).

Pregnancy and birth rates

Table 2 shows higher pregnancy rates at 46 days (P<0.05), in the CIDR-based protocols for 7 and 12 days, with 42.9% and 38.5%, compared to the CIDR protocol for days plus estradiol cypionate (21.4%), and in the CIDR group for 7 days plus estradiol cypionate, no pregnant ewe was obtained.

DISCUSSION

Estrogen administration during the phase of low progesterone concentrations induces LH release and ovulation. Thus, estradiol benzoate is effective in synchronizing ovulation in goats in short time protocols and could be an alternative to eCG (Menchaca and Rubianes, 2007). In our results when we used the progesterone device (CIDR) plus estradiol cypionate for 7 and 12 days, 93.3% and 100% estrous presentation was observed, respectively. That is to say that the addition of estradiol salts would favor estrous presentation, as was also evidenced in

goats with a 100% estrous percentage and therefore fixed-time insemination 48 hours after device removal is recommended (Maffili et al., 2005b).

In treatments with only the use of intravaginal progesterone devices (CIDR) for 7 and 12 days, 100% and 85.7% were observed, respectively; with the influence of the male effect of the rams that detected estrous and the high proportion of Creole ewes that are able to ovulate throughout the year, with less seasonality and continuous cyclic activity as other native breeds (Bravo, 1986; Arroyo et al., 2016). Compared to the use of an intravaginal progestogen of fluorogestone acetate (FGA) in Rahmani sheepheads, an estrous presentation of 66.7% was observed in a protocol of 6 days duration and 83.3% with 12 days duration, lower results, due to the geographic location of this breed and its higher seasonality (Amer and Hazzaa, 2009).

At 46 days after artificial insemination, no pregnancy is observed for the CIDR protocol for 7 days plus estradiol cypionate, due to the amount of dose used, which would produce anovulatory follicular cysts that affect the fertility of the female (Verano, 2020). While the CIDR protocol for 12 days plus estradiol cypionate, produced a pregnancy rate of 21.4%, confirming the results of other research works with the use of estradiol benzoate, which indicate that the ovulation rate in ewes is low (Castilho et al., 2015). In addition, serum estradiol levels can persist several days after administration (Larson and Kiracofe, 1995), having a negative effect on fertility, since elevated and residual serum estradiol concentrations during the first days after insemination affect embryonic development (Dieleman et al., 1993, Valenzuela-Jiménez et al., 2004). On the other hand, higher percentages in pregnancy rate were observed with the use of protocols with CIDR for 7 days (42.9%) and 12 days (38.5%). Similar percentages when flurogestone acetate was used in non-breeding season, for 6 days (50%) and for 12 days (66.7%) (Amer & Hazzaa, 2009), because the concentrations of natural progesterone or progestogens are adequate, and at the time of withdrawal the mechanism of ovulation and subsequent fertilization is induced (Knights and Singh-Knights, 2016).
The pregnancy rate at 90 days decreased to 7.14% in the CIDR group for 12 days plus estradiol cypionate, that is, when we add estradiol cypionate the pregnancy rate decreases, because it would be producing a more delayed ovulation rate unlike the use of eCG that at the end of treatment reduces the interval to estrous and ovulation and increases the diameter of the corpus luteum (Cox et al., 2012). Based on studies in cattle (Bó et al., 2003) and our findings of smaller preovulatory follicle size in BE-treated goats, we speculate that the low pregnancy rate observed in BE-treated goats could be a consequence of inadequate maturation of the ovulatory follicle and luteal development.

No birth was obtained for the CIDR device protocols for 7 or 12 days plus estradiol cypionate that is to say the use of an intravaginal progesterone device in a fixed-time insemination protocol, using estradiol cypionate to induce estrus and ovulation, does not improve ovulation rate, pregnancy rate and late embryonic loss, as also observed in high producing cows (Galvão et al., 2004). However, when we used the CIDR device for 7 and 12 days, the birth rate was 28.6% in both groups, with fetal losses of 7.1 % and 2.2 %. In addition, (Amer and Hazzaa, 2009) report a 33.3% birth rate in ewes synchronized with FGA for 6 days and 58.3% with FGA for 12 days, in non-breeding season, with 16.7% and 8.4 % of fetal embryonic loss, for FGA 6 and 12, respectively.

We must indicate that embryonic and fetal losses, after using several protocols using CIDR-G in ewes in anestrous and transition, occur in all phases of gestation; 3.8% of ewes lose one or more embryos from day 25 to 45, 6.2% lose one or more fetuses from day 45 to 65, 0.5% from 65 to 85; and 9.4% from day 85 to parturition (Dixon et al., 2007). Likewise, according to the reproductive seasonality in sheep, melatonin affects the steroidogenic capacity of the corpus luteum, blocks the generation of PGF2α by the uterus; induces differential regulation of the progesterone receptor in the endometrium; regulates the expression of some genes involved in embryonic development and implantation (Abecia et al., 2019). In addition, the nutritional status of the pregnant ewe, alters the gene expression of metabolic pathways related to energy generation in utero. An alteration in nutrient transport and metabolism in the uterus of pregnant malnourished ewes may explain the higher embryonic mortality associated with ewe malnutrition (De Brun et al., 2020), suggesting that once the estrus synchronization protocol is performed in non-breeding season, conditions such as nutrition or progesterone profiles should be as optimal as possible (Rickard et al., 2017). Therefore, we can indicate that the nutritional status of the ewe lambs in our study was not the best and also the management conditions typical of the livestock calendar, such as deworming, shearing could not be avoided in the group of ewe lambs under study, which would have had an impact on additional fetal mortality.

CONCLUSIONS

A high pregnancy rate was obtained with CIDR for 7 and 12 days, compared to the use of CIDR plus estradiol cypionate. However, no births were obtained with progesterone plus estradiol cypionate treatment.

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CONFLICT OF INTEREST
The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION
Conception and design of the study.NC; data acquisition. WG; data analysis and interpretation. UP, EC; fund acquisition. NL; writing and approval of the article. NC, WG, UP, EC, NL.

REFERENCES

- Abecia JA, Forcada F, González-Bulnes A. Hormonal control of reproduction in small ruminants. Animal Reproduction Science. 2012; 130(3–4), 173–179. https://doi.org/10.1016/j.anireprosci.2012.01.011
- Abecia JA, Forcada F, Vázquez MI, Muiño-Blanco T, Cebrián-Pérez, JA, Pérez-Pe R., Casao A. Role of melatonin on embryo viability in sheep. Reproduction, Fertility and Development. 2019; 31(1), 82–92. https://doi.org/10.1071/RD18308

- Ahmad Z, Ahmad A, Aarif O, Hasin D, Ahmad I. Physiology of reproductive seasonality in sheep – an update. Biologycal Rhythm Research. 2020; 51:4, 586-598. https://doi.org/10.1080/09291016.2018.1548112
- Amer HA, Hazzaa AM. The effect of different progesterone protocols on the reproductive efficiency of ewes during the non-breeding season. Veterinarski Arhiv. 2009; 79(1), 19–30.
- Ando H, Shahjahan M, Kitahashi T. Periodic regulation of expression of genes for kisspeptin, gonadotropin-inhibitory hormone and their receptors in the grass puffer: Implications in seasonal, daily and lunar rhythms of reproduction. General and Comparative Endocrinology. 2018; 265, 149–153. https://doi.org/10.1016/j.ygcen.2018.04.006
- Arroyo J, Sánchez-Hernández NJ, Ávila-Serrano NY, Camacho-Escobar M, Rodríguez-De-La-Torre M. Reproductive seasonality in creole hair sheep in the tropic. Tropical Animal Health and Production. 2016; 48(1), 219–222. https://doi.org/10.1007/s11250-015-0927-z
- Bó, G. A., Baruselli, P. S., & Martínez, M. F. Pattern and manipulation of follicular development in Bos indicus cattle. Animal Reproduction Science. 2003; 78(3–4), 307–326.
- Bravo Matheus PW. Factors Affecting Puberty, Estrus and Ovulation in Corriedale and Criollo Sheep of the Southern Peruvian Highlands. Master of science Thesis, Utah State University 1986. Avalaible: https://digitalcommons.usu.edu/etd/4099/
- Caballa, R. Influencia de la estacionalidad en el comportamiento reproductivo y nacimiento de corderos en ovejas de Pasco 2018. Tesis de Maestría en Producción Animal. Universidad Nacional Hemilio Valdizán. 2019. Available: http://repositorio.unheval.edu.pe/handle/UNHEVAL/6677
- Carlson KM, Pohl HA, Marcek JM, Muser RK, Wheaton JE. Evaluation of progesterone controlled internal drug release dispensers for synchronization of estrus in sheep. Animal Reproduction Science. 1989; 18(1–3), 205–218. https://doi.org/10.1016/0378-4320(89)90022-5
- Castilho C, De Almeidav MF, Giometti IC, Costa MZ, Filho LR., De Cesare AG. Use of estradiol benzoate to induce ovulation in a short-term protocol for fixed-time AI in sheep. Semina:Ciencias Agrarias. 2015; 36(3), 1419–1423. https://doi.org/10.5433/1679-0359.2015v36n3p1419
- Cox JF, Allende R, Lara E, Leiva A, Díaz T, Dorado J, Saravia F. Follicular Dynamics, Interval to Ovulation and Fertility After AI in Short-Term Progesterone and PGF2α Oestrous Synchronization Protocol in Sheep. Reproduction in Domestic Animals. 2012; 47(6), 946–951. https://doi.org/10.1111/j.1439-0531.2012.01996.x
- De Brun V, Loor J, Naya H, Vailati-Riboni M, Bulgari O, Shahzad K, Abecia, JA, Sosa C, Meikle A. The embryo affects day 14 uterine transcriptome depending on nutritional status in sheep. a. Metabolic adaptation to pregnancy in nourished and undernourished ewes. Theriogenology. 2020; 146, 14–19. https://doi.org/10.1016/j.theriogenology.2020.01.047
- De Carvalho Menezes de Almeida SF, Souza-Fabjan J, Balaro M, Bragança G, Pinto P, de Almeida J, Moura A, da Fonseca J, Brandão F. Use of two doses of cloprostenol in different intervals for estrus synchronization in hair sheep under tropical conditions. Tropical Animal Health and Production. 2018; 50(2), 427–432. https://doi.org/10.1007/s11250-017-1454-x
- De K, Kumar D, Sethi D, Gulyani R, Naqvi S. Estrus synchronization and fixed-time artificial insemination in sheep under field conditions of a semi-arid tropical region. Tropical Animal Health and Production. 2015; 47(2), 469–472. https://doi.org/10.1007/s11250-014-0735-x
- Dieleman SJ, Bevers MM, Vos PL, de Loos FA. PMSG/anti-PMSG in cattle: A simple and efficient superovulatory treatment?. Theriogenology. 1993; 39(1), 25–41. https://doi.org/10.1016/0093-691X(93)90022-W
- Dixon AB, Knights M, Winkler JL, Marsh DJ, Pate JL, Wilson ME, Dailey RA, Seidel G, Inskeep EK. Patterns of late embryonic and fetal mortality and association with several factors in sheep. Journal of Animal Science. 2007; 85(5), 1274–1284. https://doi.org/10.2527/jas.2006-129
- Evans A, Flynn J, Quinn K, Duffy P, Quinn P, Madgwick S, Crosby T, Boland M, Beard A. Ovulation of aged follicles does not affect embryo quality or fertility after a 14-day progestagen estrus synchronization protocol in ewes. Theriogenology. 2001; 56(5), 923–936. https://doi.org/10.1016/S0093-691X(01)00619-7
- Galvão, K. N., Santos, J. E. P., Juchem, S. O., Cerri, R. L. A., Coscioni, A. C., & Villaseñor, M. Effect of addition of a progesterone intravaginal insert to a timed insemination protocol using estradiol cypionate on ovulation rate, pregnancy rate, and late embryonic loss in lactating dairy cows. Journal of Animal Science. 2004, 82(12), 3508–3517. https://doi.org/10.2527/2004.82123508x
- Gómez U, Serrano N, Arroyo-Ledezma J. Reproductive response in hair sheep synchronized with medroxyprogesterone acetate impregnated in non-commercial intravaginal sponges. Tropical and Subtropical Agroecosystems. 2021; 24(2). https://www.revista.ccba.uady.mx/ojs/index.php/TSA/article/view/3423
- Hasani N, Ebrahimi M, Ghasemi-Panahi B, HosseinKhani A. Evaluating reproductive performance of three estrus synchronization protocols in Ghezel ewes. Theriogenology. 2018; 122, 9–13. https://doi.org/10.1016/j.theriogenology.2018.07.005
- Hervé, V., Roy, F., Bertin, J., Guillou, F., & Maurel, M. C. Antiequine 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. 2004; 145(1), 294–303. https://doi.org/10.1210/en.2003-0595
- Knights M., Singh-Knights D. Use of controlled internal drug releasing (CIDR) devices to control reproduction in goats: A review. Animal Science Journal. 2016; 87(9), 1084–1089. https://doi.org/10.1111/ASJ.12627
- Larson RL, Kiracofe GH. Estrus after treatment with Syncro-Mate B• in ovariectomized heifers is dependent on the

injected estradiol valerate. Theriogenology. 1995; 44(2), 177–187. https://doi.org/10.1016/0093-691X(95)00167-7

- Letelier CA, Contreras-Solis I, García-Fernández RA, Ariznavarreta C, Tresguerres JAF, Flores JM., Gonzalez-Bulnes A. Ovarian follicular dynamics and plasma steroid concentrations are not significantly different in ewes given intravaginal sponges containing either 20 or 40 mg of fluorogestone acetate. Theriogenology. 2009; 71(4), 676–682. https://doi.org/10.1016/j.theriogenology.2008.09.030
- Lozano H, Raes M, Vargas JJ, Ballieu A, Grajales H, Manrique C, Beckers JF, Kirschvink N. Onset of puberty and regularity of oestral cycles in ewe lambs of four breeds under high-altitude conditions in a non-seasonal country. Tropical Animal Health and Production. 2020; 52(6), 3395–3402. https://doi.org/10.1007/s11250-020-02372-w
- Maffili VV, Torres CA, Fonseca JF, Moraes EA, Pontes RA. Sincronização de estro em cabras da raça Saanen com esponja intravaginal e CIDR-G®. Arquivo Brasileiro de Medicina Veterinária e Zootecnia. 2005a; 57(5), 591–598. https://doi.org/10.1590/s0102-09352005000500004
- Maffili VV, Torres CA, Pontes RA, Guimarães JD, Prosperi CP. Utilização de gonadotrofina coriônica humana e cipionato de estradiol associado ao dispositivo de liberação controlada de drogas para sincronização de ovulação em cabras da raça Saanen. Arquivo Brasileiro de Medicina Veterinária e Zootecnia. 2005b; 57(2), 210–216. https://doi.org/10.1590/s0102-09352005000200012
- Martinez-Ros P, Astiz S, Garcia-Rosello E, Rios-Abellan A, Gonzalez-Bulnes A. Effects of short-term intravaginal progestagens on the onset and features of estrus, preovulatory LH surge and ovulation in sheep. Animal Reproduction Science. 2018; 197, 317–323. https://doi.org/10.1016/j.anireprosci.2018.08.046
- Menchaca A, Rubianes E. Pregnancy rate obtained with short-term protocol for timed artificial insemination in goats. Reproduction in Domestic Animals. 2007; 42(6), 590–593. https://doi.org/10.1111/j.1439-0531.2006.00827.x
- Molina, S. E., del Pilar Gamarra Reyes, Y., Ticona Huaroco, C. N., Huayta, M. Cc., Rojas, G. E., Perez Guerra, U. H., & Cruz, D. J. Evaluation of an oestrus synchronization protocol in sheep with temporary weaning for artificial insemination at fixed time. Revista de Investigaciones Veterinarias Del Peru. 2020; 31(3), 1–6. https://doi.org/10.15381/RIVEP.V31I3.16820
- Morris ST. Economics of sheep production. Small Ruminant Research. 2009; 86(1–3), 59–62. https://doi.org/10.1016/j.smallrumres.2009.09.019
- Rickard JP, Ryan G, Hall E, De Graaf SP, Hermes R. Using transrectal ultrasound to examine the effect of exogenous progesterone on early embryonic loss in sheep. PLoS ONE. 2017; 12(8), 1–18. https://doi.org/10.1371/journal.pone.0183659
- Smith JT. The role of kisspeptin and gonadotropin inhibitory hormone in the seasonal regulation of reproduction in sheep. Domestic Animal Endocrinology. 2012; 43(2), 75–84. https://doi.org/10.1016/j.domaniend.2011.11.003
- Valenzuela-Jiménez N, Hernández-Cerón J, Murcia-Mejía C, Rodríguez-Maltos R, Gutiérrez CG. The effect of estradiol benzoate on the time to the LH peak, ovulation time and fertility in melengestrol acetate synchronized goats. Agrociencia. 2004; 38(6), 603–611.
- Verano M. Evaluación de protocolos de sincronización de celos con progesterona y benzoato de estradiol para inseminación artificial a tiempo fijo en ovinos. Tesis de Doctorado en Ciencia Animal, Universidad Nacional de La Plata 2020. Avaiable: http://www.med.unlp.edu.ar/archivos/grado/medicina/medicina_interna_F_programa.pdf%5Cnhttp://www.postgradofcm.edu.ar/ProduccionCientifica/TrabajosCientificos/58.pdf
- Viñoles C, Forsberg M, Banchero G, Rubianes E. Follicular Development and Pregnancy Rate in Cyclic Ewes. Theriogenology. 2001; 55(4), 993–1004. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=11291921
- Weems PW, Goodman RL, Lehman MN. Neural mechanisms controlling seasonal reproduction: Principles derived from the sheep model and its comparison with hamsters. Frontiers in Neuroendocrinology. 2015, 37, 43–51. https://doi.org/10.1016/j.yfrne.2014.12.002
- Wildeus S. Current concepts in synchronization of estrus: Sheep and goats. Journal of Animal Science. 2000; 77(E-Suppl), 1. https://doi.org/10.2527/jas2000.00218812007700es0040x
- Yoshimura T. Thyroid hormone and seasonal regulation of reproduction. Frontiers in Neuroendocrinology. 2013; 34(3), 157–166. https://doi.org/10.1016/j.yfrne.2013.04.002.