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Factors influencing the success of an artificial insemination program in Florida goats

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Abstract

An artificial insemination (AI) program using cooled semen was evaluated over a 7-year period in Florida goats. The effect of the following variables on pregnancy rates was analysed: production system, year and season of AI, synchronisation treatment, bucks, AI technicians, semen deposition site, days in milk at AI, milk yield and parity. Animals were reared under field conditions on commercial farms in southern Spain. Semen was collected from nine bucks and cooled at 4°C until use. A total of 3,941 goats were synchronised using intravaginal progesterone sponges and inseminated 46.0 ± 0.5 h. after sponge removal. Pregnancy was diagnosed by ultrasonography 42-46 days after AI, obtaining an average pregnancy rate of 48.7%. Logistic regression showed that production system, AI year and season, bucks and semen deposition site had a significant effect ($p < 0.05$) on pregnancy rate, while the other variables analysed were removed from the model. The final statistical model accounted for 59.7% of the cases analysed, suggesting that other factors not studied here may influence pregnancy rates in Florida goats.

Additional key words: buck; fertility; fresh semen; goat; pregnancy.

Resumen

Factores que influyen en el éxito de un programa de inseminación artificial en cabras de raza Florida

Se analizaron los resultados obtenidos en un programa de inseminación artificial (IA) con semen refrigerado en cabra Florida, correspondientes a 7 años, en los que se analizó el efecto de numerosas variables (sistemas de producción, año y estación de IA, tratamientos de sincronización, machos utilizados, inseminadores, lugar de deposición del semen, días en lactación, producción de leche y número de partos) sobre la tasa de gestación. Las cabras se encontraban en granjas comerciales del sur de España y el trabajo se desarrolló en condiciones de campo. El semen fue recogido en nueve machos y seguidamente refrigerado a 4°C hasta su uso. Un total de 3.941 cabras fueron sincronizadas con esponjas intravaginales impregnadas con progestágenos y se inseminaron 46,0 ± 0,5 h. tras su retirada. El diagnóstico de gestación se realizó mediante ecografía a los 42-46 días tras la IA, obteniendo una tasa media del 48,7%. La regresión logística utilizada en el análisis estadístico de los datos identificó que las variables sistemas de producción, año y estación de IA, machos utilizados y lugar de deposición del semen afectaban significativamente ($p < 0,05$) la tasa de gestación, mientras que las restantes variables fueron eliminadas del modelo. El 59,7% de los casos analizados fueron adecuadamente explicados según el modelo estadístico final obtenido, sugiriendo que otros factores no considerados en este estudio afectan de manera importante sobre la tasa de gestación en programas de IA en la raza Florida.

Palabras clave adicionales: cabra; fertilidad; gestación; macho cabrío; semen fresco.

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Abbreviations used: AI (artificial insemination); DIM (days in milk); eCG (equine chorionic gonadotropin); FGA (fluorogestone acetate); FTAI (fixed-time artificial insemination); OR (odd ratio).

Introduction

The Florida goat is a dairy breed reared in southern Spain, with an average milk yield of 650 kg per goat per year and an average lactation period of 10 months (Sánchez, 2007). This breed was created in the early 20th century by crossing Pyrenean and Nubian goats, in a process similar to that which gave rise to several current breeds, such as Anglonubian goat. In 1997, it was included as threatened Spanish breed in the Official Catalogue of Spanish Livestock Breeds (BOE, 2009). An artificial insemination (AI) program was implemented in 2005 in Florida goats in order to optimise selection schemes and control kidding dates. This technique is an essential tool in goat breeding programs, since it increases the efficiency of sire genetic evaluation and the extension of genetic improvements; at the same time, it enables control of parturition dates with a view to meeting market demands. Insemination is usually carried out transcervically, and semen is deposited either at the entry of the cervix or, more rarely, in the uterus (Nunes & Salgueiro, 2011). However, AI is not widely used in goats. Cooled semen provides better results than frozen-thawed semen. Hormone treatments are required for fixed-time artificial insemination (FTAI) in order to avoid oestrus detection and synchronise oestrus in a group of goats. This enables more goats to be inseminated over a shorter period, as well as allowing insemination and births to be carefully scheduled; it also permits an increase in the number of kids obtained by AI at the start of the kidding season, thus enabling a more efficient use of human resources (Nunes & Salgueiro, 2011).

A number of factors are reported to affect the success of AI, including nutrition, breeding season, environmental conditions, parity, breed, farm, depth of semen deposition, extender composition and hormone treatment (Mellado *et al.*, 2004 and 2006; Salvador *et al.*, 2005; Nunes & Salgueiro, 2011). The present study sought to clarify the effect of different factors (production system, year of AI, season, synchronisation treatment, bucks, AI technicians, semen deposition site, days in milk (DIM) at AI, milk yield and parity) on pregnancy rates after AI with cooled semen in Florida goats reared under commercial farm conditions.

Material and methods

Animals, management and housing

From 2005 to 2011, a total of 3941 inseminations were carried out in goats registered in the Florida breed stud-book, as a part of a breeding and selection program. Goats were reared on commercial farms and, considering the size of the herd, the grazing surface and the concentrate consumption (Nahed et al., 2006), three different production systems were evaluated: intensive, semi-intensive and semi-extensive. Milk yield was officially recorded. All females had at least one kidding and were evaluated by ultrasonography prior to AI in order to exclude individuals with reproductive disorders such as pseudo-pregnancy. Does with previous abortions or reproductive problems were not included in the AI program.

Nine Florida bucks were housed in individual stalls at the Andalusian Government's IFAPA Research Centre (Hinojosa del Duque, Cordoba, Spain; 38.30° N, 5.09° W) in barns with a concrete floor and large roof windows with access to external paddocks, allowing some interaction with the environment. Bucks were daily fed with a commercial concentrate (0.5 kg) and given *ad libitum* access to alfalfa hay, water, and mineral supplementation blocks. They were kept in a light-controlled environment (two months of long days alternating with two months of short days). To this end, artificial light was switched on at 8:00 a.m. and off at 16:00 p.m. or 00:00 during the short and long photoperiod treatments, respectively. The supplementary light source provided a light intensity of roughly 200 lx.

Semen collection, assessment and processing

Semen was collected twice a week by artificial vagina, and volume and sperm concentration (Accucell, IMV, France) were assessed. Semen samples were diluted in a skimmed milk-based extender to a final concentration of 800×10^6 spermatozoa mL^{-1}, and then cooled at 4°C. Briefly, one sample was warmed to asses sperm motility (S.C.A., Microptic SL, Spain) and membrane integrity (hypo-osmotic swelling test) in order to determine semen quality before preparing doses. Semen was used for AI only when it complied with the following criteria: membrane integrity up to 40%, over 70% progressive motility and over 60% mass motility. Inseminating doses were packed into 0.25 ml straws with 200×10^6 spermatozoa and transported under refrigerated conditions to the farm, where insemination was performed by qualified technicians. The whole procedure was carried out early on the same day.

Synchronisation and insemination

Oestrus synchronisation was carried out using intravaginal progestagen-impregnated sponges with varying doses of fluorogestone acetate (FGA, 20 and 45 mg, Chronogest, Intervet International, Boxmeer Holland) inserted for 11 ± 1.0 days. Forty-eight hours before sponge withdrawal, goats received an intramuscular injection of 50 g cloprostenol (Estrumate, Schering-Plough Animal Health, Upper Hutt, New Zealand) and 400 UI of equine chorionic gonadotropin (eCG) (Foligon, Intervet, Spain). Only one hormonal synchronisation treatment per goat and per year was used to reduce the effects of repeated use of eCG, specifically the production of anti-eCG antibodies which reduce the fertility of artificially-inseminated females. Fixed-time artificial insemination with cooled semen was performed 46.0 ± 0.5 h after sponge withdrawal, using a lighted speculum and an ovine-caprine AI catheter. The aim was to deposit the sperm dose in the uterine cervix; information on the semen deposition site (cervical *vs.* uterine) was recorded at each insemination.

Pregnancy was diagnosed around day 42-46 after AI using an ultrasound scanner (Pie-Medical, Netherland) equipped with a 5.0 MHz linear-array transducer. Animals were restrained against railing in the standing position and scanning was performed in the inguinal region.

Statistical analysis

Records were routinely kept on farms and classified for data analysis purposes. Logistic regression analysis was performed on data for each insemination, using pregnancy diagnosis as the dependent variable (0 or 1). The following independent variables were tested: production system (intensive, semi-intensive, semi-extensive); year (2005 to 2011); AI season (cold: autumn-winter, hot: spring-summer); synchronisation treatment (20 mg or 45 mg FGA); buck (9 inseminating males); depth of semen deposition (cervical or uterine); AI technician (1, 2, 3, 4, 5); parity (1, 2, 3 and ≥ 4); days in milk at AI (DIM) (≤ 154 days, 155-181 days, ≥ 182 days) and milk yield (≤ 1.80 kg, 1.81-2.69 kg, ≥ 2.70 kg). All categorical independent variables were first screened for univariate association to pregnancy rate using the crosstabs procedure. Only variables displaying significant values were included in the logistic regression analysis, which was run using the "Forward Wald" stepwise selection method to specify how independent variables are entered into the analysis. The level of statistical significance for variables included in the model was set at $p < 0.05$. This procedure enabled calculation of the odds ratio (OR) and the 95% confidence interval as an indicator of risk or probability of pregnancy in an AI program using cooled semen. The class whose value was nearest to the mean pregnancy rate was selected in order to establish categorical variable reference values.

The Hosmer-Lemeshow method (Hosmer & Lemeshow, 1989) was used to test the goodness of fit of logistic regression models. All statistical analyses were performed using the SPSS 15.0 statistical software package.

Regression analysis of the OR for pregnancy rate on milk yield categorised in 0.5 kg increments was evaluated to determine the fitted line plot that best described this relation.

Results and discussion

A mean pregnancy rate of 48.7% was recorded over the 7-year period. The mean number of DIM and milk yield at AI were 168.66 ± 58.49 days (mean ± SD; range: 27-489 days) and 2.34 ± 1.0 kg (range: 0.18-7.04 kg), respectively. Parity ranged from 1 to 11 births.

Preliminary crosstabs procedure showed that AI technician, parity and DIM had no significant effect on fertility. Otherwise, production system, AI year, AI season, synchronization treatment, buck, depth of semen deposition and milk yield at insemination were included in the initial logistic model. After run the logistic procedure, synchronization treatment and milk yield at insemination were removed from the final model as they did not affected significantly the dependent variable. The odds ratios are shown in Table 1.

Results showed that the probability of pregnancy using cooled semen was significantly higher under the intensive production system than under the semi-intensive system. The probability of pregnancy varied significantly as a function of AI year, dropping to 35.3% in 2007 (OR = 0.56) and rising to 54.8% in 2008 (OR = 1.32). Goats inseminated in cold season (autumn and winter) recorded lower pregnancy rates (OR = 0.74). The probability of pregnancy was greater when semen from buck n.2 (OR = 1.41) and n.5 (OR = 1.42) was used. Cervical semen deposition was associated with a lower probability of pregnancy than uterine insemination (OR = 1.82). The final model correctly classified 59.7% of the cases analysed, with a sensitivity of 57.8% and a specificity of 61.8%. The Cox & Snell R Square value showed that only 4.3% of the variance in fertility was attributable to the variables included in the model for the studied population.

Data obtained from 2005 to 2011 were used to analyse the effect of several factors on fertility after AI using cooled semen in Florida goats. Results showed that the pregnancy rate was significantly affected by the production system, AI year, AI season, buck, and semen deposition site. The mean pregnancy rate (48.7%) was similar to that reported by Siqueira *et al.* (2009), but lower than described under controlled conditions (Roca *et al.*, 1997; Romano *et al.*, 2000).

The production system (intensive, semi-extensive or semi-intensive) strongly influenced the success of AI. Pregnancy rates were significantly higher under intensive systems (Table 1). This may be due in part to a greater degree of animal management in intensive production, leading to lower stress during goat-human interactions.

The marked year-on-year variations in fertility, with a sharp fall in 2007 and a marked rise in 2008, may reflect the interaction of a range of factors, including weather conditions, animal welfare, nutrition and animal breeding.

Goat reproduction is affected by changes in day length over the year. Photoperiodic control of reproductive patterns is mediated through circadian rhythmic secretions of melatonin by the pineal gland during dark-

Table 1. Odds ratios for the variables included in the final logistic regression model for pregnancy rate

Variable	n	Pregnancy rate	Odds ratio	95% CI	*p*-value
Production system					
Intensive	2,885	51.1	Reference		
Semi-extensive	594	35.1	0.53	0.42-0.67	0.0001
Semi-intensive	462	38.4	0.71	0.52-0.95	0.022
Year					
2005	219	45.6	0.89	0.58-1.35	n.s.
2006	221	43.5	0.84	0.60-1.17	n.s.
2007	380	35.3	0.56	0.41-0.76	0.0001
2008	597	54.8	1.32	1.01-1.72	0.046
2009	906	50.9	1.06	0.85-1.33	n.s.
2010	813	48.4	Reference		
2011	803	50.8	1.05	0.78-1.42	n.s.
Season					
Hot	2,649	51.0	Reference		
Cold	1,292	44.0	0.74	0.63-0.87	0.0001
Buck					
1	254	59.0	1.31	0.81-2.13	n.s.
2	364	55.1	1.41	1.05-1.90	0.024
3	450	40.0	0.82	0.63-1.09	n.s.
4	477	47.8	Reference		
5	343	60.3	1.42	1.06-1.91	0.019
6	708	46.4	1.02	0.80-1.29	n.s.
7	329	49.7	1.15	0.77-1.70	n.s.
8	680	46.7	1.14	0.88-1.48	n.s.
9	332	46.4	0.99	0.66-1.49	n.s.
Semen deposition					
Cervical	3,740	47.9	Reference		
Uterine	201	63.7	1.82	1.31-2.51	0.0001

Hosmer and Lemeshow test =16.25; 8 d.f.; $p = 0.081$ (the model fits). $R^2 = 0.043$. n.s.: not significant ($p > 0.05$); CI: confidence interval.

ness, which influences gonadotropin-releasing hormone pulse generation and the hypothalamic-pituitary-gonadal feedback loop (Fatet *et al.*, 2011). Reported goat conception rates range from 50% to 70%, depending on the season of insemination (Amoah & Gelaye, 1997). In the present study, fertility was significantly influenced by AI season. The probability of pregnancy was reduced when insemination took place during the colder seasons (autumn and winter). In contrast, the fertility of Mediterranean breeds inseminated with cooled semen is reported to be high throughout the year (Roca *et al.*, 1997). The dairy-goat market demands insemination in spring, when fertility declines due to seasonal reproductive rhythms. For this reason, oestrus synchronisation has become essential to optimise spring pregnancy rates in order to enhance milk yield in autumn.

Hormone treatments based on progestagens, eCG and/or prostaglandins, established over the last forty years, enable the synchronisation of oestrous and ovulation during both the breeding and non-breeding seasons (Fatet *et al.*, 2011). These treatments are essential for any AI program in goats cycling naturally and also in order to induce oestrus during the non-breeding season with fixed-time artificial insemination (FTAI) (Leboeuf *et al.*, 2000). Lower pregnancy rates have been reported when AI is performed in synchronised-oestrus goats than with natural oestrus (Hafez & Hafez, 2000), and also during the non-breeding with respect to the breeding season (Karatzas *et al.*, 1997), although these claims remain controversial (Summermatter, 1987; Roca *et al.*, 1997). Goats synchronised with lower FGA sponges displayed a slightly higher probability of pregnancy than those synchronised with the 45 mg FGA treatment ($p > 0.05$). These results suggest that FGA doses could be lowered in the treatment of Florida goats, thus reducing hormone residues in milk whilst ensuring good fertility results.

Although a number of authors report no difference in pregnancy rates as a function of cervical or vaginal AI (Roca *et al.*, 1997; Paulenz *et al.*, 2005), others have noted a direct correlation between depth of semen deposition and fertility (Ritar & Salamon, 1983; Salvador *et al.*, 2005; Barbosa *et al.*, 2009), uterine insemination being associated —as observed here— with increased fertility. The present study found that uterine insemination took place on only a few occasions (5.1%); similar findings are reported by Meza & Ross (2000), though higher percentages of 18% and 30% were recorded by Salvador *et al.* (2005) and Ritar & Salamon (1983), respectively. The results obtained here suggest that uterine or post-cervical deposition is to be recommended whenever it can be done easily and quickly as part of routine AI practice, since it is associated with increased pregnancy rates. Nevertheless, uterine deposition should not become a handicap for the inseminator, since it might cause a delay in insemination and/or cervical injuries that could limit the success of the AI program. For the present program, the interval elapsing between sponge withdrawal and insemination was meticulously recorded. Artificial insemination was considered to be properly performed if completed within 46.0 h ± 0.5 after sponge withdrawal. Only 10.6% of inseminations were performed incorrectly (over 47.0 h after sponge withdrawal), and this was reflected in a drop in the pregnancy rate (31.7%). This confirms the need to avoid any delay in the AI routine in order to improve pregnancy rates.

The transportation of liquid semen over long distances has been reported as a limiting factor in goat AI programs (Paulenz *et al.*, 2005). The influence of the distance between the AI centre and the farm on fertility was considered in the initial statistical model, but no significance was observed, perhaps due to good semen quality and/or the adequate transport system used. Recently, it has been suggested that liquid semen does not have to be cooled to 4°C, nor stored and transported under cooled conditions when used within a day after collection (Peterson *et al.*, 2007).

Inseminating bucks affected fertility rates, a finding also reported in other studies (Paulenz *et al.*, 2005; Salvador *et al.*, 2005). Although seminal parameters were carefully evaluated and only good ejaculates were used, variations were noted in pregnancy rates as a function of buck used. Additional sperm assessment might reveal differences between bucks which could account for this effect.

Few reports have addressed the effects of milk yield on fertility in goats (Leboeuf *et al.*, 1998). However, numerous investigations in dairy cattle have shown that milk yield at AI has negligible effects on conception rates in high-producing dairy cattle (Chebel *et al.,* 2004; Lopez-Gatius *et al.*, 2006; Garcia-Ispierto *et al.*, 2007). In the present study, the initial univariate analysis showed that OR for fertility was higher as milk production increased (Fig. 1). However, after controlling for several factors, results obtained by multivariate logistic regression model indicate that milk yield is not significantly associated with pregnancy rate, and suggest that other factors modify the relation between milk yield and pregnancy, as reported in cows (Chebel *et al.,*

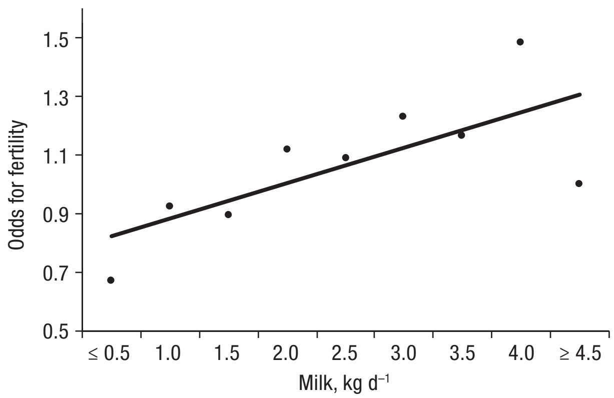

Figure 1. Relationship between odds ratio for fertility and milk yield. Odds = 0.760 + 0.061 * milk; $R^2 = 0.52$. Effect of milk yield on pregnancy according to the multivariate logistic regression model: $p > 0.05$.

2004). In addition, in the population evaluated, the probability of pregnancy did not vary significantly as a function of DIM.

As conclusions, numerous factors are involved in the success of a cooled-semen AI program in Florida goats, although here only 4.3% of variability was accounted for by the final model. Production system, AI year, AI season, buck and semen deposition site considerably influenced pregnancy rates, while the other factors analysed had no significant effect. These results show how difficult it is to monitor critical aspects in AI programs. All potentially influential factors need to be controlled, and the AI technician must be aware of any variations that could reduce the probability of pregnancy. The good fertility results obtained during the non-breeding season highlight the value of AI for improving the competitiveness of Florida dairy herds.

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References

Amoah EA, Gelaye S, 1997. Biotechnological advances in goat reproduction. J Anim Sci 75: 578-585.

Barbosa LP, Guimaraes JD, Espeschit CJB, Costa EP, Souza RS, Dutra PA, Martins LEP, Cardoso Neto BM, 2009. Influence of site of deposition of semen in the reproductive tract on gestation rate and prolificacy of artificially inseminated goats. Proc 18th Brazilian Congress of Animal Reproduction, Belo Horizonte, Brazil, Abstract 190.

BOE, 2009. Royal decree 2129/2008, of 26 December, that establishes the National Program for the conservation, improvement and promotion of livestock breeds. Boletín Oficial del Estado No. 23, 27/1/2009. [In Spanish].

Chebel RC, Santos JEP, Reynolds JP, Cerri RLA, Juchem SO, Overton M, 2004. Factors affecting conception rate after artificial insemination and pregnancy loss in latating dairy cows. Anim Reprod Sci 84: 239-255.

Fatet A, Pellicer-Rubio MT, Leboeuf B, 2011. Reproductive cycle of goats. Anim Reprod Sci 124: 211-219.

Garcia-Ispierto I, Lopez-Gatius F, Santolaria P, Yaniz JL, Nogareda C, Lopez-Bejar M, 2007. Factors affecting the fertility of high producing dairy herds in northeastern Spain, Theriogenology 67: 632-638.

Hafez ESE, Hafez B, 2000. Transport and survival of gametes. In: Reproduction in farm animals (Hafez B & Hafez ESE, eds.). Lippincott, Williams & Wilkins, Baltimore MD, USA. pp: 82-95.

Hosmer DW, Lemeshow S, 1989. Applied logistic regression. Wiley, NY, USA.

Karatzas G, Karagiannidis A, Varsakeli S, Brikas P, 1997. Fertility of fresh and frozen-thawed goat semen during the nonbreeding season. Theriogenology 48: 1049-1059.

Leboeuf B, Manfredi E, Boue P, Piacere A, Brice G, Baril G, Broqua C, Humblot P, Terqui M, 1998. L'insémination artificielle et l'amélioration génétique chez la chèvre laitière en France. INRA Prod Anim 11: 171-181. [In French].

Leboeuf B, Restall B, Salamon S, 2000. Production and storage of goat semen for artificial insemination. Anim Reprod Sci 62: 113-141.

Lopez-Gatius F, Garcia-Ispierto I, Santolaria P, Yaniz J, Nogareda C, Lopez-Bejar M, 2006. Screening for high fertility in high-producing dairy cows. Theriogenology 65: 1678-1689.

Mellado M, Valdez R, Lara LM, Garcia JE, 2004. Risk factors involved in conception, abortion, and kidding rates of goats under extensive conditions. Small Rumin Res 55: 191-198.

Mellado M, Valdez R, Garcia JE, Lopez R, Rodriguez A, 2006. Factors affecting the reproductive performance of goats under intensive conditions in a hot arid environment. Small Rumin Res 63: 110-118.

Meza CA, Ross TT, 2000. Factors affecting fertility and prolificacy of dairy goats inseminated with frozen-thawed semen. Proc 7th Int Conf on Goats. France, pp: 476-478.

Nahed J, Castel JM, Mena Y, Caravaca F, 2006. Appraisal of sustainability of dairy goat systems in Southern Spain according to their degree of intensification. Livest Sci 101: 10-23.

Nunes JF, Salgueiro CCM, 2011. Strategies to improve the reproductive efficiency of goats in Brazil. Small Rumin Res 98: 176-184.

Paulenz H, Söderquist L, Adnøy T, Soltun K, Saether PA, Fjellsøy KR, Andersen Berg K, 2005. Effect of cervical and vaginal insemination with liquid semen stored at room temperature on fertility of goats. Anim Reprod Sci 86: 109-17.

Peterson K, Kappen MAPM, Ursem PJF, Nothling JO, Colenbrander B, Gadella BM, 2007. Microscopic and flow cytometric semen assessment of Dutch AI-bucks: Effect of semen processing procedures and their correlation to fertility. Theriogenology 67: 863-871.

Ritar AJ, Salamon S, 1983. Fertility and frozen-thawed semen of Angora goats. Aust J Biol Sci 36: 49-59.

Roca J, Carrizosa JA, Campos I, Lafuente A, Vazquez JM, Martinez E, 1997. Viability and fertility of unwashed Murciano-Granadina goat spermatozoa diluted in Tris-egg yolk extender and stored at 5°C. Small Rumin Res 25: 147-153.

Romano JE, Crabo BG, Christians CJ, 2000. Effect of sterile service on estrus duration, fertility and prolificacy in artificially inseminated dairy goats. Theriogenology 53: 1345-1353.

Salvador I, Viudes De Castro MP, Bernacer J, Gómez EA, Silvestre MA, 2005. Factors affecting pregnancy rate in artificial insemination with frozen semen during non-breeding season in Murciano-Granadina goats: a field assay. Reprod Dom Anim 40: 526-529.

Sanchez M, 2007. Las razas caprinas andaluzas de fomento. In: las razas ganaderas de Andalucía. Patrimonio ganadero andaluz, vol 2. Ed. Consejería de Agricultura y Pesca. Junta de Andalucía. Sevilla, pp: 403-405. [In Spanish].

Siqueira AP, Fonseca JF, Siva Filho JM, Bruschi JH, Viana JHM, Palhares MS, Bruschi MCM, Peixoto MP, 2009. Parametros reproductivos de cabras Toggenburg inseminadas com semen resfriado, após diluicao em meio à base de gema de ovo. Arq Bras Med Vet Zootec 61: 299-305. [In Portuguese].

Summermatter P, 1987. Erkenntnisse aus der Ziegenbesamung. Reprod Dom Anim 22: 73-79. [In German].