EFFектS OF LEVAMISOLE ON PREGNANCY IN EWES

SUКRU METIN PАНСАRCI, ORSAN GUNCOR, KUTLAY GУRBULAK, METIN CENЕSIZ1, MEHMET KАYA1, SЕNA CENЕSIZ2, AND AYDIN GUZЕLOGLУ3

Department of Obstetrics, Gynaecology, and Reproduction, 1Department of Physiology, 2Department of Biochemistry, Faculty of Veterinary Medicine, University of Kafkas, 36100, Kars, Turkey
3Department of Obstetrics, Gynaecology, and Reproduction, Faculty of Veterinary Medicine, Selcuk University, 42075, Konya, Turkey
smpancarci@hotmail.com

Received for publication February 19, 2007

Abstract

The effects of levamisole, as an anthelmintic or immunomodulator, on pregnancy were investigated in ewes. Immunomodulatory and anthelmintic doses of levamisole and physiologic saline were injected on days 0 (oestrus), 7, 14, and 21 in group I (immunomodulatory dose; n=7), group II (anthelmintic dose; n=6), and group III (control; n=6), respectively. The first service pregnancy rate, tended to be lower (P<0.06) in group I (28.6%) compared to that of groups II (83.3%) and III (83.3%). Plasma progesterone (P₄) concentration was significantly higher (P<0.05) in group II compared to that of group III on day 3, and there was a treatment x day interaction (P<0.05). No effect of levamisole treatments on plasma P₄ concentrations was detected once pregnancy was established. There was a numeric decline in plasma cholesterol concentrations in group II, compared to group I or III on day 3. In conclusion, levamisole as an immunomodulator is detrimental for the establishment of pregnancy, possibly by stimulating general and intrauterine immunity.

Key words: ewes, levamisole, immunomodulation, pregnancy.

Levamisole is used as an anti-nematodal (6, 7) and immunomodulatory agent (13, 17). Weekly injections of levamisole during the dry period significantly decreased the incidences of mastitis, foetal death, and endometritis due to its immuno-potentiating activity in cows (8). Administration of levamisole has therapeutic and preventive effects on sheep pox (9). In ruminants, anthelmintic dose of levamisole is 7.5 mg/kg. Intermittent treatment with one-third of the anthelmintic dose results in an effective immune response (2, 7). A single dose (2.5 mg/kg) of levamisole results in an immunomodulatory effect for about 48 h (2).

It was shown that levamisole could change serum albumin, creatinine, cholesterol, and other biochemical parameters (19). It has been reported that even a single anthelmintic and toxic dose of levamisole administration may cause significant changes in thyroid hormone concentrations, a significant increase in serum albumin, and a slight decrease in total cholesterol concentrations in ewes (3).

It has been suggested that the use of levamisole should be avoided during the breeding season in rams, because of a harmful effect on semen’s quality (5). However, the effect of levamisole on ewes has not been investigated. Depending on the orally administered dose of levamisole used before or during the entire pregnancy in rats, decreased implantation and some growth retardation were reported (reviewed by Kazy et al. 12). The objective of this study was to investigate the effects of the administration of anthelmintic and immunomodulatory doses of levamisole following breeding, on plasma progesterone concentrations and pregnancy rates in ewes.

Material and Methods

This experiment was conducted at the Research and Training Farm of the University of Kafkas, Turkey, between November 2004 and January 2005. The Animal Handling and Ethical Board at the Faculty of Veterinary Medicine approved all the experimental design. Nineteen fat tailed (2–5-year-old) ewes were housed in flock barn with access to a feeding lot, and were fed grass hay ad libitum. Grass hay (93.3% DM, 8.8% CP, total fibre 31.1%, 2 000 kcal/kg ME as feeding basis) was served two times a day.

The ewes were observed for standing oestrus three times a day for 30 min each time, and the ones detected in standing oestrus (d 0) were separated from flock. Then, the ewes were randomly assigned into one of the treatment groups. Immunomodulatory dose of levamisole (Levamisole hydrochloride; Actipar®; Alke İlac San. 34896, Pendik, Turkey) (2.5 mg/kg; group I; n=7), anthelmintic dose of levamisole (7.5 mg/kg; group II; n=6), and physiologic saline (group III; n=6)
Injections were administered subcutaneously (sc) on days 0, 7 (early luteal phase), 14 (around the time of implantation), and 21 (early pregnancy). Following the first mating, the ewes were bred again if detected in oestrus; however, the treatment was not applied following the second breeding. Pregnancy was diagnosed with a transrectal ultrasound equipped with 6 MHz probe (Pie Medical, 100 Falko Vet Model) at 45 d after breeding.

Blood samples were collected once in three days starting from day 0 via the jugular vein puncture into tubes containing EDTA. The blood samples were immediately transferred to a laboratory for centrifugation (3,000 x g, for 15 min), and plasma samples were stored at -20°C until assay. Plasma progesterone (P₄) concentrations were determined with double antibody EIA techniques (15). Inter-assay and intra-assay coefficient of variations were 10% and 9%, respectively. The total plasma cholesterol concentrations were measured with a commercial kit (Diagnostic Systems GmbH & Co. KG, Germany).

Cholesterol and P₄ data were analysed using the repeated measures analysis of the mixed procedure of SAS. Chi-square and risk ratio analysis with 95% confidence limits of the frequency procedure in SAS were used to evaluate PR.

Results

Pregnancy rates (PR) were 28.6%, 83.3%, and 83.3% in groups I, II, and III, respectively. PR in group I was significantly lower compared to that of groups II and III (P<0.06; Table 1). However, analyses of group I versus group II indicated that ewes in group I, had 4.28 (0.67-27.24; P<0.05) times higher chance not to get pregnant than those in group II. Since this experiment was conducted close to end of the breeding season, oestrus in the ewes could only be detected one more time if they were not pregnant to the first breeding. The ewes that did not get pregnant after the second breeding entered to seasonal anoestrous period. Following the second breeding, 2/5, 1/1, and 0/1 of ewes became pregnant in groups I, II, and III, respectively. Overall PR at the end of the breeding season (the first and the second breeding) was 57.1%, 100%, and 83.3% in group I, II, and III, respectively, and PR did not differ significantly despite being numerically lower in group I.

Plasma P₄ concentrations three days after the first standing oestrus revealed that all ewes ovulated. P₄ concentration was significantly higher (P<0.05) in group II than that in group III three days after the standing oestrus (Fig. 1). Moreover, there was a day effect (P<0.01) and a treatment by day effect (P<0.05) on P₄ concentrations during the fifteen days following breeding in all ewes regardless of pregnancy status (Fig. 1). No significant effects of the treatment on plasma P₄ concentrations during the 42 d of monitored pregnancy period were found.

Plasma cholesterol concentrations did not differ between treatment groups during the fifteen days following the first breeding (Fig 2). However, there was a numeric decrease in plasma cholesterol concentration in group II compared to group I or III three days after the standing oestrus. Moreover, there was a day effect (P<0.05) on plasma cholesterol concentrations, and plasma cholesterol concentrations decreased as oestrous cycle advanced. There was a slight negative correlation (R = -0.17, P<0.08) between plasma cholesterol and P₄ concentrations. No significant effects of the treatment on plasma cholesterol concentrations during the 42 d of pregnancy period were found.

Discussion

The administration of an anthelmintic dose of levamisole, did not result in any harmful effects on the conception and maintenance of pregnancy, because no difference in PR existed after the first breeding. In contrast, lower PR existed in group treated with immunomodulatory dose of levamisole, and this could be attributable to the disruption of maternal recognition of pregnancy due to immunomodulation. The increase in P₄ concentrations following oestrus indicated that all ewes ovulated without the disruption of ovulation by levamisole. No difference in the plasma P₄ concentrations during pregnancy excluded possibility of the negative effect of immunomodulatory dose of levamisole on early pregnancy through P₄ itself.

Table 1

<table>
<thead>
<tr>
<th>Pregnancy rates</th>
<th>Group I (n=7)</th>
<th>Group II (n=6)</th>
<th>Group III (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>After the first breeding</td>
<td>28.6% (2/7)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>83.3% (5/6)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>83.3% (5/6)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>After the first and second breeding</td>
<td>57.1% (4/7)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>100% (6/6)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>83.3% (5/6)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a, b, c</sup> Superscripts within a same row indicate significance (P<0.06).
* Indicates higher chance not to get pregnant (4.28; 0.67-27.24; P<0.05).
Immunomodulatory treatment with levamisole could result in immunomodulation in utero-conceptus environment and weaken the immunosuppressive effect of conceptus, leading to a disruption in the establishment of pregnancy. Levamisole can induce cell-mediated immune reactivity and stimulate normal and depressed immune systems; therefore, it is classified as both an immunoregulator and immunostimulant (17). Since the conceptus is recognised as foreign by the dam, it likely survives by obtaining some measure of control, over the dam's immune system, corpus luteum (CL) function, uterine blood supply, and other aspects of maternal physiology for the maternal recognition of pregnancy with multiple isoforms of pregnancy-associated glycoproteins and interferons (16). At implantation, the embryo is exposed to maternal endometrial immune cells and potentially hostile cytokines. The embryo and its products establish a favourable maternal environment to promote maternal recognition, as well as an immune tolerance of pregnancy. Proximity between the putative embryo-derived compounds and the maternal immune system would allow rapid diffusion of embryonic signals resulting in local and systemic maternal immune recognition (4). Immunoregulatory pregnancy-associated molecules such as ovine uterine serpin and IFN-τ are released by the uterus and conceptus (11, 18). The early ovine conceptus has an effect on the uterine wall leading to changes in type I IFN-induced antiviral proteins, Mx proteins, expression in diverse cell types including immune cells and cells of luminal and glandular epithelium, stroma, and myometrium in the uterus (14). Probably, immunomodulatory treatment...
with levamisole compromised such immune mechanisms. Moreover, intermittent administration of immunomodulatory dose of levamisole could result in a carry-over effect of immunomodulation leading to lower PR at the end of the breeding season.

Because no difference was found among groups for plasma P₄ concentrations during the first 12 d after oestrus, immunomodulatory or anthelmintic doses of levamisole do not interfere with CL development. Moreover, no difference of plasma P₁ concentrations during the 42 d of pregnancy among treatment groups indicates any harmful effect of levamisole on CL function during pregnancy. Higher plasma P₄ concentrations in group II on day 3 implies that the administration of 7.5 mg of levamisole at standing oestrus may have accelerated steroidogenesis. Moreover, the same dose of levamisole slightly decreased the plasma cholesterol concentrations on day 3. This slight decrease in plasma cholesterol concentration was consistent with observations of Atessahin et al. (3). Furthermore, levamisole has been used as steroid-sparing agent in steroid dependent nephrotic syndrome in humans (1, 10). Perhaps, the administration of anthelmintic dose of levamisole could facilitate the bioavailability of cholesterol for P₄ synthesis. A slightly negative correlation between plasma cholesterol and P₄ concentrations, and a decrease in plasma cholesterol concentrations on oestrous cycle advances could support this result.

In conclusion, the use of levamisole as an immunomodulator should not be considered during the breeding season due to lower PR. The disruption of the establishment of pregnancy by immunomodulatory treatment with levamisole, possibly via activation of general as well as uterine immune system warrants further investigation with respect to the pregnancy, and may provide a useful tool for the investigation of immunomodulatory events during maternal recognition of pregnancy.

References