The effect of carprofen treatment on reproductive parameters following progestagen administration in lactating German Fawn × Hair crossbred goats during the transitional period

M. KUTLU[1]*, H. ALKAN[2], H. DOĞAN[3], D.A. DİNÇ[2]

[1] Department of Agriculture and Animal Services, Adana Metropolitan Municipality, Adana, Turkey, 01120
[2] Department of Obstetrics and Gynaecology, Faculty of Veterinary Medicine, Selçuk University, Konya, Turkey, 42130
[3] Department of Obstetrics and Gynaecology, Faculty of Veterinary Medicine, Tekirdag Namık Kemal University, Tekirdag, Turkey, 59030

SUMMARY

The aim of this study was determine the effects of carprofen treatment on some reproductive parameters in German Fawn (75%) × Hair (25%) Crossbred goats during the transitional period. The 60 adult goats were treated with an intravaginal sponge containing progestagen (60 mg medroxyprogesterone acetate, Esponjavet®, Hipra, Spain) for 17 days in the transitional period (June) (day -18). Two days before the sponge removal (day -3) all animals received 12.5 mg dinoprost tromethamine (Dinolytic High Concentration®, Zoetis, Spain) intramuscularly, while PMSG 400 IU (Oviser®, Hipra, Spain) was administered intramuscularly on the day of removal of the sponge (day -1). Goats in estrus were mated with a proven bucks (all goats:buck ratio of 7:1). Then animals were randomly divided into two groups. Goats in Control Group (n = 30) were not administered any nonsteroidal anti-inflammatory drug. Goats in Carprofen Group (n = 30) were given 1.4 mg/kg carprofen (Rimadyl XL®, Zoetis, Germany) on day 14 post mating (day of estrus = day 0). Pregnancies were determined with transabdominal real time B Mod ultrasonography (Hitachi EUB - 405) with convex prob (3.5 MHz) on day 40-42 post-mating. All goats showed estrus and estrus rate was 100% in all goats. The results showed that there were no statistical differences between the Control Group and Carprofen Group in pregnancy rates (90% and 93.3%), kidding rates (100% and 100%), multiple birth rates (66.6% and 78.5%) and litter sizes (2.03 and 2.32). The serum progesterone concentration on day 16 post mating of Carprofen Group (9.36 ng/ml) were significantly higher than those of Control Group (7.96 ng/ml) ($p \leq 0.05$). It is concluded that, after long term (17 day) progesterone administration during the transitional period, carprofen treatment increased P4 concentration but the increased P4 concentrations did not have a remarkable effect on the pregnancy rate.

KEY WORDS

Carprofen, goat, pregnancy rate, progesterone, litter size.

INTRODUCTION

Embryonic loss is a major constraint to reproductive performance [1]. Embryonic losses are reported to be in the range of 10.8% to 11% in goats [2,3]. In fact, there can be many causes of embryonic loss; a number of factors have been implicated including poor oocyte quality, defects within the embryo, an inadequate maternal environment. Maternal recognition is important for the establishment of a pregnancy. Maternal recognition of pregnancy in goats occur between day 15-17 after mating [4]. During maternal recognition of pregnancy, the embryo cannot develop sufficiently due to the conceptus or asynchronization between the uterus and the embryo. As a result, the embryo cannot synthesis interferon tau (IFN-τ) synthesis, which is required for maternal recognition [5]. Insufficient IFN-τ cannot avoid the secretion of luteolytic PGF2α from the endometrium [6,7]. The following luteolysis break up the required secretion of progesterone and results in embryonic death during early pregnancy [8].

Nonsteroidal anti-inflammatory drugs (NSAID) could be injected after mating to prevent embryonic losses and also increase fertility [9]. NSAID block the synthesis of prostaglandins via prevention of the two isoforms of cyclooxygenase (COX) COX-1 and COX-2 [10]. PGF2α release continues for 2-3 days during luteolysis [11]. Single dose administration of carprofen, a COX 2 selective NSAID, could provide longer time for the embryo to secrete sufficient IFN-τ for maternal recognition of pregnancy. In previous studies COX-2 has been found to be produced by the uterus luminal epithelium and stroma surrounding the blastocyst during implantation in rats [12,13]. In another previous study, COX-1 deficient female rats were found to have normal fertility and litter size. Because in the presence of COX-1 deficiency, COX-2 fills this gap [12]. However, female rats which have COX-2 deficiency are infertile. Because COX-2 deficiency causes ovulation, fertilization, implantation and decidualization defects [14]. COX 2 selective NSAIDs (meloxicam, carprofen, tolfenamic acid) suppress the COX-2, but they also suppress the COX-1 when the treatment doses are exceeded [5].

Corresponding Author:
Metehan Kutlu (metehankutlu2@hotmail.com).

Carprofen provides an advantage over other NSAID due to its long-acting effect with its ease of subcutaneous injection [15]. Carprofen was reported to have relatively longer half-life of 33.93 ± 7.48 h in Alpine goats [16]. However, there is no study in the present literature on carprofen treatment and its effect(s) on fertility and reproduction performance in goats. The presented study was, therefore, undertaken to determine if carprofen would affect reproductive performance throught fertility by preventing early embryonic deaths with a single dose of long-acting NSAID carprofen treatment after mating in goats during the transitional period.

MATERIAL AND METHODS

Animals

This study was carried out in the Adana City during the transitional period. This region is situated at 37°43'66.7" N latitude and 35°62'15.8" E longitude and altitude of 120 m. A total of 60 adult lactating German Fawn (75%) X Hair (25%) Crossbred goats (2-5 years old and 45-50-kg body weight) having 180-210 days in milk were used. Goats were fed with pasture-based system (12h) and not given any compound feed except for natural pasture and water was offered ad libitum. Goats (n = 60) were milked by hand once a day in which almost totally 60 kg of milk were obtained.

Synchronization protocol and treatment groups

An intravaginal sponge containing 60 mg medroxyprogesterone acetate (Esponjavet®, Hipra, Spain) for 17 days in the transitional period (June) was applied (day -18) in 60 goats. Two days before the sponge removal (day -3) all animals received 12.5 mg dinoprost tromethamine (1 ml) (Dinolytic High Concentration®, Zoetis, Spain) intramuscularly (im), while PMSG 400 IU (4 ml) (Oviser®, Hipra, Spain) was administered im on the day of removal of the sponge (day -1). Goats were randomly divided into two groups. In Control Group (n = 30) were not treated to any nonsteroidal anti-inflammatory drug. In Carprofen Group (n = 30) goats were given 1.4 mg/kg carprofen (Rimadyl XL®, Zoetis, Germany) by subcutaneous injection on day 14 post mating (day of estrus = day 0).

Estrus detection

One teaser buck was used twice a day for 1 h duration during 24 h after removal of sponges for estrus detection. Sixty goats that were determined to be in estrus were controlled and mated with one of the proven bucks (all goats: buck ratio of 7:1) under the supervision of a veterinarian.

Blood collection and hormonal assessment

On the 14th and 16th days post mating, blood samples were taken from the jugular vein into non-heparinized tubes. Serum was obtained by centrifugation of samples at 4.000 rpm for 15 minutes and were stored at at −18 °C for pending analysis.

Serum progesterone (P4) concentrations was measured by chemiluminescence immunoassay (Abbot Architect- I2000SR) with a sensitivity of ≤ 0.1 ng/ml, a specificity of 90% and intra-assay and interassay coefficients of variation 3.4-5.5% and 1.6.-2.2% for samples between 0.1 and 36 ng/ml.

Ultrasonography

In all goats, transabdominal ultrasound examination (Hitachi EUB-405, 3.5 MHz convex probe) was performed to diagnose pregnancy on day 40-42 post mating. The number of kids were determined after kidding.

The reproductive parameters

The reproductive parameters were calculated according to following methods; estrus rate (the number of goats showing estrus / the number of goats receiving intravaginal sponge × 100), pregnancy rate (the number of pregnant goats / the number of goats receiving intravaginal sponge in each group × 100), kidding rate (the number of kidding goats / the total number of pregnant goats in each group × 100), multiple birth rate (the number of multiple kidding goats / the number of pregnant goats in each group × 100), litter size (the total number of kids born/ the total number of kidding goats in each group × 100). Serum progesterone concentrations were compared between the groups for the same days. The effects of the treatments on the serum P4 concentrations were investigated.

Statistical analysis

The SAS (Version: 8.0) was used for all the the statistical analyses. Reproductive parameters were analysed using the Chi-squared test, Fisher's exact test and PROC GENMOD procedure. Comparison of serum progesterone concentrations on different days was made using the Independent Samples Test. The results were given as the percentage or mean ± standard error of the mean (± SEM). Calculated P values less than 0.05 were considered significant.

RESULTS

Results for estrus rate, pregnancy rate, kidding rate, multiple birth rate, number of kids and litter size are given in Table 1. Serum progesterone concentrations of pregnant goats are shown in Table 2 and Figure 1 according to the groups. The change in serum P4 level was determined between on the days 14 and 16 days post mating due to carprofen treatment. There were significant differences between groups on the day 16 post mating in the study ($p \leq 0.05$).

DISCUSSION

In recent years, it has been reported that exogenous nonsteroidal anti-inflammatory drugs could be administered after mating to reduce embryonic losses and increase fertility [5,9]. In fact, the presented study was aimed to prolong the life of the corpus luteum (CL) by suppressing PGF2α release at maternal recognition period with the injection of carprofen, which is a COX-2 inhibitor, and to provide longer time for the weak/slow developing embryo to produce sufficient IFN-τ.

Previous studies of carprofen treatment in dairy cattle have reported varying degrees of positive responses differing from 12% [on day 5 after insemination [17]] to 17% [on day 14 after insemination [15]] in pregnancy rate, while some others have reported decrease at about 2.5% to 10% [18-20], compared to the controls. Dogruer et al. [9] reported that double-dose diclofenac sodium injections on 15-16 days post mating increased the pregnancy rate by 25.9% in lactating goats during the transition-

Table 1 - Reproductive parameters in carprofen and control groups at the end of the study.

	Control Group (n = 30)	Carprofen Group (n = 30)	P-value
Estrus Rate	100% (60/60)		-
Pregnancy Rate	90% (27/30)	93.3% (28/30)	0.643
Kidding Rate	100% (27/27)	100% (28/28)	0.685
Multiple Birth Rate	66.6% (18/27)	78.5% (22/28)	0.487
Number of Kids	55	65	0.294
Single	9	6	-
Twin	9 (18)	13 (26)	-
Triplets	8 (24)	5 (15)	-
Quadruplets	1 (4)	2 (8)	-
Quintuplets	-	2 (10)	-
Litter Size	2.03 (55/27)	2.32 (65/28)	0.841

Table 2 - Serum progesterone concentrations (ng/ml) on different days following mating in pregnant goats.

	Control Group	Carprofen Group	P-value
Day 14 Post Mating	9.35 ± 0.67	9.62 ± 0.68	0.653
Day 16 Post Mating	7.96 ± 0.47	9.36 ± 0.66*	0.050

* Different between groups ($p \leq 0.05$).

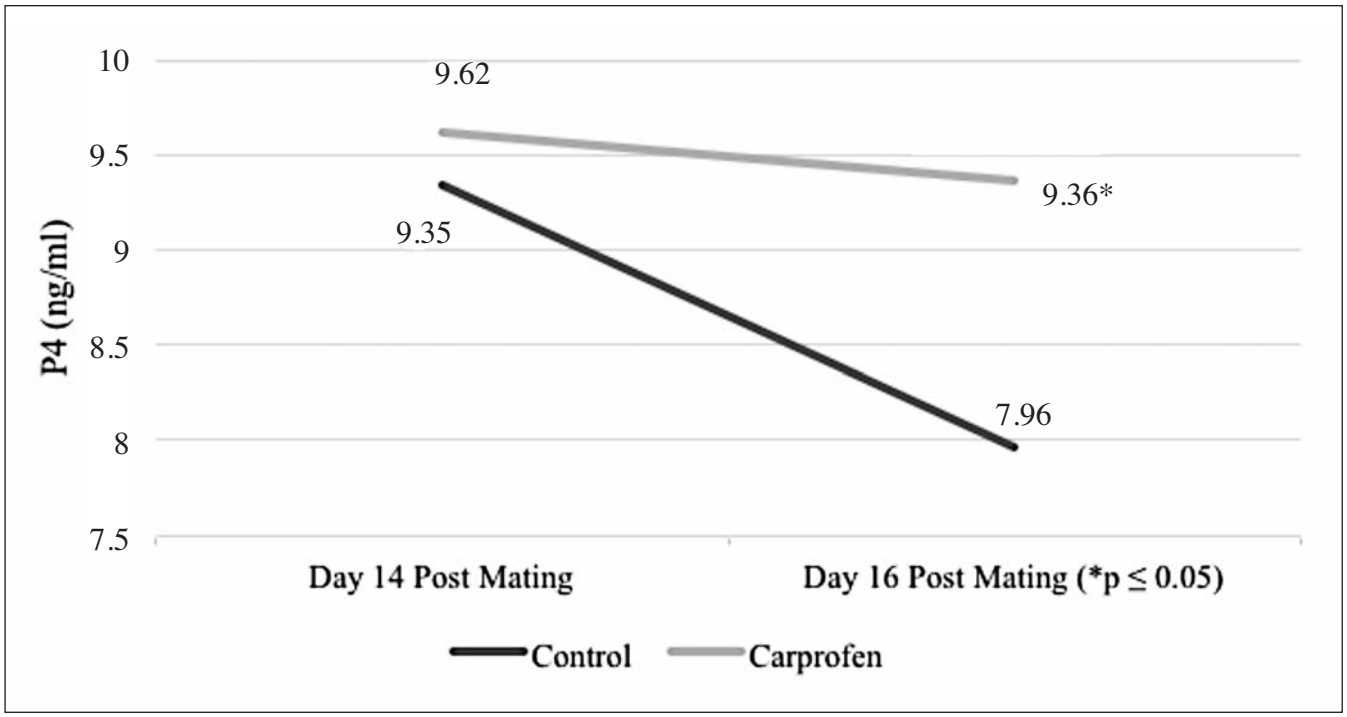

Figure 1 - Serum P4 concentrations in pregnant goats (ng/ml).

al period. In the present study, the pregnancy rates of goats were found to be 90% and 93.3% in the control group and carprofen group, respectively. There were no differences between groups ($p > 0.05$). While these results are in agreement with some of the studies, they do not comply with the values transferred from some others. The variation between the results could be attributed to the differences in animal material, animal breeds, differences in nonsteroidal anti-inflammatory drug used, route of administration, the days of treatment and NSAID dosage. Moreover, it is thought that the reason why no significant difference was found in the pregnancy rates may be due to the insufficient number of animals in this study.

Litter size is also one of the most important reproductive parameters that determines economic profitability. In this study, the litter size of goats were found to be 2.03 and 2.32 in the control group and carprofen group, respectively. There were no differences between groups ($p > 0.05$). It is thought that the reason for the numerical increase in litter size in the carprofen group is due to the reduction of embryonic mortality rate and the maintenance of multiple pregnancies, especially in goats with multiple pregnancies. In addition, it is believed that some embryos probably lost in the early period in the control group. Because, embryonic deaths can occur when there is a difference of more than 12-24 hours between the stages of embryo development and the maternal environment. Because the asynchrony between the maternal environment and the embryo weakens the development of the embryo [15,21]. Poorly developing embryos produce small amounts of IFN-τ and cannot prevent PGF2α release during maternal recognition, resulting in embryonic deaths [15]. Moreover, a rising in P4 concentration may increase the IFN-τ and prevent PGF2α release and luteolysis of CL [22], resulting in the formation of healthy embryos [23]. For this reason, it is thought that one or more of the embryos may have lost in the early period, especially in goats with multiple embryos in the control group. Because carprofen treatment prevented the decrease of progesterone level by suppressing luteolysis, and it is thought that low quality or slow/weak developing embryos that do not secrete enough IFN-τ allow longer time to continue their development, and as a result, litter size is thought to be increased.

Having a certain level of progesterone after mating is very important for the establishment and maintenance of pregnancy [24]. The 15th day post mating is a very important period for the mother to recognize the pregnancy in goats [25] which coincides with the onset of the regression of the CL [22]. Delayed increase or an insufficient secretion of progesterone during the early luteal phase may cause decelerated embryonic growth [18]. Ewes with lower progesterone concentration after mating causes embryonic loss [24]. In ewes, the progesterone obtained as a result of GnRH/hCG treatment performed at different times from the 36th hour after the removal of the sponge to day14 after mating may increase the production of interferon-τ [23,26]. This prevents luteolysis by inhibiting the secretion of PGF2α and thus maintains the pregnancy [27]. In this study, the carprofen treatment was preferred on the day 14 after mating; as the CL regression starts on the days 15-17 post mating (critical maternal recognition days cited in the literatures) and the undesired decrease in P4 concentration reaches the baseline 72 h after injection.

In fact, the previous literatures with respect to serum P4 concentration of ewes and cows injected NSAID showed variable results. Ake-Lopez et al. [28] reported that the period of the luteal phase (depend on P4 concentration) for ewes treated with flunixin meglumine (2.2 mg/kg) twice a day on the days 11-19 post mating was greater ($p \leq 0.05$) than for control group (15.3 ± 0.40 days vs 12.2 ± 0.28 days). Aiumlamai et al. [29] treated with flunixin meglumine (2.2 mg/kg) four times daily from day 15 of the estrous cycle during 7 days (a total of 28 injections) through the vena jugularis on cows in their studies. The results of Aiumlamai et al. [29] showed that after flunixin treatment the levels of 15-ketodihydro-PGF2α, quickly decreased to baseline and that the pulsatile release during the expected luteolytic period was delayed. PGF2α release started about one day after ending of flunixin meglumine treatments and then luteolysis happened. Progesterone concentrations were normal during the flunixin meglumine treatments and decreased concurrently with the pulsatile release of PGF2α. However, Merril et al. [30] treated with single administration 1.1 mg/kg of flunixin meglumine approximately 14 d after artificial insemination on cows in their study. Serum progesterone levels differed ($p \leq 0.05$) by treatment for transported group (3.4 ± 0.2 ng/ml), transported + flunixin meglumine group (3.3 ± 0.2 ng/ml), and nontransported group (2.9 ± 0.2 ng/ml), respectively. In the present study, serum P4 concentrations in carprofen group increased P4 concentration on the day 16 post mating (9.36 ng/ml vs 7.96 ng/ml), due to the carprofen treatment ($p \leq 0.05$) as expected.

CONCLUSIONS

Long term progestagen receiving with PMSG + PGF2α to lactating German Fawn × Hair crossbred goats could stimulate estrus during the transitional period. Carprofen treatment increased serum progesteron level but had no differences on reproductive parameters between groups.

Acknowledgments

The authors thank Prof. Dr. Hasan Rustu Kutlu for the reading and correction of the manuscript. We also thank the Zoetis Animal Health Turkey (Süleyman Cömertpay) for donation of Dinolytic High Concentration and Rimadyl XL. A limited part of this article was presented orally at the 1st International Cukurova Agriculture and Veterinary Congress, on 9-11 October 2020.

Financial Support

This research received no grant from any funding agency/sector.

Ethics approval

Prior to the execution of study, an approval was obtained from Adana Veterinary Control Institute Ethics Committee, Adana, Turkey (5-1094, 2020). All institutional and national guidelines for the care and use of laboratory animals were followed.

References

1. Wang X., Wu G., Bazer F.W. (2016). mTOR: The master regulator of conceptus development in response to uterine histotroph during pregnancy in ungulates. In: Molecules to Medicine with mTOR Translating Critical Pathways Into Novel Therapeutic Strategies Book, Ed. Maiese K, 25-35, Elsevier, New York.
2. Engeland I.V., Waldeland H., Andresen E., Loken T., Bjorkman C., Bjerkas I. (1998). Foetal loss in dairy goats: An epidemiological study in 22 herds. Small Rumin Res, 30: 37-48.
3. Anwar M., Ahmad K.M. (1999). Ovulation rate, number of fetuses and embryo loss in Teddy goats of Pakistan. Small Rumin Res, 31: 281-283.
4. Gnatek G.G., Smith L.D., Duby R.T., Godkin J.D. (1989). Maternal recognition of pregnancy in the goat: effects of conceptus removal on interestrus intervals and characterization of conceptus protein production during early pregnancy. Biol Reprod, 41: 655-663.
5. Alkan H., Erdem H. (2018). Reproductive use of nonsteroidal antiinflammatory drugs in cows. Atatürk Üniversitesi Vet Bil Derg, 131: 112-120.
6. Diskin M.G., Murphy J.J., Sreenan J.M. (2006). Embryo survival in dairy cows managed under pastoral conditions. Anim Reprod Sci, 96: 297-311.
7. Mann G.E., Lamming G.E. (2001). Relationship between maternal endocrine environment, early embryo development and inhibition of the luteolytic mechanism in cows. Reproduction, 121: 175-180.

8. Thatcher W.W., Moreira F., Santos J.E.P., Mattos R.C., Lopes F.L., Pancarci S.M., Risco C.A. (2001). Effects of hormonal treatments on reproductive performance and embryo production. Theriogenology, 55: 75-89.
9. Dogruer G., Karaca F., Sarıbay K., Ate C.T. (2012). The effect of postmating diclofenac sodium injections on the pregnancy rate of the lactating goats synchronized by buck effect at the initiation of breeding season. YYU Vet Fak Derg, 23: 19-22.
10. Anderson K.L., Neff-Davis C.A., Davis L.E., Bass V.D. (1990). Pharmacokinetics of flunixin meglumine in lactating cattle after single and multiple intramuscular and intravenous administrations. Am J Vet Res, 51: 1464-1467.
11. Guzeloglu A., Erdem H., Sarıbay K., Thatcher W.W., Tekeli T. (2007). Effect of the administration of flunixin meglumine on pregnancy rates in Holstein heifers. Vet Rec, 160: 404-406.
12. Reese J., Brown N., Paria B.C., Morrow J., Dey S.K. (1999). COX-2 compensation in the uterus of COX-1 deficient mice during the pre-implantation period. Molecular and Cellular Endocrinology 150: 23-31.
13. Chakraborty I., Das S.K., Wang J., Dey S.K. (1996). Developmental expression of the cyclooxygenase- 1 and cyclo-oxygenase-2 genes in the peri-implantation mouse uterus and their differential regulation by the blastocyst and ovarian steroids. Journal of Molecular Endocrinology 16: 107-122.
14. Lim H., Paria B.C., Das S.K., Dinchuk J.E., Langenbach R., Trzaskos J.M., Dey S.K. (1997). Multiple female reproductive failures in cyclooxygenase 2-deficient mice. Cell, 91: 197-208.
15. Alkan H., Satilmis F., Yesilkaya Ö.F., Çiftçi M.F., Alkan K.K., Erdem H. (2021). The effect of carprofen treatment on conception rate after insemination in cows with increased days in milk. Kocatepe Vet J, 14: 83-90.
16. Özsemerci M. (2017). Comparative pharmokinetics of carprofen oral, intravenous and subcutaneous administration in goats. Master Thesis, Adnan Menderes University Health Science Institute, Aydın, Turkey.
17. Özkök S. (2019). The effect of carprofen on the conception rate on the 5th day after insemination in holstein breed cows. Master Thesis, Selçuk University Health Science Institute, Konya, Turkey.
18. Von Krueger X., Heuwieser W. (2010). Effect of flunixin meglumine and carprofen on pregnancy rates in dairy cattle. J Dairy Sci, 93: 5140-5146.
19. Heuwieser W., Iwersen M., Goetze L. (2011). Efficacy of carprofen on conception rates in lactating dairy cows after subcutaneous or intrauterine administration at the time of breeding. J Dairy Sci, 94: 146-151.
20. Satilmis F., Alkan H., Yesilkaya Ö.F., Çiftçi M.F., Erdem H. (2020). Efficacy of carprofen on conception rates in lactating dairy cows after subcutaneous or intrauterine administration at the time of breeding age. Eurasian J Vet Sci, 36: 306-311.
21. Lonergan P., Forde N. (2014). Maternal-embryo interaction leading up to the initiation of implantation of pregnancy in cattle. Animal, 8: 64-69.
22. Bazer F.W., Ott T.L., Spencer T.E. (1998). Maternal recognition of pregnancy: comparative aspects. Placenta, 19: 375-386.
23. Khan T.H., Beck N.F.G., Khalid M. (2007). The effects of GnRH analogue (buserelin) or hCG (chorulon) on day 12 of pregnancy on ovarian function, plasma hormone concentrations, conceptus growth and placentation in ewes and ewe lambs. Anim Reprod Sci, 102: 247-257.
24. Ashworth C.J., Sales D.L., Wilmut I. (1984). Patterns of progesterone secretion and embryonic survival during repeated pregnancies in Damline ewes. Page 741-743 in Proc 10th International Congress Animal Reproduction and Artificial Insemination, Urbana-Champaign, USA.
25. Weise D.W., Newton G.R., EmesiIh G.C. (2003). Effects of day of the estrous cycle or pregnancy on protein secretion by caprine endometrial tissues. Biol Reprod, 49: 522-527.
26. Beck N.F.G., Jones M., Davies B., Mann G.E., Peters A.R. (1996). The effect of GnRH analogue (buserelin) treatment on day 12 post mating on ovarian structure and plasma progesterone and oestradiol concentration in ewes. Anim Sci, 63: 407-412.
27. Khan T.H., Beck N.F.G., Mann G.E., Khalid M. (2006). Effect of postmating GnRH analogue (buserelin) treatment on PGF2a release in ewes and ewe lambs. Anim Reprod Sci, 95: 107-115.
28. Ake-Lopez R., Segura-Correa J.C., Quintal-Franco J. (2005). Effect of flunixin meglumine on the corpus luteum and possible prevention of embryonic loss in Pelibuey ewes. Small Rumin Res, 59: 83-87.
29. Aiumlamai S., Odensvik K., Stabenfeldt G., Kindhal H. (1990). Regulation of prostaglandin biosynthesis with flunixin meglumine in the bovine species. J Vet Med A, 37: 16-22.
30. Merril M.L., Ansotegui R.P., Burns P.D., MacNeil M.D., W. G.T. (2007). Effects of flunixin meglumine and transportation on establishment of pregnancy in beef cows. J Anim Sci, 85: 1547-1554.