Research Article

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Comparison between the CIDR or sponge with hormone injection to induce estrus synchronization for twining and sex preselection in Naimi sheep

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Abstract: The management of sheep reproduction requires the induction and synchronization of the estrus cycle and ovulation for the ewe out-of-season and/or synchronized lambing. These managements are based on the insertion of an intravaginal device of controlled internal drug release (CIDR or sponge) and then the administration of a hormonal injection, such as PMSG, eCG, hCG, or GnRH. This study aimed to compare the impact of CIDR or sponges, with PMSG and GnRH injection, respectively, for inducing estrus synchronization, lambing rate, twining, and sex preselection rate in Naimi sheep. A total of 86 Naimi sheep ewes and six male rams with proven fertility were used in this study. The results showed that the first ewes or CIDR group had the highest ewe fertility rate of 26/28 (92.59%) with a 35/92 (38.04%) lamb production ratio. The offspring sex ratio was 22/35 males (62.85%) and 13/35 female lambs (37.14%). Their twin ratios were 9/22 (40.91%), producing 14 males (77.77%) and 13 females (37.14%). The second or sponge ewe group’s fertility ratio was 66/78 (84.42%) with a 35/79 (44.35%) lamb production ratio. The offspring sex ratio was 29/35 males (82.86%) and 16/35 females (45.71%). They sired the lowest offspring ratio of 20/92 total lambs (21.73%) and had sex ratios of 11/20 males (55.00%) and 9/20 (45.00%) female lambs. The twin production ratios were 4/22 (25%) with two males and two males with female twins). Their twin sex ratio was six males (55.00%) and two females (45.00%). The third or control ewe group’s fertility rate was 29/33 (87.88%). They produced 37/92 (40.27%) lambs, and the total sex ratios were 22/37 male lambs (59.45%) and 15/35 (40.45%) female lambs. They produced eight twins (27.58%), and their twins’ sex was equal to six male and six female (50%) lambs. Regarding the progesterone and testosterone hormone levels, no differences during pre- or post-intravaginal insertion were observed, but the estrogen level showed some differences during pre-insertion. In conclusion, the use of CIDR with hormone injection is better for reproduction management, male lambing, and sex preselection rate. The control group produced approximately the same fertility rate with equal male and female lambing sex preselection rates.

Keywords: estrus synchronization, sheep, CIDR, sponge, PMSG, GnRH

1 Introduction

The management of sheep reproduction requires the induction of the ewe estrus cycle and lambing synchronization out-of-season [1,2]. This induction management is based on either prostaglandin F2 (to eliminate the corpora lutea and subsequent estrus) or progesterone, or its analog administration. Then, a single intramuscular dose of equine chorionic gonadotropin (eCG) was injected to mimic the preovulatory luteinizing hormone (LH) surge and ovulation during seasonal anestrus to increase the percentage of twin pregnancies throughout the entire year. Another protocol is to inject the gonadotropin-releasing hormone (GnRH) [3–5]. Alternatively, the injection of human chorionic gonadotropin (hCG) or human menopausal gonadotropin for inducing ovulation during estrus synchronization in sheep, was related to a high occurrence of abnormal follicular growth patterns, disturbances, and retardments of ovulation and concomitant formation of follicular cysts in the treated females [6,7].

The impact of GnRH, PMSG, and hCG treatments on follicular diameter, conception, and lambing rates of Egyptian ewe lambs using an intravaginal sponge has
also been studied [8]. In a study on the effect of prostaglandin and gonadotropins (GnRH and hCG) injection combined with the ram effect on progesterone concentrations and reproductive performance of Karakul ewes during the non-breeding season, researchers found that the conception rate was 93.8, 90, and 87.1% in the hCG, GnRH, and control groups, respectively; the lambing rate increased in the hCG group compared with the control group (87.1% versus 58.1%; \( P < 0.01 \)) [9].

The injection of prostaglandin and hCG in combination with the ram effect decreased the lambing period but increased the lambing rate in the first cycle [9]. Recently, the comparison of five protocols to enhance the estrous synchronization on reproductive performance of Hu sheep in China was performed, and ewes underwent insertion of fluorogestone acetate (FGA, 45 mg) sponge for 11 days in Group I, while Groups II and III ewes were treated with the FGA sponge for 15 days [10]. Before 2 days of the withdrawal of the sponge, the ewes were injected with PGF2α 0.1 intramuscularly and 36 h after were injected with different hormones (6 μg GnRH, Group I; 330 IU PMSG Group II; and a combination of 6 μg of GnRH and 330 IU of PMSG was treated in Group III), and groups VI and V were injected with 330 IU PMSG. Then, all ewes were artificially inseminated with freshly diluted semen. Their results showed no significant difference in the percentages of ewes’ sponge loss rate, vaginitis rate, total percentage of estrous ewes, conception rate, single lambing rate, twinning rate, and multiple lambing rates of ewes among the five protocols. In addition, the total estrous ewe rate and conception rate were approximately 80% in Groups II and III, and the twinning lamb rate of the Group II protocol was 70%. In addition, there was no difference in the lambing rate of ewes among Groups II, III, IV, and V [10].

In farm animals, CIDR can synchronize estrus and increase reproduction rates [11]. Estrous synchronization in ewes can also be achieved with the use of short-term progesterone treatment [12]. Estrus in sheep can be induced and synchronized by intravaginal P4 therapy for brief durations (5–7 days) in both the breeding and nonbreeding seasons [13–15].

A rise in pregnancy hormones can result from the use of PMSG, which promotes follicle growth, ovulation, and the production of more CL. Success with inducing ovulation with PMSG in sheep and goats after progesterone therapy intense prenatal development, aided by more gestational hormones, leads to heavier lambs at delivery. [16]. It has been studied in bovine and goat subjects whether the injection of hCG or other oocyte inducers (such as GnRH or LH) can result in the production of auxiliary (CL) and an increase in P4 concentration [17,18]. It is crucial to clarify the potential benefits of hCG treatment across a variety of sheep breeds, climates, and management regimes. In ruminants, a rise in P4 has been shown in several studies to enhance embryonic survival rates and minimize the number of embryos that are lost [19].

Our study aimed to compare the impact of controlled internal drug release CIDR or sponge with PMSG and GnRH injection, respectively, to induce estrus synchronization and the subsequent lambing rate, twining, and sex preselection rate in Naimi sheep.

2 Materials and methods

2.1 Animal treatment

All animal treatments were performed according to the regulations and guidelines of the ethics committee, the Institutional Animal Care at King Saud University, and the Collaborative Institutional Training Initiative (CITI) program.

Naimi sheep ewes and male rams aged 2–3 years were used in this study. All ewes used were fertile and sired at least one time before starting this study. Additionally, male fertility and semen analysis were performed by EVOS system computer-assisted semen analysis for ram fertility properties. The sheep were housed in yards under a roof in an open-sided barn. Sheep were fed commercial pellets from the Arabia Agriculture Service Company (ARASCO Riyadh, Saudi Arabia) (energy 2.6 M cal. ME kg−1 dam, 14% crude protein, 2.78 M cal., fibers = 12%, fat = 2%, Ca = 0.8%, K = 0.55%, P = 0.5%, Na = 0.25%, Cu = 7/mil, Se = 300/109, Vit A = 900 IU, Vit D = 1,400 IU) to meet daily energy and protein requirements. The body weight of ewes was recorded at the beginning of the experiment, and they were randomly allocated into one of the following three groups: (1) a new vaginal controlled internal drug release CIDR (intravaginal progesterone release device each device contains 0.3 g progesterone P4 [Eazi-breed™ CIDR® Pfizer Animal Health, New Zealand]) was used for 14 days to synchronize estrus. Then, the ewes were injected with 500 IU PMSG (Sergon, Serum gonadotropin ad.us.vet, Bioveta, Czech Republic). Then, on the same day, the rams were introduced to ewes. (2) An intravaginal progesterone polyurethane sponge soaked with 60 mg of medroxyprogesterone acetate/Flushing Sponge Esponjavet (Manufacturer by Laboratorios, Hipra S.A. Avda, La Selva, Girona, Spain) was inserted into ewes to synchronize estrus for 12–14 days. At the time of sponge withdrawal, the ewes were injected with Gonasyl (gonadorelin
acetate 50 μg/mL and benzyl alcohol 9 mg/mL) and 2 mL GnRH per ewes (Manufacturer Laboratories, Syva s.a.u., Leon, Spain). The rams were introduced to ewes on the same day of sponge withdrawal. (3) Ewes without any intravaginal sponge or hormone injection treatments were considered the control group. Later, the rams were kept with ewes continuously. Blood samples were collected via the jugular vein of all ewes into plain vacutainer tubes from the treated group at the time of CIDR or sponge insertion and in control ewes.

### 2.2 Hormone blood analysis

Serum from 86 ewes was separated by centrifugation at 2,000×g for 10 min, transferred into 1.5 mL Eppendorf tubes, and stored at −20°C until analysis. Serum estradiol (E2) and P4 concentrations were measured using commercial ELISA kits (HUMAN Gesellschaft für Biochemica und Diagnostica mbH, Germany). The assays were performed according to the manufacturer’s instructions, and absorbance readings were taken using an automated spectrophotometer (Moleculle-On, New Zealand). The analytic sensitivity of the E2 and P4 ELISA tests were 3–6 pg/mL (range of 0–2,000 pg/mL) and 0.03–0.07 ng/mL (range of 0–40 ng/mL), respectively. The intra- and inter-assay coefficients of variation were 3.32 and 6.58% for P4 and 11.93 and 18.65% for E2, respectively.

### 2.3 Pregnancy diagnosis

About 25–30 days after mating, the pregnancy was confirmed using an ultrasound (Prosound 2, Aloka, Japan). The insemination date was used to determine the gestational age. Ewes were monitored throughout their pregnancies and documented if they had abortions.

### 2.4 Data collection

The number of single and twin lambs, along with the sex of newly borne lambs, was recorded and the twinning rate (ewes that lambed twins/pregnant ewes × 100) was calculated.

### 2.5 Statistical analysis

Comparisons between groups were conducted using SAS (Statistical Analysis System, version 9.2). Analysis of variance was used to analyze the mean body weight and steroid hormone concentrations E2 and P4. The frequency numbers of lambs with their sex ratio were analyzed using a Chi-square ($\chi^2$) test between the three different groups to determine the effect of CIDR or sponge. A difference was considered significant at $P < 0.05$. Data are expressed as percentages or means, with ± standard error mean.

### 3 Results

#### 3.1 Animal body weight

Table 1 illustrates the results of the different groups of ewe numbers used with their mean body weight before insertion of the intravaginal device CIDR or sponge and after their respective withdrawal. Before CIDR insertion, 28 ewes had a mean body weight of 62.37 ± 1.98 kg and 59.62 ± 2.00 kg after, compared to the second pre-intravaginal sponge-treated group (26 ewes) mean body weight was 60.75 ± 2.93 kg and after withdrawal 57.15 ± 2.55 kg. The control group (33 ewes) weighed 55.30 ± 4.22 kg at the time of the other groups treatment, and after 14 days, weighed 55.30 ± 3.90 kg. The mean body weight results showed no significant differences between or within the same treated groups or control groups.

#### 3.2 The fertility or pregnancy rate

The ewes in the first group treated with CIDR and injected with PMSG and the control group showed statistically higher fertility rates (92.59 and 87.87%, respectively, $P < 0.05$) than the ewes in the second group (treated with the sponge and injected with GnRH) (61.53%). All three animal groups produced a total of 92 lambs (1.29 lambs per ewe) from 71/86 pregnant ewes (82.35%), with 55 male lambs (59.78%), which is higher than the female lamb total of 37 (40.22%). The first group (CIDR and PMSG) sired 35/92 lambs (38.04%), which was significantly higher ($P < 0.05$) than the second group (sponge and GnRH), which produced 20/92 lambs (21.73%). Additionally, the second treated group produced 37/92 (40.21%) of total sired lambs, which was higher than the control group (Table 1).

#### 3.3 Regarding the sex ratio

Of the lambs produced, the first group produced 22 of the 35 males (62.85%), which was higher ($P < 0.05$) than the
female 13/35 lambs (37.15%). The second group produced close sex ratios of 11/20 (55%) males and 9/20 (45%) females, while the third control group produced 22/37 (59.45%) males, which was higher than 15/37 (40.45%) female lambs. There were no significant differences between the male or female lamb sex ratios of the three groups (Figure 1).

3.4 Regarding twins production and their sex preselection

All ewe groups produced 21 twins (42 lambs = 45.65% of the total lambs produced), eight of which were male (38.09%). The first group produced one set of male + female twins (4.76%), and the second group produced four sets of male + female twins (21.73%), of which two were male (50%) and two were male + females (50%). The third group (control) sired six sets of twins (27.36%), of which one set was males (12.5%), females (12.5%), and four sets were male + females (44.5%), with no female twins (0%). The first group was statistically (P < 0.05) lower than the two treated groups. While the third control group produced the six sets of males + females (75%), which is higher than the two treated groups, and one set was female twins (12.5%). In an analysis of pooled twin sex ratio for lambs (33.34%). The sex ratio of the total lamb production, there were 28 male lambs (66.66%) and 16 females (33.34%).

<table>
<thead>
<tr>
<th>Ewes group and no.</th>
<th>Mean body weight kg ± SE before and after CIDR or Sponge injection</th>
<th>Intravaginal insertion and hormone injections</th>
<th>Pregnant ewes no and fertility rate (%)</th>
<th>Lamb delivered (%)</th>
<th>Twins (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 CIDR and PMSG 28 ewes</td>
<td>62.37 ± 1.98</td>
<td>CIDR and PMSG</td>
<td>26 (92.59%)*</td>
<td>35 (38.04%)</td>
<td>9 twins = 18 lambs (34.4%)</td>
</tr>
<tr>
<td>Group 2 Sponge and GnRH 26 ewes</td>
<td>60.75 ± 2.93</td>
<td>Sponge and GnRH</td>
<td>16 (61.53%)*</td>
<td>20 (21.73%)</td>
<td>4 twins = 8 lambs (25%)</td>
</tr>
<tr>
<td>Group 3 Control untreated 33 ewes</td>
<td>55.30 ± 4.22</td>
<td>Control</td>
<td>29 (87.87%)*</td>
<td>37 (40.52%)</td>
<td>8 twins = 16 lambs (27.58%)</td>
</tr>
<tr>
<td>Total 86 ewes</td>
<td>55.30 ± 3.90</td>
<td>3 group</td>
<td>71 (82.85%)</td>
<td>92 lamb (1.3/ewe)</td>
<td>M = 55 (59.78%)* F = 37 (40.21%)</td>
</tr>
</tbody>
</table>

*Significantly different at P value <0.05 within the same column. M = male lamb, F female lamb, M + F = twins male with female.

Figure 1: The offspring production of the three treated ewes groups (CIDR + PMSG, Sponge + GnRH, and control) with their lamb sex ratio.

Table 1: Results of fertility and lambing rate of ewes with the twins production and the sex of the offspring in the three groups of ewes inserted with intra-vaginal CIDR or sponge and treated with PMSG or eCG hormone compared to the untreated control group.
each group, the first group produced the highest male rate production of 77.77% \( (n = 14) \) males compared to the female ratio of 22.23% \( (n = 4) \). The second twin group produced six males \( (66.66\%) \) compared to the total two females produced \( (33.34\%) \). While the third control group produced an equal sex ratio of eight males and eight females \( (50\%) \) (Figure 3).

Table 2 shows the statistical analysis of estrogen E, progesterone P, and testosterone hormone levels in treated ewes. The statistical analysis of estrogen levels in the pre-sponge insertion in ewes was significantly different \( (P < 0.01) \) compared to the first and control ewes group. The estrogen level after sponge insertion was higher \( (420.27\ pg) \) compared to the pre-insertion group \( (417.71\ pg) \) and the control ewes \( (409.01\ pg) \). The progesterone level showed no significant differences between the pre- or post-sponge group compared to the control group of ewes, varying between 14.609 and 14.970 ng in the pre-and post-vaginal insertion groups, respectively, compared to the control ewes \( (13.691\ ng) \). The testosterone hormone level also showed no significant differences, varying from 21551.095 pg before intravaginal insertion and 19126.723 pg in post-vaginal withdrawal compared to the control ewe group with 20190.106 pg (Table 2).

4 Discussion

The results of this study illustrate that, in terms of the fertility rate, lambing numbers, and twinning rates, the group which underwent administration of CIDR with PMSG injection and the control group performed better than the group with sponge and GnRH injection. This finding agrees \([20]\) that the pregnancy, fertility, twinning rates, and fecundity were significantly \( (P < 0.05) \) higher in the CIDR group \( (77.86, 75.57, 34.34, \) and 1.02, respectively) than in the FGA group \([20]\). Additionally, another study \([21]\) found that the overall fertility recorded was 80

### Table 2: Statistical analysis of estrogen, progesterone, and testosterone levels in ewes before or after intra-vaginal insertion compared to the control

<table>
<thead>
<tr>
<th>Hormones type before or after intravaginal insertion in ewes and ewes number</th>
<th>Mean hormone levels (pg/mL or ng/mL)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estrogen level before vaginal insertion 10 ewes</td>
<td>417.71 ± 3.444 pg</td>
<td>*0.0194</td>
</tr>
<tr>
<td>Estrogen level after vaginal insertion 10 ewes</td>
<td>420.27 ± 3.631 pg</td>
<td>0.10</td>
</tr>
<tr>
<td>Estrogen level control no vaginal insertion 10 ewes</td>
<td>409.01 ± 9.228 pg</td>
<td>0.10</td>
</tr>
<tr>
<td>Progesterone level before intravaginal insertion 10 ewes</td>
<td>14.406 ± 0.759 ng</td>
<td>0.10</td>
</tr>
<tr>
<td>Progesterone level after sponge insertion 10 ewes</td>
<td>14.970 ± 0.5930 ng</td>
<td>0.10</td>
</tr>
<tr>
<td>Progesterone level control no sponge 10 ewes</td>
<td>13.691 ± 1.230 ng</td>
<td>0.10</td>
</tr>
<tr>
<td>Testosterone level before sponge insertion 10 ewes</td>
<td>21251.095 ± 819.92 pg</td>
<td>0.10</td>
</tr>
<tr>
<td>Testosterone level after sponge insertion 10 ewes</td>
<td>19126.723 ± 850.13 pg</td>
<td>0.10</td>
</tr>
<tr>
<td>Testosterone level control no sponge 10 ewes</td>
<td>20190.106 ± 1404.6 pg</td>
<td>0.10</td>
</tr>
</tbody>
</table>

*Significantly different at \( P < 0.01 \) within the same column.
and 93% in response to estrus when ewe lambs were synchronized with intravaginal progesterone (40 mg MPA medroxyprogesterone acetate) for 14 days followed by intramuscular injection of 200 or 400 IU PMSG after sponge withdrawal. This could also be due to the impact of GnRH, PMSG, and hCG treatments on follicular diameter, conception, and lambing rates of Egyptian ewe lambs using an intravaginal sponge [8]. Their results showed that the follicular diameter tended to increase significantly in treated groups compared with the control, and the administration of PMSG had the best response compared to other hormonal treatments [8]. Although all progeny sex ratios of singletons with twin lambing rates showed no differences between the three groups, the first and second treated groups produced a higher total male twin lambing rate (77.77%) than the total males produced by twins in the control group (50%). This is attributed to the hormonal administration leading to ovulation, then fertilization occurring directly after ovulation, and the Y sperms that are faster but survive for less time in the female genital tract. This might give a better chance to the Y sperm that first reach the ovulation site (~70%) than the X sperm (30%). The X sperm are slower, but they are more resistant and therefore survive longer.

The lower fertility rate in the second group could be due to sponges, which increase vaginitis and drawstring breakage rates [22]. Additionally, this vaginitis also results from the use of sponges, which could negatively affect the sexual attraction of rams to mate with ewes in estrus [23].

Regarding the sex preselection rate of the ewes offspring, the result of this study agrees with our previous study [24], where we used three feeding formula (group A food formula for male sex preselection, Group B for female sex preselection and group C control group), by altering the presence of minerals in diet content before breeding the embryo sex preselection ratio for male offspring production group A (77.27%) and females 22.72%. The pregnancy rate in group B is male offspring (27.27%) and females (72.72%). In addition, the control group (C) had male offspring (54.41%) and female offspring (44.83%).

The progesterone hormone levels in our study were the same before or after the insertion of CIDR or sponge and did not show any differences, which disagrees with a previous study [25] that found that the progesterone serum concentration was higher in sheep from the 10 day CIDR groups. Additionally, in other studies [26,27], the P4 concentration increased rapidly in the blood after CIDR insertion and dropped rapidly after CIDR removal, with no cumulative effect on serum P4 concentrations [26,27]. This might be due to the differences in the ewe strains. Additionally, this study agrees with others [28,29] that the use of intravaginal devices (silicone or polyurethane sponges) impregnated with progesterone (P4) were the most efficient hormonal treatment in ovine reproduction programs.

5 Conclusion

The use of CIDR with hormone injection is better for reproduction management, male lambing, and the sex preselection rate. The control group produced approximately the same fertility rate with equal male and female lambing sex preselection rates.

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Conflict of interest: The authors of the manuscript declare that they have no competing or financial interests.

Ethical approval: All of the experiments were conducted according to the Guidelines for the Institutional Animal Care and Use Committee of the Zoology Department, College of Science at King Saud University.

Data availability statement: The authors confirm that the data supporting the findings of this study are available within the article.

References


