Effect of flunixin meglumine or prostaglandin E2 treatment 15 days after breeding on fertility in Saanen does

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Abstract

The objective of this study was to determine the effects of timely injections of flunixin meglumine (FM) or vaginal application of prostaglandin E2 (PgE2) on pregnancy, fertility, fecundity, and prolificacy rates in Saanen goats. One hundred and sixty-three nonlactating Saanen does were treated with a flugestone acetate (20 mg)-containing intravaginal sponge for 12 days. They also received eCG (400 IU) and a PGF2α analogue (50 μg) 10 days after progestagen priming. Does detected in estrus were mated and assigned randomly to one of three treatment groups. The PgE2 group (N = 40) received PgE2 (2.5 mg) intravaginally 15 days after mating. The FM group (N = 54) received flunixin meglumine (total dose, 100 mg) intramuscularly 15 days after mating. Flunixin meglumine was administered at 9:00 AM. Animals in the control group (N = 69) received no treatment. Pregnancy was diagnosed using transrectal ultrasonography (B-mode at 8 MHz) 30 days after mating. The pregnancy rate was significantly greater (P < 0.01) after 30 days in goats treated with PgE2 and also in the control group than in those treated with FM (67.5%, 59.4%, and 42.5%, respectively). The pregnancy rate did not differ between the PgE2 and the control group. The pregnancy and fertility rate were lowest in the FM group compared with the other groups. There was no significant difference in the prolificacy rate among experimental groups. In conclusion, our results showed that FM administration during a late luteal phase is detrimental to early pregnancy in goats.

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1. Introduction

Early pregnancy loss in dairy goats can negatively affect animal production. Nearly 40% of all pregnancy losses occur 15 to 17 days after estrus, a critical period during which the conceptus must produce sufficient quantities of IFN-τ (interferon-tau) to prevent pulsatile prostaglandin secretion and to maintain the corpus luteum (CL) [1,2]. In nonpregnant ruminants, the CL is broken down by a series of events initiated by the pulsatile release of prostaglandin \( \text{PGF}_{2\alpha} \) from the uterine endometrium during the late luteal phase of the estrous cycle. The uterus has only a few days to recognize the presence of an embryo, and any factor affecting this synchrony could affect embryo survival and ultimately fertility [3].

Prostaglandin E2 (PgE2), a lipid mediator produced by most mammalian tissues, regulates multiple biological processes in normal and pathological conditions. In addition to being a key mediator of inflammation, PgE2 was recently demonstrated to play an important role in the establishment of pregnancy. The embryo itself secretes and/or stimulates the endometrium to secrete PgE2 to prevent luteolysis during early pregnancy [4]. It is generally...
accepted that PgE2 and PGF2α are involved in the life span of the CL but with opposite actions. In contrast to PGF2α, PgE2 has a luteotropic role in ruminant reproduction [5]. Endometrial production of the luteotropin PgE2 in ewes increases during maternal recognition of pregnancy beginning on Day 13, thus increasing the ratio of PgE2 to PGF2α secreted by the endometrium [6]. In the uterine luminal epithelium, the biosynthesis of PgE2 and PGF2α is achieved by the sequential actions of three groups of enzymes. First, membrane-bound and secretory phospholipase A2 isoforms convert phospholipids to arachidonic acid. Next, the cyclooxygenases (COXs) convert arachidonic acid into prostaglandin H2, the substrate for specific isomerases that generate biologically active prostaglandins. Finally, terminal PgE2 synthase or prostaglandin F synthase enzymes isomerize prostaglandin H2 into PgE2 or PGF2α, respectively. Numerous studies have demonstrated the existence of two distinct genes encoding isoforms of COX, namely COX-1 and COX-2 [2,7].

Currently there are several strategies, such as lengthening the life span of the CL, being used to prevent early embryonic loss. One of these strategies involves the use of flunixin meglumine (FM), a potent nonsteroidal anti-inflammatory drug that inhibits the synthesis of the COX enzyme [8]. The rationale for administering FM is to inhibit the activity of prostaglandin G/H synthase-2 and to reduce uterine synthesis of PGF2α, which both contribute to antiluteolysis during early pregnancy. Previous reports showed FM to inhibit PGF2α secretion by bovine [9], porcine [10], mare [11], and goat [12] uteri. Guzeloglu et al. [13] reported that timely injections of FM to heifers increase pregnancy rates.

We hypothesized that support of the CL with vaginal application of PgE2 or indirect inhibition of PGF2α synthesis using FM treatment can increase the pregnancy rate by reducing embryonic loss in Saanen goats. To the best of our knowledge, there is no previous report on the effect of PgE2 on the pregnancy rate in goats.

2. Materials and methods

This study was carried out at a commercial dairy goat farm located in Isparta, Turkey. The climate in the region is continental, with an average annual temperature of 12.1 °C and an average annual rainfall of 498 kg/m². These experiments were approved by the Animal Ethics Committee of the Faculty of Veterinary Medicine.

2.1. Animals

One hundred and sixty-three multiparous, nonlactating Saanen does 2 to 4 years of age (2.8 ± 0.05, mean ± SEM) and weighing between 45 and 75 kg (55.2 ± 0.7) with good body condition score (BCS) (3.0 ± 0.05) were used in this study. The BCS was assessed on a 1 to 5 scale at the time of enrollment. All animals were maintained in paddocks and allowed access to a mixture of oats and alfalfa, water, and a mineral lick ad libitum. Animals also received a commercial supplement (250 g) containing 14% crude protein daily.

2.2. Estrus synchronization

All does were treated with 20 mg flugestone acetate sponges (Chronogest CR, Intervet, Turkey) for 12 days. They also received 400 IU of eCG (Intervet) and 50 µg of a PGF2α analogue (d-cloprostenol; Dalmazin, Vetas, Turkey) 10 days after progestagen priming. Does in estrus were mated (hand mating) and the dates of mating were registered. Does that showed no estrus after sponge removal were excluded from the study.

2.3. Treatments

The mated does were divided randomly into three groups. The groups were approximately equal with regard to average BCS and body weight. One group, the PgE2 group (N = 40) received 2.5 mg PgE2 (one-quarter of a silicone implant, Propess; Controlled Therapeutics) intravaginally 15 days after mating. The second group, the FM group (N = 54), received 100 mg flunixin meglumine (Finadyne; Schering-Plough) intramuscularly 15 days after mating. The FM group was administered only one dose at 9:00 AM. Animals in the control group (N = 69) received no treatment. Pregnancy was diagnosed using transrectal ultrasonography (B-mode at 8 MHz) 30 days after mating.

2.4. Statistical analysis

Pregnancy rate (goats pregnant/goats presented to bucks), fertility (goats kidding/goats presented to bucks), prolificacy (kids born/number of kidings), and fecundity (kids born/goats presented to bucks) were determined. The difference between two population proportions, p1 and p2, was compared by using the z test. All analyses were performed using the SAS statistical package (Version 8.1, Cary, NC, USA).

3. Results

All results are shown in Table 1. There was no statistical difference in the age, BCS, and body weight among groups. The pregnancy rate was significantly greater (P < 0.01) in goats treated with PgE2 than in those receiving FM (67.5%)

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Age, mean ± SEM</th>
<th>BCS, mean ± SEM</th>
<th>BW, mean ± SEM</th>
<th>PR, % (N)</th>
<th>Fertility, % (N)</th>
<th>Prolificacy</th>
<th>Fecundity</th>
</tr>
</thead>
<tbody>
<tr>
<td>PgE2</td>
<td>40</td>
<td>2.70 ± 0.11</td>
<td>2.95 ± 0.09</td>
<td>53.6 ± 1.38</td>
<td>67.5% (27/40)</td>
<td>57.5% (23/40)</td>
<td>1.95% (45/23)</td>
<td>1.12% (45/40)</td>
</tr>
<tr>
<td>FM</td>
<td>54</td>
<td>2.85 ± 0.11</td>
<td>3.00 ± 0.09</td>
<td>54.0 ± 1.30</td>
<td>42.5% (23/54)</td>
<td>38.8% (21/54)</td>
<td>1.95% (41/21)</td>
<td>0.75% (41/54)</td>
</tr>
<tr>
<td>Control</td>
<td>69</td>
<td>2.95 ± 0.07</td>
<td>3.21 ± 0.08</td>
<td>57.3 ± 1.19</td>
<td>59.4% (41/69)</td>
<td>56.5% (39/69)</td>
<td>1.79% (70/39)</td>
<td>1.01% (70/69)</td>
</tr>
</tbody>
</table>

Within a column, means without a common superscript letter differ significantly. a,b; j,k; h,j = P < 0.01; b,c; d,e; f,g; h,k = P < 0.05.

Abbreviations: BCS, body condition score; BW, body weight; FM, flunixin meglumine; PgE2, prostaglandin E2; PR, pregnancy rate.
vs. 42.5%, respectively). There was no difference in pregnancy rate between the PGE2 group and the control group. The fertility rate was lower in the FM group than in the other groups (P < 0.05). There was no significant difference in the prolificacy rate among experimental groups. The fecundity rate was greater in the PGE2 group than in the FM and the control group.

4. Discussion

The prevalence of embryonic loss in goats is approximately 11%. Most embryonic losses are likely to occur between 8 and 17 days of pregnancy. In goats, the incidence of early embryonic loss is greater after estrus synchronization treatments which have been widely used in small ruminant reproduction [14]. A significant portion of this loss is thought to result from asynchrony between the conceptus and uterine endometrium and/or inadequate production of IFN-γ, an antiluteolytic signal, by the conceptus [15]. Guzeloglu et al. [13] reported that poorly developed embryos are viable but develop slower, indicating that maternal recognition of pregnancy induced by IFN was not initiated at the appropriate time. One of the aims of this study was to diminish embryonic loss using FM administration on Day 15 after mating with the help of COX inhibition also temporarily suppressing the intra-uterine PGF2α. Flunixin meglumine is sometimes used to increase the pregnancy rate in cattle. Scenna et al. [16] reported an improvement in the pregnancy rate in cows receiving FM compared with the control group (64.5% vs. 53.5%, respectively). These authors suggest that developmentally compromised embryos (fair quality) are most susceptible to deleterious effects of PGF2α when compared with good quality embryos [16]. Likewise, Guzeloglu et al. [13] reported a greater pregnancy rate in heifers treated with FM than in control heifers (76.9% vs. 50%, respectively). Doğruer et al. [17] showed that the pregnancy rate was also greater in repeatedly bred heifers that were treated with FM than in control heifers. Ake-Lopez et al. [8] reported that administration of FM (daily from Day 11 to 18) effectively increases the length of the estrous cycle and luteal phase but has no effect on lambing rate in ewes. In the current study, the pregnancy rate of the FM-treated group was lower than the control group (42.5% vs. 59.4%, respectively). Our results are in contrast to these reports concerning pregnancy rates in cows and heifers, after treatment with FM. In this study, administration of a single intramuscular injection of FM at the recommended anti-inflammatory dose significantly decreased pregnancy, fertility, and fecundity rates in goats. The difference between our study and the other studies might be attributed to diverse mechanisms used by different species to intervene in maintenance of the CL function. Another probable reason for the adverse effect we have found, might be the inhibitory effect of FM, a nonselective cyclic oxygenase inhibitor, on COX-1 and COX-2 enzymes. Inhibition of COX-2 during early pregnancy might decrease PGE2 production by the endometrium. Prostaglandin E2 is a putative luteotrophin and luteoprotectant, and its production by the endometrium and conceptus probably serves as an alternative mechanism for sustaining CL function during early pregnancy [18]. Several investigators have reported an alteration in the PGE2:PGF2α ratio in favor of PGE2 during pregnancy [6,19]. Others have demonstrated in the pig and the ewe that PGE2 directly inhibits PGF2α-induced regression of the CL [20,21]. In the present study, pregnancy, fertility, and fecundity rates were significantly greater in the PGE2 group than in the FM group. However there was no difference between the PGE2 group and the control group. The fecundity rate was significantly greater in the PGE2 group than in the FM and the control group. Our results illustrate that PGE2 might play an important role in early pregnancy in goats. The cause of detrimental effects of FM on the pregnancy rates in our study was unclear. However, prostaglandins are one of the most important mediators of embryo-uterine communication during the initiation of implantation. Reese et al. [22] demonstrated that simultaneous inhibition of COX-1 and COX-2 more severely affects early pregnancy than inhibition of either isoflurane alone in mice. Similarly, anti-implantation effects of various nonsteroidal anti-inflammatory drugs were also reported in rats [23]. Nonselective inhibition of COX-1 and COX-2 using FM treatment might be cause of the lower pregnancy rates in the current study.

On the basis of maternal recognition days reported in the literature, FM was administered 15 days after mating in the current study. However, timing of the FM application for blocking of luteolytic process is controversial. Some authors [8] argued that the luteolytic process had already started at Day 11 after mating in ewes. Ake-Lopez et al. [8] suggested that the most appropriate time to administer FM to ewes is on Day 9 or 10 of the estrous cycle before the onset of CL lysis. In another study, Battye et al. [12] found that luteal regression can be observed as early as 2 days after their formation and treatment with FM prevents luteal regression in superovulated goats. The establishment of the most appropriate time, if any, to administer FM for the inhibition of the luteolytic process needs more research.

Ake-Lopez et al. [8] reported that prolificacy is not affected by FM treatment in ewes because FM affects the COX enzyme which inhibits the secretion of PGF2α by the uterus and not the ovulation rate itself. In agreement with our results, PGE2 or FM treatment did not affect the prolificacy rate in Saanen goats.

Goat production concentrated in developing countries contributes largely to the livelihoods of low- and medium-income farmers [24]. In these countries, the dairy goat sector requires a systemic approach, whereby nutrition, animal health, breeding, know-how, inputs, and technologies must be assembled [24]. We hope that the results of this study will help dairy goat breeders to improve the reproductive efficiency in dairy goat production systems.

4.1. Conclusions

Our results showed that FM administration during a late luteal phase is detrimental to early pregnancy in goats. In contrast, the intravaginal administration of PGE2 can be used to increase the fecundity of goats.
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