Prevention of embryonic death using different hormonal treatments in ewes

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Abstract: The purpose of this study was to evaluate different treatment protocols to prevent embryonic death in ewes. A total of 180 Akkaraman crossbred ewes and 10 healthy rams were used as material. The ewes were divided into 3 equal groups, with each of the 3 groups then separated into 3 subgroups. Ewes in estrus, determined with teaser rams, were exposed to mating. Three different treatment protocols of gonadotropin-releasing hormone (GnRH) analog (buserelin, intramuscularly) at a dose of 20 µg, vaginal sponges containing 30 mg fluorogestone acetate (FGA), and saline at a dose of 1 mL (control, intramuscularly) were applied on days 4, 12, and 16, respectively, for each subgroup after mating. No significant differences were observed in the pregnancy or multiple birth rates among any of the treatment groups. In the groups treated on days 4 and 12 after mating, the hormonal treatments gave lower rates of embryonic death compared to the control group (P < 0.05). In conclusion, the application of GnRH or FGA on days 4 and 12 after mating was found to be effective in preventing embryonic death in ewes.

Key words: Ewe, hormonal treatment, embryonic death

1. Introduction
Embryonic losses are observed within the first 3 weeks of pregnancy in ewes. The proportion of synchrony between the uterus and the developmental stage of the conceptus is one of the basic factors influencing fecundity. Embryonic losses are reported to be 20%–30% in ewes. Of this total loss, 70%–80% occurs between days 8 and 16 after insemination (1–3). The major reason for embryonic mortality is likely to be inadequate luteal function (4).

To reduce embryonic losses and also to increase fertility, progesterone administration during early pregnancy has been applied to sheep and cattle (5–8). Progesterone usage after mating or insemination increases pregnancy rates and induces fetal growth. Additionally, progesterone administration may compensate for luteal insufficiency. The use of human chorionic gonadotropin (hCG) and gonadotropin-releasing hormone (GnRH) is an alternative method to reduce embryonic losses and hence to increase the pregnancy rate after different times of mating or insemination. The injection of GnRH between days 10 and 13 after mating has been shown to increase embryonic survival and pregnancy rate (5,7,8). These hormones can be used on different days of the cycle after mating, such as on days 4, 5, 11, and 12. Administration on day 12 after mating is suggested to be the critical period for maternal recognition of pregnancy in the ewe, because the corpus luteum starts to regress on day 12 in the estrous cycle. Hormonal treatments to increase progesterone production on day 12 after mating may prevent luteolysis of the corpus luteum and prostaglandin F2α (PGF2α) secretions (9).

The objective of this study was to determine the effects of different hormones applied after mating on the prevention of embryonic losses in ewes.

2. Materials and methods
This study was performed at a private farm in Konya during the sheep breeding season of 2007. A total of 180 Akkaraman crossbred ewes and 10 healthy rams were used as material. The ewes were divided into equal 3 groups, with each group then separated into 3 treatment subgroups. Each subgroup consisted of a total of 20 ewes. Ewes in estrus, determined with teaser rams, were exposed to mating. Mated ewes were then selected, and 3 different treatment protocols of GnRH analog (buserelin, intramuscularly) at a dose of 20 µg, vaginal sponge containing 30 mg fluorogestone acetate (FGA), and saline at a dose of 1 mL (control, intramuscularly) were applied on days 4, 12, and 16, respectively, for each subgroup.
after mating. Pregnancy and the number of embryos were detected on days 24, 30, and 36 after mating using real time B-mode ultrasound.

3. Results
As shown in the Table, no significant differences were observed in the pregnancy and multiple birth rates among any of the treatment groups. Hormonal treatments applied on days 4 and 12 after mating gave lower rates of embryonic death compared to the control group (P < 0.05). The rates of pregnancy, multiple births, and embryonic losses are illustrated in the Table.

3.1. Statistical analysis
The results of study were evaluated by the chi-square test. Differences with values of P < 0.05 were considered to be statistically significant. Statistical analyses were performed by using SPSS 11.5.

4. Discussion
The results of the present study indicated that the usage of GnRH and progesterone sponges on days 4 and 12 after mating did not improve the pregnancy rates but did reduce embryonic losses. Luteal function is the most important factor affecting embryo survival (10). Embryonic mortality is manifested as a constant loss of 20% to 30% of eggs in sheep. In an open breeding system, this situation may not present a problem, as nonpregnant ewes will ultimately return to service. Prolificacy may be lowered, however, if there is a significant partial loss of multiple ova with the female remaining pregnant. Embryo loss consists of fertilization failure, observable genetic damage, and idiopathic loss (11–13).

Progesterone is essential for pregnancy maintenance (14) and one of the important functions of the blastocyst is to ensure that the uterine luteolytic mechanism is counteracted (15). Progesterone and estrogen determine the proper function of the uterus in preparation for embryo development and implantation (16–18). Steroid imbalance may induce asynchrony between the embryo and uterus, i.e. alteration of embryonic growth rates relative to uterine timing (19–21). This could be viewed as a mechanism to reestablish synchrony, but generally asynchrony results in embryonic mortality (10,21). FGA and GnRH administration in the present study reduced the embryonic death rate through enhanced luteal function. These effects of the GnRH and FGA on embryonic survival are in agreement with the previous findings of Beck et al. (5) and Drew and Peters (22) in cattle but contradicted with the findings of Beck et al. (23), who did not detect any stimulatory effect of GnRH/FGA on the plasma progesterone in sheep. Dutt (24) strongly suggested that variability in progesterone concentration through estrus, and the timing and extent of the luteal rise, account for a proportion of embryonic mortality. Progesterone is necessary at a certain level after mating, as ewes with lower progesterone levels suffer greater embryo loss (25). Low periovulatory progesterone secretion may result in abnormal uterine function or poorly developed oocytes, and low steroid secretion may originate from follicles rapidly undergoing atresia (26).

Days 12 and 13 after mating are the critical period of maternal recognition of pregnancy, which coincides with the beginning of the regression of the corpus luteum in the estrous cycle (9). Progesterone provided by GnRH and FGA on day 12 may increase interferon-T production (27), which in turn prevents luteolysis by preventing PGF2α secretion (9). Luteolysis may also be prevented by GnRH administration, because GnRH will ovulate or luteinize any developing follicles and hence decrease the estradiol production of the follicles. The decrease in estradiol production and increase in progesterone prevent the synthesis of PGF2α, providing pregnancy maintenance (28). The GnRH and FGA treatments may have increased plasma progesterone levels, which may in turn stimulate the secretion of embryonic substances from

### Table
Effect of different hormone administrations applied after mating on the rates of pregnancy, multiple births, and embryonic death.

<table>
<thead>
<tr>
<th>Day</th>
<th>Group</th>
<th>Pregnancy rate (%)</th>
<th>Multiple birth rate (%)</th>
<th>Embryonic death rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 4</td>
<td>GnRH</td>
<td>85</td>
<td>41.2</td>
<td>17.6*</td>
</tr>
<tr>
<td></td>
<td>FGA</td>
<td>90</td>
<td>33.3</td>
<td>5.5*</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>90</td>
<td>33.3</td>
<td>38.8*</td>
</tr>
<tr>
<td></td>
<td>GnRH</td>
<td>90</td>
<td>50.0</td>
<td>5.6*</td>
</tr>
<tr>
<td></td>
<td>FGA</td>
<td>90</td>
<td>38.8</td>
<td>5.6*</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>95</td>
<td>26.3</td>
<td>31.6*</td>
</tr>
<tr>
<td>Day 12</td>
<td>GnRH</td>
<td>85</td>
<td>29.4</td>
<td>29.4</td>
</tr>
<tr>
<td></td>
<td>FGA</td>
<td>90</td>
<td>38.8</td>
<td>22.2</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>80</td>
<td>37.5</td>
<td>31.2</td>
</tr>
</tbody>
</table>

a, b, c: Different superscripts within the same column show significant differences (P < 0.05).
the uterus. Wilmut et al. (29) demonstrated that a uterine environment advanced by treatment with the exogenous progesterone accelerated conceptus development. Fluctuations in the concentration of plasma progesterone appear to have implications for embryo survival (25). In this study, on postmating day 12, hormonal treatments such as GnRH and FGA decreased the embryonic death rate. In conclusion, the application of GnRH or FGA on days 4 and 12 after mating was found to be effective in preventing embryonic death in ewes.

References


