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Original article

Effects of tocolytic drugs (*isoxsuprine hydrochloride*) during the implantation period in pregnant sheep

N.K. Akbulut, Y. Kal

Bahri Dağdaş International Agricultural Research Intitute. Ereğli street number: 10Y Karatay / Konya/Türkiye

Abstract

In this study, the effects of isoxsuprine hydrochloride applied 14 and 15 days after insemination in Anatolian Merino Sheep on lamb yield and some blood parameters were investigated. The research was conducted during the breeding season and 54 ewes inseminated on the same day were used. The ewes were assigned to three groups. Group I: For the placebo effect, physiological saline was injected on the 14th day into half of the control group and on the 15th day into the other half after insemination (n=18). Group II: Tocolytic drug was injected on the 14th day after insemination (n=18). Grop III: Tocolytic drug was injected on the 15th day after insemination (n=18). As the tocolytic drug, isoxsuprine hydrochloride (HCl) 3 ml (Utelax, Sanovel, Türkiye) was used intramuscularly. The number of pregnant and viviparous ewes, single and multiple birth ewes, lambs per viviparous ewes were determined as reproductive parameters. Blood progesterone, cortisol and calcium concentration were determined. As a result, it was determined that the single application of isoxsuprine hydrochloride (3 ml) in the implantation period did not have a positive or negative effect on reproductive parameters, and did not change the blood progesterone, calcium and cortisol concentration in ewes.

Key words: isoxsuprine hydrochloride, reproductive parameters, progesterone, cortisol, calcium

Introduction

Isoxsuprine hydrochloride is a beta-adrenergic agonist, an effective vasodilator and the first active ingredient which was reported as a tocolytic agent to prevent uterine contractions. Tocolytic drugs are used in preterm labor, threatened abortion, controlled calving and nocturnal delivery, reduction of neonatal morbidity and mortality in dystocia, obstetrical operations (cesarean section and fetotomy), uterine prolapse, uterine torsion, and embryo biotechnology in animal reproduction (Bhoi 2022). There is no literature information about its use in early gestational period.

In sheep, embryonic deaths cause significant economic losses due to the delay of conception during the breeding season, therefore the prolongation of the

Correspondence to: N.K. Akbulut e mail: nkakbulut@gmail.com

lambing season and the decrease in the number of offspring in multiple pregnancies (Saribay and Erdem 2007). Embryonic deaths often occur during critical periods such as maternal recognition of pregnancy, nidation, implantation, and the first stage of placentation (Yotov 2012). It is reported that the embryonic mortality rate is between 20-30%, most of these deaths occur before the 30th day and the losses after the 30th day are between 1-4% in sheep (Wilkins 1997, Saribay and Erdem 2007).

In addition to embryonic deaths, one of the most important causes of infertility in humans (Macklon et al. 2002) and animals (Diskin and Morris 2008) is failure in embryo implantation (Marei et al. 2017). Embryo implantation is considered as the first step towards embryo-maternal interaction and it is critical for further embryo development (Chen et al. 2013). Implantation begins with close contact between the apical plasma membranes of the conceptus trophectoderm and the uterine luminal epithelium, and ends with a placenta formation (Bowen and Burghardt 2000). For embryo nidation and decidual invasion, it is a prerequisite that surface the uterus must be relaxed, when implantation occurs and the embryo interacts with the luminal epithelium (Bulletti and De Ziegler 2005). In sheep, the embryo transforms from a spherical form to a tubular and filamentous structure between the 12th and 15th day and adhesion occurs until the 16th day (Spencer et al. 2004). Our hypothesis is the use of tocolytic drugs during the embryo implantation period can provide a better embryo implantation by reducing uterine contractions. In this study, the effects of isoxsuprine hydrochloride applied on the14th and 15th day after insemination in Anatolian Merino Sheep on lamb yield and some blood parameters were investigated.

Materials and Methods

Animals

As animal material, 2-4 years old Hasak ewes breed in the Konya Bahri Dağdaş International Agricultural Research Institute, Small Ruminant Department were used. The research was conducted during the breeding season and 54 ewes inseminated on the same day were used. The ewes were assigned to three groups. Group I: For the placebo effect, physiological saline were injected at the 14th day into half of the control group and at the 15th day into the other half after insemination (n=18). Group II: Tocolytic drug was injected on the 14th day after insemination (n=18). Grop III: Tocolytic drug was injected on the 15th day after insemination (n=18). As the tocolytic drug, isoxsuprine hydrochloride (HCl) 3 ml (Utelax, Sanovel, Türkiye) was administered intramuscularly. The pregnancy statuses of ewes were examined by determining whether was an embryo with a heartbeat 27 days after mating (Akbulut and Çelik 2021) and non-pregnant ewes were excluded from the project. The ethics committee permission for the study was obtained from the Animal Experiment Local Ethics Committee of the Bahri Dağdaş International Agricultural Research Institute (28.10.2021-127).

Reproductivre parameters

The number of pregnant ewes, viviparous ewes, single birth and twin birth, lambs per viviparous ewes were determined as reproductive parameters.

Blood samples

Blood samples from all ewes were collected from the jugular vein into vacutainers containing sodium EDTA, 2 hours after the injections. Blood samples were centrifuged at 3000 *g* for 10 min at 5°C and aliquots were stored in a freezer at -20°C until analysis. Plasma progesterone (Artitect i1000 SR Abbott U.S.) and cortisol (Abbott Architect-8D15, U.S.) concentrations were determined using commercial kits utilizing the Chemiluminescence Enzyme Immunoassay (CLIA) method (Abbott Architect I 1000 SR). Calcium was analyzed by colorimetric method using an autoanalyzer (Abbott Architect C 8000).

Statistical analysis

One-way analysis of variance (One Way Anova) was used to evaluate blood parameter data. The SPSS 23 (Statistical Package for the Social Sciences) program was used for data analyses.

In the analysis of intergroup reproductive parameters, the hypothesis test for differences between proportions (Z test) was used. The following formula was used for the z test:

$$z = \frac{(\hat{P}_1 - \hat{P}_2) - (P_1 - P_2)}{\sqrt{P_0 - q_0\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}} \sim N_z(0;1)$$

The statistics in the equation are calculated as follows:

$$\hat{P}_1 = \frac{x_1}{n_1} \qquad \hat{P}_2 = \frac{x_2}{n_2} \qquad P_0 = \frac{x_1 + x_2}{n_1 + n_2} \qquad q_0 = 1 - P_0$$

in the equations

x_1 – shows the number of samples drawn from group 1 with n_1 sample width

x_2 – shows the number of samples drawn from group 2 with n_2 sample width

Table 1. Some reproductive parameters in the sheep groups.

	Number of pregnant ewes	Number of viviparous ewes	Number of single birth	Number of twin birth	Total number of lambs	Lambs per viviparous ewes
Grup I	18	16	12	4	20	1.25
Grup II	18	16	14	2	18	1.125
Grup III	18	17	16	1	18	1.05

Differences between groups were found to be statistically insignificant ($p>0.05$).

Table 2. Progesterone, calcium and cortisol plasma concentrations in the sheep.

	Progesterone Mean S.D.	Calcium Mean S.D.	Cortisol Mean S.D.
Grup I (n =18)	2.27 ± 0.15	8.00 ± 0.30	0.17 ± 0.08
Grup II (n =18)	2.38 ± 0.20	8.25 ± 0.22	0.37 ± 0.11
Grup III (n =18)	2.39 ± 0.23	8.43 ± 0.46	0.29 ± 0.11

Differences between groups were found to be statistically insignificant ($p>0.05$).
S.D. Standard deviation.

Results

In Table 1 some reproductive parameters in the sheep groups are given.

The blood progesterone, calcium and cortisol concentration in the groups are given in Table 2.

Discussion

Relaxion of the uterine surface is an important event for better embryo implantation during the early pregnancy in sheep (Bulletti and De Ziegler 2005). Therefore, it is predicted that the use of tocolytic drugs in this period will lead to better implantation and less embryonic losses.

Although 18 pregnant sheep were found in each group by the ultrasonographic method, the numbers of lambing sheep were 16 (Group I), 16 (Group II) and 17 (Group III) respectively in groups. The differences between groups in terms of other characteristics (Table 1) were found statistically insignificant ($p>0,05$). Therefore, isoxsuprine hydrochloride has not had a positive or negative impact on the reproductive parameters. Some studies have shown that the plasma half life of intraveneous isoxsuprine hydrochloride is lower than 3 hours (Erkert and Macallister 2002). In this study, it was thought that the dose and single application of isoxsuprine hydrochloride did not have a positive effect on implantation.

β-adrenergic agonists not only inhibit uterine contractions, but also increase progesterone and maintain pregnancy (Viswanathan and Chaudhari 2006). Progesterone is strictly necessary for the survival and elongation of the conceptus in sheep (Burns et al. 2018). Csapo and Herzczeg reported that isoxsupine administered increased progesterone levels significantly ($p<0.001$) compared to the placebo group in patients with premature birth symptoms (Csapo and Herzczeg 1977). Fujimoto et al. reported that ritodrine hydrochloride, a β-adrenergic agonist, did not cause any change in plasma progesterone levels in their study in sheep (Fujimoto et al. 1983). In our study, isoxsuprine hydrochloride did not cause any change in blood progesterone concentration in sheep (Table 2).

β-adrenergic agonists exert their effects via cAMP. Increasing the intracellular concentration of cAMP increases the intracellular binding of Ca++ and decreases the activation of myosine light chain kinase. (Altıntaş 1986). Vural et al. (2016) examined the effect of isoxsuprine hydrochloride on blood calcium levels in sheep and reported that, the calcium level increased in the isoxsuprine group, but the difference was statistically insignificant ($p>0.01$), similar to our study (Table 2).

At the same time, the effect of isoxsuprine hydrochloride on blood cortisol levels was investigated in the present study. Cortisol is a glucocorticoid hormone produced by the adrenal cortex in response to stress (Medan et al. 2015). Cortisol causes an increase in estrogen concentration and a decrease in progesterone concentration. Thus, by stimulating the release of PGF2α, it causes an increase in the oxytocin, which increases myometrial contractions (Nagel et al. 2019, Akbulut et al. 2021) and may adversely affect embryo implanta-

tion. In our study, no statistically significant differences were found in blood cortisol levels between the groups (Table 2).

Conclusion

As a result, it was determined that the single application of isoxsuprine hydrochloride (3 ml) in the implantation period did not have a positive or negative effect on reproductive parameters, and did not change the blood progesterone, calcium and cortisol concentration in sheep. We think that new studies are needed on this subject.

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