The effect of hCG or GnRH administration on pregnancy rates in Holstein heifers when used to induce ovulation as part of a 5-day Co-Synch + Progesterone-Releasing Intravaginal Device protocol

M. KURU*, H. ORAL1, A. ÇOLAK2, K. GÜRÇÜLAK3, T. BEKYÜREK2

1Department of Obstetrics and Gynecology, Faculty of Veterinary Medicine, Kafkas University, Kars - TURKEY
2Department of Obstetrics and Gynecology, Faculty of Veterinary Medicine, Atatürk University, Erzurum - TURKEY
3Department of Obstetrics and Gynecology, Faculty of Veterinary Medicine, Erciyes University, Kayseri - TURKEY

*Corresponding author: moshapkuru@hotmail.com

ABSTRACT

The aim of this study was to investigate how administering human chorionic gonadotropin (hCG) or gonadotropin-releasing hormone (GnRH) effects pregnancy rates in heifers when used to stimulate ovulation as part of a 5-day Co-Synch + Progesterone-Releasing Intravaginal Device (PRID) protocol. In this study, Holstein heifers (n=242) were randomly divided into 4 groups. In Group GPG (GnRH-PG-GnRH, n=60), GnRH was administered on day 0, and a PRID was inserted. After the PRID was removed on day 5, progstaglandin F2 alpha (PGF2α) was administered, and 72 h later fixed-time artificial insemination was performed with sexed semen, and GnRH was administered. Group PG (NoGnRH-PG-GnRH, n=59) was given the same protocol as Group GPG, except no GnRH was administered on day 0. Group GPH (GnRH-PG-hCG, n=62) differed from Group GPG in that hCG was administered at artificial insemination. Group PH (NoGnRH-PG-hCG, n=61) did not receive GnRH treatment on day 0, but like Group GPH, hCG was administered to induce ovulation. Based on progesterone levels measured 10 days prior to the application of the protocol and on day 0 of treatment, it was determined that all of the heifers included in the study were cyclic. Pregnancy rates on day 30 were 48.3%, 54.2%, 53.2% and 45.9% in Groups GPG, PG, GPH and PH, respectively. Pregnancy rates on day 60 were 45%, 50.8%, 50% and 44.3% in Groups GPG, PG, GPH and PH, respectively. Furthermore, gestational losses in these groups were 6.8%, 6.2%, 6.1% and 3.6%, respectively (P>0.05). In conclusion, in the present study, similar pregnancy rates (P>0.05) were achieved in all groups. Furthermore, it was determined that similar pregnancy rates were also achieved with no GnRH treatment on day 0 and with administration of hCG or GnRH for induction of ovulation (P>0.05).

Keywords: Heifer, GnRH, hCG, PRID, 5-Day Co-Synch

RÉSUMÉ

Effet de l'administration d’ hCG ou de GnRH sur le taux de gestation chez les génisses Holstein dans le cadre d’une co-synchronisation de 5 jours associée à une libération de progestérone par dispositif intravaginal

Deux cent quarante-deux génisses Holstein ont été réparties au hasard en 4 groupes de 60. Le groupe GPG (GnRH-PG-GnRH, n=60) a reçu une injection de GnRH le jour 0 et un PRID qui a été retiré le jour 5 alors qu’une injection de prostaglandine F2 alpha (PGF2α) était effectuée et qu’une insémination artificielle était réalisée 72 heures plus tard avec du sperme sexué, et une nouvelle injection de GnRH était réalisée. Le groupe PG (NoGnRH-PG-GnRH, n = 59) a reçu le même protocole que le groupe GPG, sauf qu’aucune GnRH n’a été administré le jour 0. Le groupe GPH (GnRH-PG-hCG, n = 62) a reçu le même protocole que le groupe GPG, sauf que de l’hCG a été administré lors de l’insémination artificielle à la place de la GnRH. Le groupe PH (NoGnRH-PG-hCG, N = 61) a reçu le même protocole que le groupe PG, sauf que de l’hCG a été administré lors de l’insémination artificielle à la place de la GnRH. Sur la base des niveaux de progesterone mesurés 10 jours avant l’application du protocole et le jour 0 de traitement, il a été déterminé que toutes les génisses incluses dans l’étude étaient cyclées. Les taux de gestation au jour 30 ont été de 48,3%, 54,2%, 53,2% et 45,9% dans les groupes GPG, PG, GPH et PH, respectivement. Les taux de gestation au jour 60 étaient de 45%, 50,8%, 50% et 44,3% dans les groupes GPG, PG, GPH et PH, respectivement. En outre, les pertes gestationnelles dans ces groupes étaient de 6,8%, 6,2%, 6,1% et 3,6%, respectivement (P>0,05). En conclusion, dans la présente étude, des taux de gestation similaires (P>0,05) ont été observés dans tous les groupes. En outre, il a été déterminé que des taux de gestation similaires ont également été observés sans GnRH le jour 0 et avec administration de hCG ou GnRH pour l’induction de l’ovulation (P> 0,05).

Mots-clés : génisse, GnRH, hCG, PRID, synchronisation des chaleurs, éponge vaginale

Introduction

Reproduction is one of the major factors affecting profitability in cattle feedlots and dairy holdings, so one calf in a year and maximum milk production are required [17]. Increased milk production significantly lowers fertility. Because estrous detection is problematic in cattle with high yields, many operations suffer serious economic losses. Failure to detect estrus in a timely and accurate manner is especially common with heifers [36].

High pregnancy rates have been reported especially in cows using ovulation synchronization techniques, but these rates are lower in heifers. Therefore, synchronization protocols have been developed that do not require observation to induce ovulation [11, 30]. Since the targeted results cannot be achieved with most of these protocols in heifers, more efficient protocols (i.e. 5-day Co-Synch) are being developed [19, 22-24, 35].

In a previous study, the application of a 5-day Co-Synch + Progesterone protocol achieved estrus synchronization in dairy heifers. According to this protocol, one of the study groups was administered gonadotropin-releasing hormone (GnRH) on day 0, and the other group was not given GnRH. Pregnancy tests conducted on the animals on day 32 showed
EFFECT OF hCG OR GnRH ON PREGNANCY RATES IN HOLSTEIN HEIFERS

that the pregnancy rate of the group that had not received GnRH (54.1%) was similar to that of the group that was given GnRH (52.5%) [24].

Depending on market conditions, dairy operations might want female calves sometimes and males at others. When milk prices are high, female calves might be desired while demand for males would rise when the price of meat goes up. In situations like this, the operation will want to design the new crop of calves based on economic conditions. Dairy operations generally prefer female calves due to both milk and for the sale of breeding heifers [2, 18]. It has been reported that when cows or heifers are inseminated artificially with conventional semen or sexed semen, pregnancy rates may be similar and that pregnancy rates may be higher with artificial insemination using classic techniques [15, 16]. Studies in this vein have also been conducted on heifers [15].

The ovarium is directly affected by the administration of human chorionic gonadotropin (hCG) to cows or heifers [14, 34]. After administration of hCG, ovulation of mature follicles reportedly stimulates a follicular wave and formation of the corpus luteum [7, 10, 29]. In heifers, this hormone synchronization can be used on the first day of the protocols to stimulate ovulation [37]. Schmitt et al. [37] used hCG hormone to induce ovulation in heifers with the Co-Synch protocol, achieving a pregnancy rate of 52.9% at the end of the study.

The aim of this study was to investigate the effect of hCG or GnRH on pregnancy rates when used to induce ovulation in Holstein heifers as part of a 5-day Co-Synch + Progesterone-Releasing Intravaginal Device (PRID) protocol. This study also investigated whether GnRH had to be administered to heifers on day 0 as part of a 5-day Co-Synch + PRID protocol, as well as how progesterone levels on the first day of the protocol affected the pregnancy rates achieved.

Material and method

This study was conducted pursuant to the approval of the Local Ethics Board for Animal Experiments at Erciyes University (Erciyes University - HADYEK 2013 – Meeting number: 06 – Decision number: 13/18).

ANIMAL MATERIAL AND FEED RATION

The study was carried out between June and September of 2014 on heifers that were fed a mixed ration, provided with ad libitum water and housed in a semi-open barn located in the Kayseri province, Turkey. The mixed ration contained maize silage, dry alfalfa, maize hay and wheat hay as roughage, barley, maize, bran, sunflower oil cake, calcium carbonate and salt as concentrates, and manganese, iron, zinc, copper, cobalt, selenium, calcium, magnesium, phosphorus, and Vitamins A, D and E as a vitamin-mineral premix. The ration was prepared so that the percentage of dry matter was 90%, metabolic energy content was 2550 kcal/kg and the crude protein content was 16%. The 242 Holstein heifers included in the study were randomized into 4 groups.

SYNCHRONIZATION PROTOCOLS

Group GPG (GnRH-PG-GnRH, n=60): This group was treated with 100 µg of gonadorelin diacetate tetrahydrate (2 mL, im, GnRH, Ovarinel®, CEVA, Turkey) and fitted with a Progesterone-Releasing Intravaginal Device (1.55 g, progesterone, PRID, PRID-Delta®, Ceva, Turkey) on day 0. On day 5, the PRID was removed and the animals were treated with 25 mg of dinoprost tromethamine (5 mL, PGF₂α, im, Dinolytic®, Zoetis, Turkey). Seventy-two h after prostaglandin F₂ alpha (PGF₂α) administration, fixed-time artificial insemination was performed on the animals and they were also given GnRH.

Group PG (NoGnRH-PG-GnRH, n=59): The PRID was inserted in the vagina on day 0. On day 5, the PRID was removed, and the heifers were given a PGF₂α injection. Seventy-two h after PGF₂α administration, fixed-time artificial insemination was performed and they were also given GnRH.

Group GPH (GnRH-PG-hCG, n=62): GnRH was injected and the PRID was inserted in the vagina on day 0. On day 5, the PRID was removed and the animals were given PGF₂α. After seventy-two h after PGF₂α administration, the animals were given 1500 IU of human chorionic gonadotropin (hCG, 5 mL, im, Chorulon', Intervet, Turkey) when the fixed-time artificial insemination was performed.

Group PH (NoGnRH-PG-hCG, n=61): The PRID was inserted in the vagina on day 0. On day 5, the PRID was removed, and the heifers were injected with PGF₂α. Seventy-two h after PGF₂α administration, fixed-time artificial insemination was performed, and the heifers were also given hCG (Figure 1).

ARTIFICIAL INSEMINATION

In all groups, prior to the fixed-time artificial insemination performed on day 8, the number and size of the ovarian follicles were determined by ultrasonography (5-7.5 MHz, Honda HS-2100®, Honda Electronic, Japan). Subsequently, semen was deposited into the uterine horn with the Graafian follicle. Sexed semen (2 x 10⁶ motile spermatozoa/straw, Plushansky Farsano, Egevet, Turkey) was used for the artificial insemination of the heifers. After being thawed in a water bath at a temperature of 37°C for 30 sec, the semen was deposited into the uterine horn [38] using the recto-vaginal method. The heifers included in this study were artificially inseminated only once. In order to avoid any differences due to application, the artificial insemination procedures were performed by the same veterinary practitioner.

PROCEDURES PERFORMED ON THE DAY OF ARTIFICIAL INSEMINATION

All of the artificially inseminated heifers were measured for wither height and body weight. Furthermore, starting 24 h prior to artificial insemination, the animals were observed at 4-h intervals for signs of estrous. Shortly before artificial insemination, an ultrasonographic (5-7.5 MHz, Honda HS-2100®, Honda Electronic, Japan) examination was performed to determine the presence of ovarian follicles, the diameter of ovarian follicles and the location of ovarian follicles (in the left or right ovary). Furthermore, the presence of the corpus luteum, increase in uterine tone and the presence of estrual mucus discharge were also observed. The vulvar and vaginal mucosa were inspected for the presence or absence of hyperaemia. The body condition scores (BCS) of the heifers were graded using a scale of 1-5 with 0.25 increments as described by Edmonson et al. [13].

PREGNANCY DIAGNOSIS

Pregnancy examinations were performed 30 ± 2 d and 60 ± 2 d after artificial insemination using ultrasonography. The rates of gestational loss that occurred within these time periods were compared between the study groups.

ASSESSMENT OF BLOOD SAMPLES

All of the heifers included in the study were sampled for blood on days -10, 0, 5 and 8 from the vena coccigaea using sterile holders (BD Vacutainer®, Tipkimsan, Turkey). The blood samples were transferred into 8.5-mL dry vacuum gel-coated tubes (BD Vacutainer®, Tipkimsan, Turkey). All samples were centrifuged at 4000 rpm for 10 min (Hettich Universal 320®, Hettich, Germany). The serum samples were stored at -18°C until they were used. Hormone analyses were performed at the Biochemistry Laboratory of Veterinary Medicine at the Kafkas University. Progesterone levels were measured using the Enzyme-Linked Immunosorbent Assay (ELISA) commercial kits (EIA 1561®, DRG International, Germany) and with the aid of an ELISA reader (Epoch®, Biotek, USA). All samples were evaluated in a single batch to eliminate interassay variation. The range of the assay is between 0 – 40 ng/mL. Assay sensitivity was 0.045 ng/mL and intraassay variation was 5.4%.

Progesterone (P₄) levels in the blood samples collected from the heifers on days -10 and 0 were measured to determine the cyclicity of the animals (P₄ ≥ 1 ng/mL on at least one of these days). On the basis of the results obtained, the effect of progesterone levels on the pregnancy rate was investigated. The corpora lutea detected at the time of artificial insemination and the serum progesterone levels were comparatively evaluated for each animal. The active corpora lutea (P₄ ≥ 1 ng/mL) were determined for luteolysis failure [6].

STATISTICAL ANALYSIS

Statistical analysis of the data was performed with the SPSS 20 (SPSS®, Chicago, IL, USA) statistical software package. The proportional evaluation of the pregnancy rates achieved by the end of the study and the estrous signs observed at the time of artificial insemination was performed using the chi-square method and the logistic regression test. Analyses of the group for progesterone levels were performed using one-way analysis of variance and Tukey's honestly significant difference (HSD) test. We tested the difference between groups for all the variables (body weight, wither height, body condition score, largest follicle diameter, and progesterone level) by fitting a logistic regression model to the data using software program R [31]. The results were expressed as mean ± standard error (SE). The level of statistical significance was set at values of P<0.05 and below.

Results

DISTRIBUTION OF THE GROUPS FOR AGE, WITHER HEIGHT, BODY WEIGHT AND BCS

It was determined that, on the day of artificial insemination, the distribution of the groups for mean age, wither height, body weight and BCS did not significantly differ from each other (P>0.05). These data compared between the groups are presented in Table I.

SIGNS OF ESTROUS OBSERVED IN THE GROUPS ON THE DAY OF ARTIFICIAL INSEMINATION

Table II provides vaginal hyperaemia, uterine tone, estrual mucus discharge and estrous signs present at the time of artificial insemination. On the basis of these data it was determined that the study groups did not statistically differ in terms of vaginal hyperaemia or signs of estrous (P>0.05). On the other hand, it was determined that the differences between the study groups for the increase in uterine tone detected by transrectal palpation prior to artificial insemination, and for estrual mucus discharge were statistically significant (P<0.05). The evaluation of the study groups for increase
in uterine tone demonstrated that the increase observed in Groups GPG and GPH was significantly lower than that observed in Group PH (P<0.05). Furthermore, we found that the percentage of estrual mucus discharge in Group PH was lower than that detected in Groups GPG, PG and GPH (P<0.05).

ULTRASONOGRAPHY FINDINGS AT THE TIME OF ARTIFICIAL INSEMINATION

The ovary bearing the largest follicle and follicle diameters <10 mm or ≥10 mm are presented in Table III. No statistically significant difference was found between the study groups for the ovary bearing the largest follicle (LF) or for the LF diameters <10 mm or ≥10 mm (P>0.05). The largest follicle diameter in Group GPH was statistically different from the LF diameters detected in Groups PG and PH (P<0.05).

The ultrasonographic examination performed on day 8 of the synchronization protocol (the day of artificial insemination) also revealed the presence of corpora lutea. The number of heifers found to have a corpus luteum was 2 in Group GPG, 1 in Group PG, 3 in Group GPH, and 5 in Group PH. No statistically significant difference was found between the groups (P>0.05).

**TABLE I:** Distribution of the study groups by age, wither height, body weight and body condition scores (BCS) (mean ± SE).

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Age (day)</th>
<th>Wither height (cm)</th>
<th>Body Weight (kg)</th>
<th>BCS</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPG</td>
<td>60</td>
<td>447.6 ± 2.9</td>
<td>136.4 ± 0.4</td>
<td>384.7 ± 4.0</td>
<td>3.07 ± 0.04</td>
</tr>
<tr>
<td>PG</td>
<td>59</td>
<td>439.0 ± 2.7</td>
<td>138.3 ± 0.4</td>
<td>386.9 ± 4.3</td>
<td>3.14 ± 0.05</td>
</tr>
<tr>
<td>GPH</td>
<td>62</td>
<td>447.6 ± 2.8</td>
<td>135.9 ± 0.4</td>
<td>383.8 ± 4.6</td>
<td>3.20 ± 0.04</td>
</tr>
<tr>
<td>PH</td>
<td>61</td>
<td>445.5 ± 3.1</td>
<td>136.3 ± 0.4</td>
<td>390.5 ± 4.9</td>
<td>3.07 ± 0.03</td>
</tr>
</tbody>
</table>


**Table II:** Distribution of estrous signs observed in the study groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Vaginal hyperemia</th>
<th>Uterine tone</th>
<th>Estrual mucus</th>
<th>Estrous detection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% (no/total no)</td>
<td>% (no/total no)</td>
<td>% (no/total no)</td>
<td>% (no/total no)</td>
</tr>
<tr>
<td>GPG</td>
<td>70.0 (42 / 60)</td>
<td>71.6* (43 / 60)</td>
<td>60.0* (36 / 60)</td>
<td>41.7 (25 / 60)</td>
</tr>
<tr>
<td>PG</td>
<td>67.8 (40 / 59)</td>
<td>55.9ab (33 / 59)</td>
<td>50.8a (30 / 59)</td>
<td>40.7 (24 / 59)</td>
</tr>
<tr>
<td>GPH</td>
<td>71.0 (41 / 62)</td>
<td>75.8a (47 / 62)</td>
<td>66.1a (41 / 62)</td>
<td>43.7 (27 / 62)</td>
</tr>
<tr>
<td>PH</td>
<td>70.5 (43 / 61)</td>
<td>47.5b (29 / 61)</td>
<td>32.7b (20 / 61)</td>
<td>32.8 (20 / 61)</td>
</tr>
</tbody>
</table>

* Different superscripts within column were significant (P < 0.05). * Show the P-value: P > 0.05. GPG: GnRH-PG-GnRH, PG: NoGnRH-PG-GnRH, GPH: GnRH-PG-hCG, PH: NoGnRH-PG-hCG.

**Table III:** Findings obtained by ultrasonography shortly before artificial insemination.
Table IV: Pregnancy rates (PR) and gestational losses in the study groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Day 30 PR</th>
<th>F ≥ 10 mm PR</th>
<th>Day 60 PR</th>
<th>Gestational losses</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPG</td>
<td>48.3 (29 / 60)</td>
<td>63.0 (29 / 46)</td>
<td>45.0 (27 / 60)</td>
<td>6.8 (2 / 29)</td>
</tr>
<tr>
<td>PG</td>
<td>54.2 (32 / 59)</td>
<td>74.4 (32 / 43)</td>
<td>50.8 (30 / 59)</td>
<td>6.2 (2 / 32)</td>
</tr>
<tr>
<td>GPH</td>
<td>53.2 (33 / 62)</td>
<td>67.3 (33 / 49)</td>
<td>50.0 (31 / 62)</td>
<td>6.1 (2 / 33)</td>
</tr>
<tr>
<td>PH</td>
<td>45.9 (28 / 61)</td>
<td>60.8 (28 / 46)</td>
<td>44.3 (27 / 61)</td>
<td>3.6 (1 / 28)</td>
</tr>
</tbody>
</table>

P value


Table V: The change in serum progesterone levels (ng/mL) in the groups with the day of blood sampling.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Day -10 Mean ± SE</th>
<th>Day 0 Mean ± SE</th>
<th>Day 5 Mean ± SE</th>
<th>Day 8 Mean ± SE</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPG</td>
<td>3.3 ± 0.2a</td>
<td>3.2 ± 0.2a</td>
<td>4.3 ± 0.2b</td>
<td>0.3 ± 0.03c</td>
<td>0.000</td>
</tr>
<tr>
<td>PG</td>
<td>3.3 ± 0.2a</td>
<td>4.0 ± 0.3b</td>
<td>4.8 ± 0.2b</td>
<td>0.3 ± 0.03c</td>
<td>0.000</td>
</tr>
<tr>
<td>GPH</td>
<td>3.5 ± 0.2a</td>
<td>3.7 ± 0.3b</td>
<td>4.4 ± 0.2b</td>
<td>0.3 ± 0.02c</td>
<td>0.000</td>
</tr>
<tr>
<td>PG</td>
<td>3.4 ± 0.3a</td>
<td>3.9 ± 0.3b</td>
<td>4.3 ± 0.2b</td>
<td>0.2 ± 0.02c</td>
<td>0.000</td>
</tr>
<tr>
<td>P value</td>
<td>0.712</td>
<td>0.059</td>
<td>0.321</td>
<td>0.403</td>
<td></td>
</tr>
</tbody>
</table>


Table VI: Pregnancy rates (PR) in the study groups based on serum progesterone levels on day -10.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Day 0 Mean ± SE</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPG</td>
<td>13.3 (8 / 60)</td>
<td>*</td>
</tr>
<tr>
<td>PG</td>
<td>16.9 (10 / 59)</td>
<td>*</td>
</tr>
<tr>
<td>GPH</td>
<td>12.9 (8 / 62)</td>
<td>*</td>
</tr>
<tr>
<td>PH</td>
<td>13.1 (8 / 61)</td>
<td>*</td>
</tr>
</tbody>
</table>

P value


Table VII: Pregnancy rates (PR) in the study groups based on serum progesterone levels on day 0.
PREGNANCY RATES AND GESTATIONAL LOSSES IN THE GROUPS

The pregnancy rates on days 30 ± 2 and 60 ± 2, and the gestational losses in each group are shown in Table IV. Evaluation showed no statistically significant difference between the groups with regard to these parameters (P>0.05).

The number of pregnancies detected in the heifers by presence of estrual mucus discharge was 24 in Group GPG (29 pregnancies), 23 in Group PG (32 pregnancies), 29 in Group GPH (33 pregnancies) and 10 in Group PH (28 pregnancies). The difference detected between Groups GPG, PG and GPH and Group PH were statistically significant (P<0.05).

The results showed that all of the heifers included in the study were cycling. The serum progesterone levels measured in the study groups on days -10, 0, 5 and 8 are presented in Table V.

The pregnancy rates determined for the study groups based on serum progesterone levels on days -10 and 0 lower or higher than 1 ng/mL are provided in Tables VI and VII.

Discussion

Cows and heifers in estrous display vaginal hyperaemia, increased uterine tone and estrual mucus discharge [3, 12]. In a study conducted on heifers by Lima et al. [24] using the 5-day Co-Synch + Progesterone protocol, the percentage of animals in estrous on the day of artificial insemination was 64.5% in the group that did not receive GnRH on day 0, and 69.2% in the group that received GnRH on day 0. Estrous monitoring was continued from the administration of PGF2α to the performance of artificial insemination, which was 3 days. In the present study, estrous percentages were 41.7% and 40.7%, respectively, in Groups GPG and PG. The estrous rates detected in previous studies appear to be higher than those detected in the present study. This difference was attributed to estrous monitoring having been continued for only 24 h (until artificial insemination) in the present study.

In a study carried out on dairy heifers by Colazo and Ambrose [5], the largest follicle diameter on the day of artificial insemination was 13.9 mm and 13.5 mm, respectively, in animals that were given the 5-day Co-Synch + PRID protocol and the 7-day Co-Synch + PRID protocol. With the use of protocols similar to those applied in the present study (GPG/PG), Kasimanickam et al. [21] reported that the diameter of the largest follicle ranged between 13.2-16.4 mm. In the present study, the mean LF diameters were determined to range between 12.0-13.1 mm. The LF diameters we obtained were smaller than that in other studies.

In previous research where protocols similar to those used in the present study were tested, pregnancy rates ranged between 39% and 66% [1, 6, 19, 25, 26]. Studies that used the 5-day Co-Synch + progesterone (GnRH/PGF2α/GnRH) protocol on beef and dairy heifers achieved pregnancy rates ranging between 48.8% and 62.1% on day 30 ± 2 [5, 6, 20, 21, 24, 32]. In the present study, the pregnancy rate on day 30 in Group GPG was 48.3%. There are reports in the literature of pregnancy rates ranging from 44.8% to 58.4% using protocols with no GnRH injection at the beginning (No GnRH/PGF2α/GnRH) [6, 20, 21, 24, 26-28].

When heifers were treated with the 5-day Co-Synch + Progesterone protocol (GnRH/PGF2α/GnRH), pregnancy rates between days 45 and 60 have been found to range from 49.8% to 59.6% [24, 32, 33]. In the present study, pregnancy examinations on day 60 revealed a pregnancy rate of 45% in Group GPG. The differences observed between pregnancy rates in different studies are attributed to several factors, including among others, the practitioner performing the insemination procedure, the season and the use of sexed semen. The literature indicates that pregnancy rates on day 60 in heifers treated with the 5-day Co-Synch + Progesterone protocol without receiving GnRH on day 0 range between 42%-51.6% [24, 26, 27]. In the present study, the pregnancy rate in Group PG on day 60 was 50.8%. It has been reported that gestational losses in heifers treated with the 5-day Co-Synch + Progesterone protocol may range between 4.6% and 5.8% [20, 24, 25, 32]. The gestational losses determined in the present study ranged between 3.6% and 6.8%.

The administration of hCG to cows and heifers directly affects the ovaries [15, 34]. In heifers, this hormone can be used on the first day of ovulation synchronization protocols and at the time of artificial insemination [37]. When using hCG to induce ovulation as part of the Co-Synch protocol they applied to heifers, Schmitt et al. [37] achieved a pregnancy rate of 52.9%. Our study also indicates that hCG used the first time in the 5-day Co-Synch protocol could be an alternative to GnRH for inducing ovulation. In the present study, pregnancy rates in Groups GPH and PH, both of which were administered hCG to induce ovulation, were 53.2% and 45.9%, respectively.

In previous research conducted using sexed semen, pregnancy rates ranging between 41%-53% were reported on day 30 [4, 8, 9, 15]. Melleion et al. [27] reported achieving a pregnancy rate of 50% with the use of sexed semen. The pregnancy rates achieved in previous research were similar to those achieved in the present study (45.9%-53.2%). The low pregnancy rates in some studies may have been caused by the motility of the sexed semen and the sperm count.

Seidel et al. [38] established study groups, which were inseminated with semen deposited in different areas of the uterus (the corpus uteri or the cornu uteri). In their study, these researchers achieved pregnancy rates of 54% and 51%, respectively, with semen deposited into the cornu uteri and corpus uteri. In the present study, at the time of artificial insemination, semen was deposited into the cornu uteri in all groups, and an average pregnancy rate of 50.4% was achieved.
In conclusion, of the heifers treated with the 5-day Co-Synch + PRID protocol, those that did not receive GnRH on day 0 presented with less evident signs of estrous (increase in uterine tone and estrual mucus discharge). It was determined that the administration of GnRH at the beginning of the protocol could increase the diameter of the largest follicle. The similarity of the pregnancy rates achieved in all of the study groups suggest that protocols with no GnRH administration on day 0 might be preferable. It was determined using hCG or GnRH to induce ovulation had a similar effect on pregnancy rates. The serum progesterone levels on day 0 did not affect the pregnancy rate in this study, but more comprehensive studies should be conducted. Therefore, the use of these protocols on farms encountering problems with estrous detection and on farms using sexed semen would be beneficial.

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Conflict of interest

None of the authors declared having any conflict of interest.

References

EFFECT OF hCG OR GnRH ON PREGNANCY RATES IN HOLSTEIN HEIFERS