Comparison of the endocrinological and immunological results of different induction of parturition methods in ewes

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Keywords
Induction of parturition, endocrinology, immunology, colostrum, lamb, ewe

Summary
Objective: This study aimed to compare different induction of parturition (IP) methods in terms of endocrinological and immunological parameters in ewes and lambs. **Materials and methods:** A total of 24 ewes and their respective 24 lambs were studied. Groups of six ewes were induced on the 138th day: groups I (control), II (dexamethasone), III (aglepristone) and IV (aglepristone + dexamethasone). Blood samples were taken from the ewes from IP to 2 days postpartum at 12-hour intervals for the prolactin and oxytocin analyses. Colostrum and blood samples were collected from the lambs at 0, 12, 24, 36 and 48 hours for the IgG analysis. The prolactin, oxytocin and IgG levels were measured by ELISA. **Results:** A significant difference was found in prolactin levels in all groups and in the colostral IgG level in group IV with respect to the sampling time. Additionally, a significant difference in prolactin level was found at 24 hours postpartum between group IV and groups I and III. A significant difference in the colostral IgG level was determined at 24 hours after parturition between group III and the other groups and at 48 hours after parturition between groups II and III. A positive and significant correlation between the colostral and serum IgG levels of the lambs was found at 24 hours in groups I and II. **Conclusion and clinical relevance:** Although varying individual results were found within the groups, the endocrinological and immunological results did not suggest any superiority among the IP methods. Considering the presented study results, a single dose of aglepristone may be used alone or in combination with dexamethasone for IP.

Schlüsselwörter
Geburtseinleitung, Endokrinologie, Immunologie, Kolostrum, Lamm, Mutterschaf

Zusammenfassung
Ziel: Vergleich verschiedener Methoden der Geburtseinleitung in Bezug auf endokrinologische und immunologische Parameter bei Mutterschafen und Lämmern. **Material und Methoden:** Untersucht wurden 24 Mutterschafe und ihre 24 Lämmer. Jeweils sechs Mutterschafe bildeten eine Gruppe. In den vier Gruppen kamen am 138. Tag nach der Bedeckung verschiedene Protokolle der Geburtseinleitung zur Anwendung: Gruppe I (Kontrolle), II (Dexamethason), III (Aglepriston) und IV (Aglepriston + Dexamethason). Blutproben der Mutterschafe wurden vom Zeitpunkt der Geburtseinleitung bis 2 Tage nach der Geburt in 12-Stunden-Intervallen zur Messung der Prolaktin- und Oxytozinkonzentration entnommen. Kolostrum und Blutproben von den Lämmern wurden 0, 12, 24, 36 und 48 Stunden nach der Geburt für die IgG-Bestimmung gesammelt. Die Messung der Prolaktin-, Oxytozin- und IgG-Konzentration erfolgte mittels ELISA. **Ergebnisse:** Die Prolaktinkonzentrationen veränderten sich über die Zeit signifikant in allen Gruppen. Die IgG-Konzentration im Blut zeigte nur in Gruppe IV eine signifikante Veränderung im zeitlichen Verlauf. Im Gruppenvergleich differierte die Prolaktinkonzentration zwischen Gruppe IV und I sowie III 24 Stunden post partum signifikant. Signifikante Unterschiede hinsichtlich der IgG-Konzentration des Kolostrums fanden sich zwischen Gruppe III und den anderen Gruppen 24 Stunden nach der Geburt sowie zwischen Gruppe II und III 48 Stunden nach der Geburt. In den Gruppen I und II ergab sich eine positive Korrelation zwischen der IgG-Konzentration im Kolostrum und im Serum 24 Stunden nach der Geburt. **Schlussfolgerung und klinische Relevanz:** Bei Schafen und Lämmern waren nach verschiedenen Protokollen der Geburtseinleitung keine relevanten endokrinen und immunologischen Unterschiede nachweisbar. Basierend auf den Resultaten der Studie kann zur Geburtseinleitung eine Einzeldosis von Aglepriston allein oder in Kombination mit Dexamethason verwendet werden.

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Introduction

Induction of parturition (IP) is used to terminate the gestation of ewes with pathological conditions, such as metabolic, traumatic or respiratory diseases. IP also provides well-timed manipulation of the newborn lambs and the ewes during parturition (24, 33, 34, 40). Many hormones and medications are used for IP in ewes, including adrenocorticotropic hormone (ACTH) (8), glucocorticoids, $PGF_{2\alpha}$ and analogues, 3β-hydroxy steroid dehydrogenase enzyme inhibitors (epostane and trilostane) (24, 33), progesterone receptor blockers (21, 29), and synthetic PGE_1 analogues (misoprostol) (36). Glucocorticoid usage is a well-known conventional choice to terminate gestation in the veterinary field (1, 5, 26). Because progesterone plays a primary role in continuing gestation, the application of antiprogestine drugs (mifepristone or aglepristone) has been popular in recent studies (3, 12, 19, 21).

During the prenatal period, housing problems, release of hormones due to stress and inductive drug usage indirectly affect lactation and the quality of the colostrum (immunoglobulins) (11). Colostral feeding in newborn lambs is suggested for passive immune transfer, because immunoglobulins do not transport across the placenta from the ewe to the lamb. Hence, an early intake of colostrum is crucial (11, 39).

The hormonal mechanisms of the inductive drugs and their physiological effects in the newborns have been discussed in the literature (3, 10, 19, 20, 29, 30, 34, 40); however, the advantages and disadvantages of the IP methods have not been studied thoroughly. Here, we aimed to compare the prolactin and oxytocin levels of ewes induced with different IP methods as well as the IgG levels of the colostrum and the serum IgG levels of lambs fed colostrum.

Materials and methods

Animals and experimental design

This study received the approval of the ethics research committee at Uludag University (no: 2013–12/02). Twenty-four Turkish Kivircik breed ewes (clinically and hematologically healthy) ranging between 3 and 4 years of age at the third gestation period with breeding records and their 24 healthy newborn lambs were included in the study.

The ewes were randomly divided into four groups. Each group consisted of six ewes and six of the newborn lambs. At day 138 of the pregnancy, IP was performed as follows:

- group I (GR I; control): 0.9% NaCl (1 ml, i. m.)
- group II (GR II) dexamethasone (total 16 mg, i. m.)
- group III (GR III) aglepristone (5 mg/kg, s. c.)group IV (GR IV) aglepristone (2.5 mg/kg, s. c.) + dexamethasone (total 8 mg, i. m.).

All induced ewes were separated into different boxes and closely monitored during the study. After parturition, general examinations (body temperature, heart and respiratory rate, color of mucous membranes and capillary filling time) and the sucking reflexes of the lambs were evaluated to ascertain their health status. All lambs were separated into a box together with their mothers. The first colostrum was given to the lambs after blood sampling, and they were fed ad libitum. Single birth lambs were included in the study; if twinborn lambs were present, the healthy and larger lambs were selected for the study. Six of the lambs were grouped as described for their mothers.

Sampling and analysis

Blood samples were taken from the ewes from the jugular vein using plain vacuum tubes at 12-hour intervals from IP to the second day postpartum to analyze the serum prolactin and oxytocin levels. To measure the serum IgG levels of the lambs, blood samples were taken from the jugular vein in sterile tubes following parturition, before feeding with colostrum at the zero hour and at hours 12, 24, 36 and 48. The colostrum samples from the ewes were collected into sterile tubes to measure the colostral IgG levels at 0, 12, 24, 36 and 48 hours postpartum.

The blood samples were centrifuged at 1000 rpm for 15 minutes; then, the serum was removed for analysis. The serum and colostrum samples were stored at –20 °C. The colostrum samples were thawed prior to analysis and centrifuged at 1000 rpm for 15 minutes to separate the upper layer. After 1 ml of colostrum was collected into a sterile tube, 50 µl was used for the analysis. Commercial ELISA kits specific for sheep (CUSABIO®, Cusabio Biotech Co., Ltd., Wuhan, China) were used to analyze the prolactin (CSB-E13161Sh), oxytocin (CSB-E13236Sh), and IgG levels (CSB-E14400Sh). The hormone concentrations were measured with an ELISA reader (Biotek ELx808®, USA). The IgG levels of the colostra and sera were determined with an ELISA reader (Thermo Multiscan FC®, USA).

Statistical analysis

Descriptive statistics were calculated for the data. All statistical analyses were performed using a statistical package program (IBM SPSS 20.0 Statistics®, USA), with $p < 0.05$ considered significant. The graphics were drawn using Microsoft Office 2007 Excel.

In each group, the prolactin, oxytocin and IgG levels were analyzed using the Friedman test according to the sampling time points. Significant differences at the sampling time points were analyzed using the Wilcoxon test based on the 138th day and parturition for the prolactin and oxytocin levels and based on parturition for the colostral and lamb serum IgG levels.

The comparative statistical analyses among the groups were evaluated using the Kruskal-Wallis test. The test was performed for the prolactin and oxytocin levels based on the 138th day and parturition and for the IgG levels in the colostrum and serum

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samples based on parturition. In case of significance, the Mann-Whitney U test was used to compare the groups in pairs.

The correlation between the colostral and serum IgG levels in each group was determined by Spearman's correlation test.

Results

The details concerning the acquired number of lambs, mean gestation length (day) and mean interval from induction to parturition (MI) (hour) for the groups are given in ▶Table 1. The differences between the gestation lengths and the MIs were significant (p = 0.006; p = 0.009). The gestation length was significantly higher in the control group than in the other groups (GR II, p = 0.026; GR III, p = 0.004; GR IV, p = 0.004). No dystocia was observed in the groups, and the mothering ability was present in all ewes.

The mean prolactin and oxytocin concentrations in the groups following IP are given in ▶Fig. 1 and ▶Fig. 2, respectively. The mean IgG levels of the colostral and serum samples from all groups are detailed in ▶Fig. 3 and ▶Fig. 4, respectively.

Statistical analysis results within the groups

In each group, the prolactin concentrations changed significantly during the examination period [GR I (p = 0.001), GR II (p = 0.050), GR III (p = 0.001) and GR IV (p = 0.001)]; however, no significant differences were observed among the oxytocin sampling time points within the groups. For the colostral IgG levels at the different sampling time points, only GR IV exhibited a significant difference (p = 0.033). No significant difference in the serum IgG levels of the lambs was found among the groups at the different sampling time points.

Table 1 Summary of the results following induction of parturition in ewes in the four groups.

Tab. 1 Zusammenfassung der Ergebnisse nach der Geburtseinleitung bei Schafen in den vier Gruppen

Groups	Number of lambs	Mean gestation time (days)	Mean interval from induction to partus (hours)
GR I	8	145.6 ± 2.94	177.5 ± 71.29
GR II	6	142.3 ± 1.21	98.66 ± 31.55
GR III	7	141.3 ± 0.81	74.83 ± 17.31
GR IV	6	141.3 ± 0.51	73.33 ± 13.89

Based on the 138th day for prolactin:

- In GR I, a significant difference was found at prepartum 12 (p = 0.028) and postpartum 12 (p = 0.028), 24 (p = 0.028), 36 (p = 0.028) and 48 hours (p = 0.028).
- In GR II, a significant difference was found at parturition (p = 0.046) and at postpartum 12 (p = 0.046), 24 (p = 0.046) and 36 hours (p = 0.046).
- In GR III, a significant difference was found at parturition (p = 0.028) and at postpartum 12 (p = 0.046), 24 (p = 0.028), 36 (p = 0.028) and 48 hours (p = 0.046).
- In GR IV, a significant difference was found at prepartum 12 hours (p = 0.028), at parturition (p = 0.028), and at postpartum 12 (p = 0.028), 24 (p = 0.028), 36 (p = 0.028) and 48 hours (p = 0.046).

Based on parturition for prolactin:

- In GR I, a significant difference was found only at prepartum 48 hours (p = 0.046).

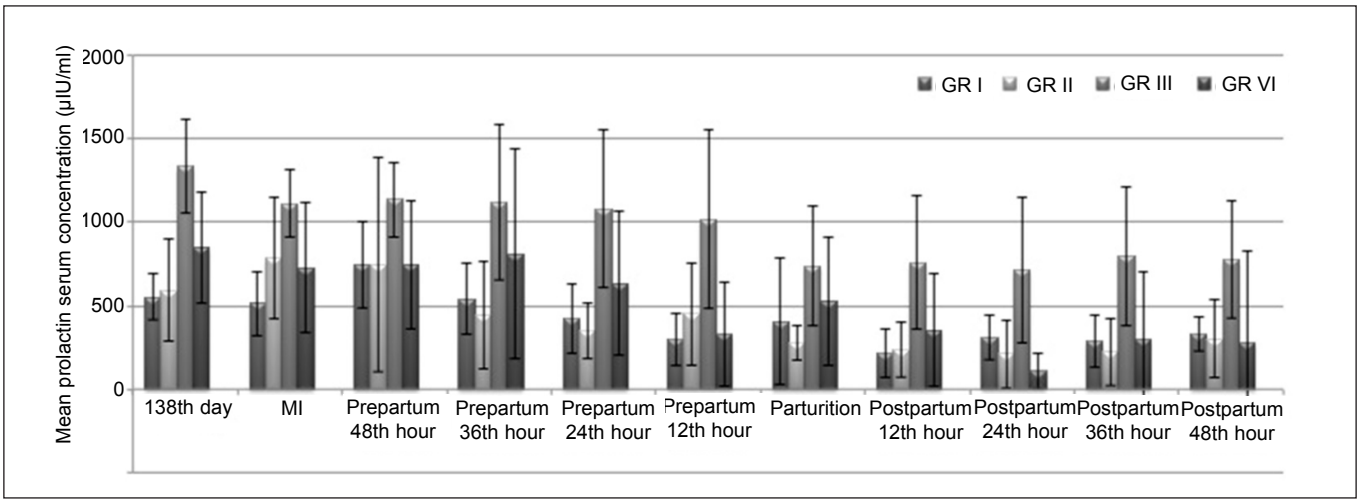


Fig. 1 Mean prolactin serum concentrations (µIU/ml) after different methods of induction of parturition in ewes. MI = mean interval from induction to parturition (hours).

Abb. 1 Mittlere Prolaktinkonzentationen im Serum (µIU/ml) nach verschiedenen Methoden der Geburtsinduktion bei Mutterschafen. MI = mittlere Dauer zwischen Geburtsinduktion und Partus (Stunden).

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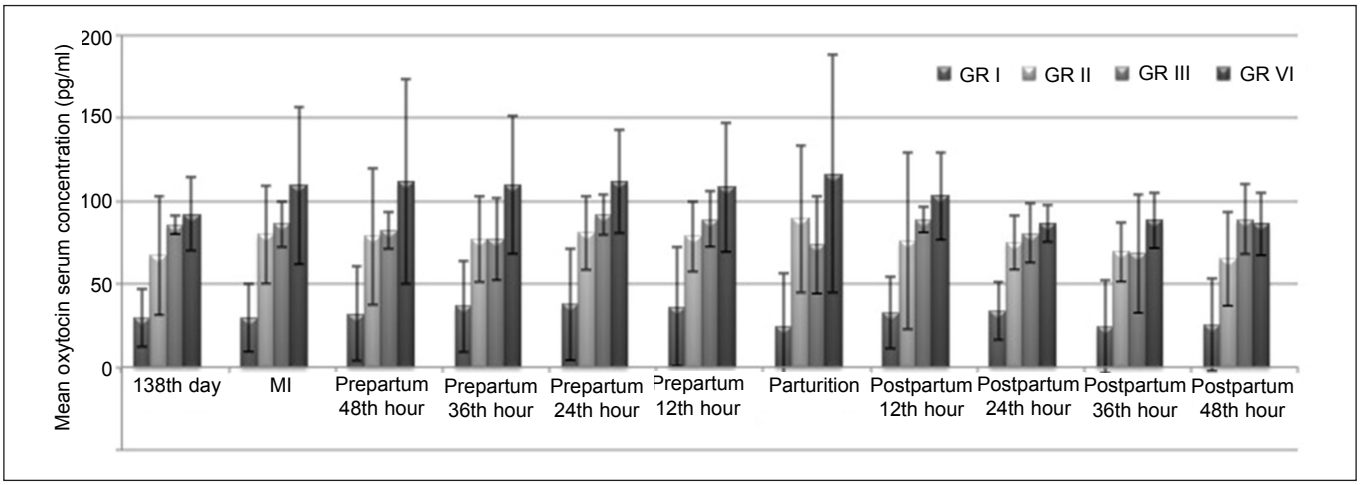


Fig. 2 Mean oxytocin serum concentrations (pg/ml) after different methods of induction of parturition in ewes. MI = mean interval from induction to parturition (hours).

Abb. 2 Mittlere Oxytozinkonzentationen im Serum (pg/ml) nach verschiedenen Methoden der Geburtsinduktion bei Mutterschafen. MI = mittlere Dauer zwischen Geburtsinduktion und Partus (Stunden).

- In GR II, a significant difference was found on the 138th day (p = 0.046) and at prepartum 48 hours (p = 0.028).
- In GR III, a significant difference was found on the 138th day (p = 0,028) and at prepartum 48 (p = 0.028), 36 (p = 0.028) and 24 hours (p = 0.028).
- In GR IV, a significant difference was found on the 138th day (p = 0.028) and at postpartum 24 (p = 0.028) and 36 hours (p = 0.028).

Based on parturition for IgG levels of the colostrum:

- In GR IV, a significant difference was found at postpartum 12 (p = 0.046), 24 (p = 0.028), 36 (p = 0.046) and 48 hours (p = 0.046).

Statistical analysis among the groups

Based on the 138th day, statistical comparison of the **prolactin levels** between the groups showed a significant difference only at postpartum 24 hours (p = 0.049). Comparisons of the groups at postpartum 24 hours revealed a significant difference between GR I and GR IV (p = 0.009) and between GR III and GR IV (p = 0.041). Based on parturition, no significant difference in the prolactin levels was found between the groups.

For **oxytocin**, no significant differences were found among the groups based on both the 138th day and parturition.

Based on parturition, a significant difference in the **colostral IgG levels** was found only at 48 hours (p = 0.049). This difference was only between GR II and GR III (p = 0.004).

Based on parturition, a significant difference in the **serum IgG levels** of the lambs was found only at 24 hours (p = 0.040). This

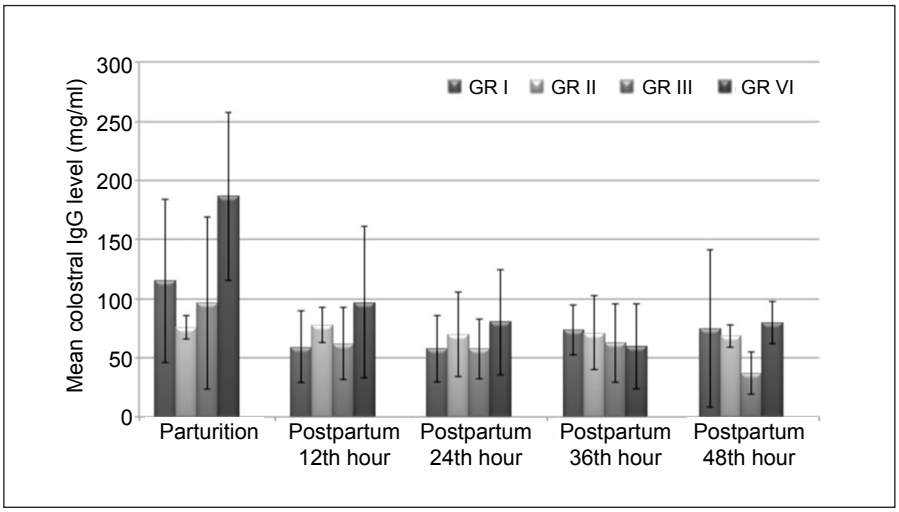


Fig. 3
Mean colostral IgG concentrations (mg/ml) after different methods of induction of parturition in ewes.

Abb. 3
Mittlere IgG-Konzentrationen (mg/ml) im Kolostrum bei Mutterschafen nach verschiedenen Methoden der Geburtsinduktion

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significant difference was between GR III and GR I (p = 0.026), GR II (p = 0.026), and GR IV (p = 0.015).

In the groups, correlations between the **colostral and serum IgG levels** of the lambs based on parturition were as follows. A positive and significant correlation was found only at 24 hours in GR I (R = 0.886; p = 0.019) and GR II (R = 0.886; p = 0.019). In GR III, a negative and significant correlation was found at 48 hours (R = –0.886; p = 0.019). The correlations were not significant in GR IV.

Discussion

In ewes, IP at the 138th gestation day results in uncomplicated birth and non-premature lambs (18, 26, 34). Although IP at this gestation time point is not preferred by some authors due to premature birth (1, 30), many studies have investigated the timing of IP before the 138th gestation day (26, 40). Therefore, the 138th gestation day was planned for IP in this study, and all parturitions were normal. No dystocia and no premature lambs were found in the groups.

Clinical and experimental studies note many methods for IP in ewes (4, 21, 25). IP with glucocorticoids is a conventional method that imitates the effects of fetal cortisol and contributes to early organ maturation in the fetuses, such as lung development (1, 33, 40). At the 142nd gestation day, IP with dexamethasone synchronized parturition without dystocia (38). Repeated doses (15 mg/kg) of aglepristone or its combinations have been used for IP in dogs (19), and this drug has also been applied as a unique dose (5–3.3–2.5–1.5 mg/kg) in goats at the 145th gestation day for IP (3). Because the incidence of retained fetal membranes and neonatal mortality was high at a 1.5 mg/kg dose in goats (3), we used 2.5 mg/kg of aglepristone in combination with dexamethasone in our study. Therefore, the present study was planned with four groups. GR I was the control. IP was performed with 16 mg of dexamethasone (i. m.) in GR II, 5 mg/kg of aglepristone (s. c.) in GR III and 2.5 mg/kg of aglepristone and 8 mg of dexamethasone in GR IV.

The MI varies according to the inductive drug dose and induction day (5). IP with 16 mg of dexamethasone at the 141st gestation day has shown MIs of 45 ± 8.4 hours (22). The MIs were 115.10 ± 32.62 and 56.25 ± 11.62 hours following IP with 1 and 2.5 mg of corticosteroids at the 138th gestation day, respectively (18). The MI was 98.66 ± 31.55 hours in GR II after IP with 16 mg of dexamethasone. Batista et al. (3) applied s. c. aglepristone to goats on the 145th gestation day for IP and achieved MIs of 29.5 ± 2.7 hours (5 mg/kg), 33.7 ± 2.1 hours (3.3 mg/kg), 32.9 ± 2.5 hours (2.5 mg/kg) and 46.8 ± 3.5 hours (1.5 mg/kg) based on the dose ranges compared to the MI of 112.7 ± 5.7 hours in the control group (1 ml s. c. of 0.9% NaCl). These authors also observed dystocia in the study groups. In this study, the MIs were 98.66 ± 31.55 and 74.83 ± 17.31 hours in GR II and GR III, respectively. The MI of GR IV (73.33 ± 13.89 hours) was close to the MI of GR III. Furthermore, the parturitions occurred spontaneously without any complications.

The usage of some drugs for IP may indirectly affect the hormonal status and lactation period (11, 20). Although some researchers have suggested that IP does not affect lactation (4, 17, 30), lactation does not start normally if IP is performed (31). A successful induction method should initiate the parturition and lactation period normally (33). Prolactin and oxytocin play key roles in milk ejection and secretion (27). Thus, prolactin and oxytocin analyses were planned to evaluate the lactation status of the ewes and the passive transfer of IgG from the colostrum to the lambs in the present study.

The peak status of prolactin can only be determined if the sampling time intervals are short. In non-induced ewes, the serum prolactin levels range from 281–727 ng/ml at 24 hours before parturition, 426.2 ± 151.8 ng/ml on the 145th gestation day and 495.8 ± 142.8 ng/ml 24 hours after parturition (7). Another report showed that the serum prolactin levels rose at the 1st or 2nd day before parturition, peaked on the parturition day, and decreased

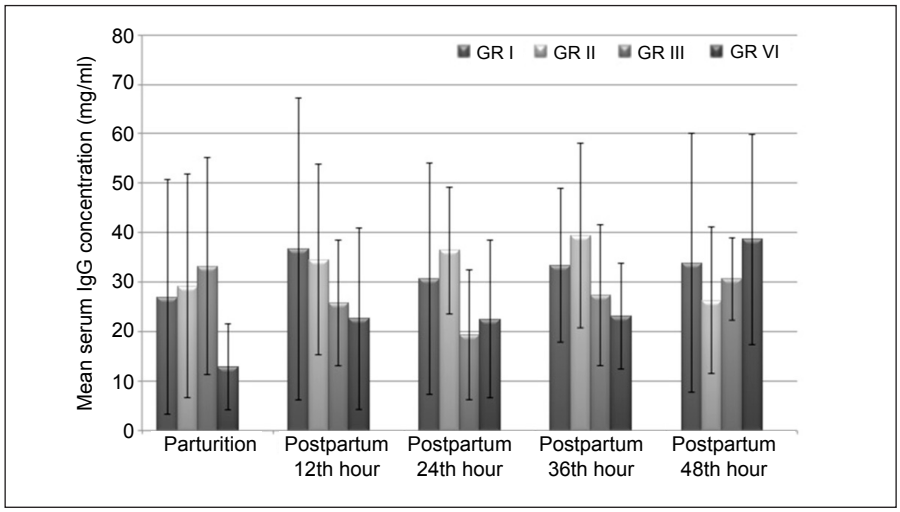


Fig. 4
Mean IgG serum concentrations (mg/ml) of the lambs after different methods of induction of parturition in ewes.

Abb. 4
Mittlere IgG-Konzentration im Serum (mg/ml) von Lämmern nach unterschiedlichen Methoden der Geburtsinduktion bei Mutterschafen

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gradually during the early stage of lactation (13). These levels sometimes fluctuate, with increases and decreases during the early lactation period due to stress factors (14). In this study, the differences in the prolactin levels at sampling time points within the groups were significant. Taking the pre- and postpartum sampling time points into account, we observed fluctuating prolactin levels within the groups. Based on the 138th day and parturition, significant differences were also found at the different sampling time points between the groups. Only GR I and GR IV had high prolactin levels at parturition, although the prolactin levels had not peaked markedly.

A 3–5 pg/ml concentration of oxytocin is sufficient for milk ejection and has a 2–5 minute half-life (27). The oxytocin levels measured at the 93rd–99th days of lactation were 27.5 ± 1.9 pg/ml in the autumn and 12 ± 1.4 pg/ml in the spring. The level increases to 158–214 pg/ml during milking (28). The oxytocin level is 29.1 ± 2.6 pg/ml on average at the final stage of gestation and 20.1 ± 3.3 pg/ml at the 136th–142nd days of gestation (16). In the present study, although the oxytocin level was higher in the induced groups than in the control, the difference was not significant based on the 138th day and parturition. Additionally, no significant difference was found among the oxytocin sampling time points within the groups.

The colostral IgG levels are lower in preterm births. Hormonal alterations following IP may negatively affect the colostral IgG level (10, 11, 23). IP with 10, 17.5 and 25 mg doses of dexamethasone at the 142nd-145th days of gestation causes a decrease in the colostral IgG levels in ewes (15). In goats, IP with a $PGF_{2\alpha}$ analog resulted in higher colostral IgG levels in the control group than in the induced group, because prolactin reached its peak level 24 hours before parturition, stimulated lactogenesis and terminated the transfer of IgG to mammary secretions (11). Comparison of the colostral IgG levels among the groups showed significant differences between GR II and GR III at 48 hours, which was not clinically important. In contrast to the results of the present study, some researchers (11, 15) have determined that IP reduces the colostral IgG levels. At parturition, the colostral IgG level was highest in GR IV, but the increase was not significant.

The serum IgG levels of the lambs at 24 and 48 hours following parturition represent a valuable method to determine the passive immune status. If the IgG level in lamb serum is lower than 0.5 g/100 ml (5 mg/ml) at postpartum 24 hours, the vitality of the lambs will be lower (37). Thus, the IgG level must be higher than 1000 mg/dl (10 mg/ml) at 24 or 48 hours after parturition to protect the lambs from failure of passive immune transfer (6). In our study, the mean IgG level in the groups was higher than 0.5 g/100 ml in the following 48 hours. No passive transfer failure was noted in the newborn lambs in the groups.

The pre-colostral serum immunoglobulin concentrations are usually changeable, and lambs are not entirely agammaglobulinemic at birth. The ovine fetus is immunologically competent as early as the 66th day of gestation. As it has been seen possible with the presented study results, relatively high IgG and IgM levels may be observed in certain lambs prior to suckling (35). Although the serum IgG levels differ, they generally rise 24 hours after colostrum intake (2, 6). In a non-induced parturition, the serum IgG levels of the lambs after colostrum intake at 24 hours are 41.7 ± 4.4 mg/ml (9). In the present study, changes in the serum IgG levels of the lambs were not significant among the sampling time points. However, in GR IV, the serum IgG levels of the lambs increased based on the sampling time, although the difference was not significant.

In pigs, IP with alfaprostol caused no difference in the IgG levels of the piglets at 24 hours between the induced and non-induced groups (20). In the present study, a significant difference in this factor was only found between GR III and the other groups. For 30 hours after the first colostrum feeding, a positive correlation exists between the consumed colostrum and IgG levels in the lambs (32). However, in another study, no correlation was found between the colostral and serum IgG levels at 24 hours (2). A positive correlation was observed in GR I and GR II at 24 hours, and a negative correlation was observed at 48 hours in GR III. GR IV had no correlation.

In conclusion, because there was no distinctive significant difference in the prolactin, oxytocin, and colostral and serum IgG levels within and between the groups, we can emphasize that a unique dose of dexamethasone, aglepristone or a combination of these drugs can be chosen for IP in ewes.

Conclusion for practice

A successful induction of parturition (IP) method must start lactation normally and should not have any adverse effect on the neonatal or maternal health status. However, IP hormonally affects lactation by affecting the colostral IgG levels and thus indirectly influencing the IgG intake of the newborn lambs. This study is the first to report the prolactin, oxytocin, colostral IgG and lamb serum IgG levels following IP. According to the present study results, IP by different methods (unique doses of dexamethasone, aglepristone or a combination of these drugs) can be chosen for ewes.

Conflict of interest

The authors confirm that they do not have any conflict of interest.

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