Synchronization of estrus in cows using double PGF$_{2\alpha}$, GnRH-PGF$_{2\alpha}$ and hCG-PGF$_{2\alpha}$ combination

° K. ÇOYAN, ° M.B. ATAMAN, °° H. ERDEM, ° A. KAYA and °°° G. KASIKCI

SUMMARY

The aim of this study was to compare the effectiveness of treatments combining GnRH and PGF$_{2\alpha}$, hCG and PGF$_{2\alpha}$, combinations, and double PGF$_{2\alpha}$ administration for synchronization of estrus in cows.

This study was carried out in 30 Brown Swiss cows, aging 3 - 5 years. The cows were randomly divided into three groups. In group I (n = 10), the cows were treated with an intramuscular injection of 20 µg GnRH (day = 0) at a random stage of the estrous cycle followed by intramuscular injection of 0.150 mg PGF$_{2\alpha}$ 7 days later (day = 7). In group II (n = 10), the cows were treated with an intravenous injection of 3000 IU hCG (day = 0) at a random stage of the estrous cycle followed by intramuscular injection of 0.150 mg PGF$_{2\alpha}$ 7 days later (day = 7). In group III (n = 10), the cows were received two injections of 0.150 mg PGF$_{2\alpha}$ 11 day apart (day= 0, and 11).

GnRH (10 µg) was injected intramuscularly to the cows 48 hours after the injection of PGF$_{2\alpha}$ in the groups I and II, and after the second PGF$_{2\alpha}$ in group III. The cows were inseminated 12 hours after the GnRH injections.

Blood samples were collected daily to determine plasma progesterone levels on day 0 and at estrus were similar among the three groups, but it was significantly higher (p < 0.01) in the groups I and II than in the group III on the day of PGF$_{2\alpha}$ administration. The mean GnRH injections-estrus, injections-ovulation, synchronization and pregnancy rates of the groups were determined.

Plasma progesterone levels on day 0 and at estrus were similar among the three groups, but it was significantly higher (p < 0.01) in the groups I and II than in the group III on the day of PGF$_{2\alpha}$ administration. The mean GnRH injections-estrus, injections-ovulation interval in the groups I, II and III were 52.2 ± 1.69, 48.4 ± 1.34 and 68.2 ± 1.31 hours; 70.4 ± 1.17, 67.3 ± 1.36 and 90.6 ± 2.15 hours, respectively and were significantly different (p < 0.01). The synchronization rates tend to be higher in the groups I and II (100 %) than in the group III (80 %), and pregnancy rates of the groups were determined.

As a conclusion, a combination of GnRH and hCG analogue prior to synchronization of estrus with an injection of PGF$_{2\alpha}$ may provide better results than two injections of PGF$_{2\alpha}$ for estrus synchronization in cows.

KEY-WORDS : Cow - estrus synchronization - GnRH - hCG - PGF$_{2\alpha}$

RÉSUMÉ

Synchronization de l’œstrus chez la vache à l’aide soit de deux doses de PGF2α, soit l’association GnRH - PGF2α, soit hCG - PGF2α. Par K. ÇOYAN, M.B. ATAMAN, H. ERDEM, A. KAYA et G. KASIKCI.

Le but de cette étude était de comparer l’efficacité, dans la technique de synchronisation de l’œstrus chez la vache, de traitements associant soit du GnRH et PGF2α, soit hCG et PGF2α, soit deux injections de PGF2α.

Cette étude a été entreprise sur 30 vaches de race Brune, randomisées, âgées de 3 à 5 ans, réparties en 3 groupes. Le groupe I (10 animaux) recevait une injection intramusculaire de 20 µg de GnRH au jour 0 suivie d’une injection intramusculaire de 0,150 mg de PGF2α 7 jours plus tard (J7). Le groupe II (n = 10) recevait une injection intraveineuse de 3000 IU d’hCG (J = 0) suivie d’une injection intramusculaire de 0,150 mg de PGF2α à J7. Les animaux du groupe III (n = 10) reçurent deux injections de 0,150 mg de PGF2α, 11 jours d’intervalle.

Tous les animaux ont reçu 10 µg de GnRH par voie intramusculaire 48 heures après le PGF2α.

Les inséminations ont été pratiquées 12 heures après les injections de GnRH.


Les niveaux de progestéronémie plasmatique au jour 0 de l’œstrus ont été comparables dans les trois groupes, mais ils furent plus élevés (p < 0.01) dans les groupes I et II le jour de l’injection de PGF2α. Les intervalles injection de GnRH-œstrus et injections-ovulations dans les groupes I, II et III furent de 52.2 ± 1.69, 48 ± 1.34 et 68.2 ± 1.31 heures ; 70.4 ± 1.17, 67.3 ± 1.36 et 90.6 ± 2.15 heures (différences significatives p < 0.01). Le taux de synchronisation était supérieur dans les groupes I et II (100 %) à celui du groupe III (80 %) et les taux de gestation ont été respectivement de 60 %, 60 % et 30 % dans les groupes I, II, III.

En conclusion, l’utilisation de GnRH et de hCG avant la synchronisation par le PGF2α peut donner des résultats supérieurs à ceux obtenus avec deux injections de PGF2α.


Introduction

Poor rates of estrous detection combined with poor conception rates make management of reproduction in lactating dairy cows a challenge in most dairy herds. Synchronization of estrus have been developed to help farmers manage reproduction more efficiently [10].

Estrous synchronization programs commonly involve the synchronization of luteal regression using prostaglandin treatment in cows. A treatment with 2 administration of PGF2α 10 to 12-day apart results in estrus occurring over a 5-day period [2, 10, 18]. The variability in the occurrence of estrus is due to the maturity of the ovulatory follicle at luteolysis, which in turn reflects the stage of the wave of follicular
growth [8]. In addition, when cows were inseminated 72-80 hr after the second injection of PGF$_{2\alpha}$, conception rate was lower compared to conception rate of cows mated to a detected estrus [2, 21, 25]. Therefore, improved synchrony of estrus could be achieved by the synchronization of the follicular waves in addition to the synchronization of luteolysis [6].

In the cyclic animals, a follicular wave terminates when the dominant follicle either regresses or ovulates, leading to start another wave of follicular growth [13]. An injection of GnRH analogues 6 days prior to an injection of PGF$_{2\alpha}$, enhanced conception rate [26], increased number of synchronized animals and reduced variability of time to estrus in cows and heifers [28, 29]. This decrease may be explained by the initiation of a new follicular wave following injection of GnRH, which resulted in a new dominant follicle being present at the time of PGF$_{2\alpha}$ injection [20, 22].

Treatment of heifers with GnRH at random stages of the estrous cycle for lactating cows or on day 3 to 5 of cycle following single injection of PGF$_{2\alpha}$ generally stimulates formation of a new luteal structure or secondary corpus luteum by inducing luteinization or ovulation of the dominant follicle in most cases [26]. The resetting follicular development could produce a new dominant follicle that contains an oocyte of greater potential fertility [16], which would lead to greater embryonic survival [1].

Human chorionic gonadotropin (hCG) is produced by the cytotrophoblast of the chorionic villi in the human placenta. When administered to cows, hCG exerts primarily an LH effect accompanied with very little FSH effect. Clinically, hCG is a good exogenous source of LH activity [4]. GnRH and hCG are commonly used in bovine practice to treat follicular cysts [5], to accelerate ovulation [3], and to initiate new follicular wave [20].

The objective of this study was to compare the fertility rate at the induced oestrus after three schedules of treatments: the hCG + PGF$_{2\alpha}$, GnRH + PGF$_{2\alpha}$ and PGF$_{2\alpha}$ + PGF$_{2\alpha}$ combined with GnRH.

Materials and methods

Thirty Brown Swiss lactating and cycling cows, aging between 3-5 years old with known fertility were used as a material, belonging to Animal Central Research Institute, Konya, TÜRKİYE. The animals were cycling and the interval between calving and treatment of synchronization was 60-90 days.

Treatment of cows

Cows were randomly divided into three groups. hCG at the dosage of 3000 IU (Pregnyl, Organon, Istanbul) was injected intravenously to the cows in the first group (n = 10). Seven days after hCG injection, (Day 0 = injection of hCG), 0.150 mg d-cloprostenol (Dalmazine, Vetas, Istanbul) was injected to the same group of cows intramuscularly. Busereline at the dosage of 20 µg (Receptal, Hoescht, Istanbul) was intramuscularly injected to the cows of the second group (n = 10). The same dosage of d-cloprostenol was applied to the cows of the second group seven days after GnRH injection. (Day 0 = injection of GnRH). D- cloprostenol (0.150 mg), an analogue of PGF$_{2\alpha}$, was intramuscularly injected two times (At day 0 and day 11) at 11-day interval to the cows of the third group (n = 10). GnRH (10 µg) was also injected to the cows 48 hours after the injection of PGF$_{2\alpha}$ in the groups I and II and after the injection of second PGF$_{2\alpha}$ in group III (Ovulation induction). The cows have been inseminated at fixed time (12 hours later) after the GnRH injections. The treatment protocol for the cycling animals was summarised in Table I.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
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<tbody>
<tr>
<td>GnRH+PGF$_{2\alpha}$ ( n:10 )</td>
<td>hCG+PGF$_{2\alpha}$ ( n:10 )</td>
<td>PGF$<em>{2\alpha}$+PGF$</em>{2\alpha}$ ( n:10 )</td>
<td></td>
</tr>
<tr>
<td>Day 0</td>
<td>20 µg busereline ( IV )</td>
<td>3000 IU hCG ( IM )</td>
<td>0.150 mg d-cloprostenol ( IM )</td>
</tr>
<tr>
<td>Day 7</td>
<td>0.150 mg d-cloprostenol ( IM )</td>
<td>0.150 mg d-cloprostenol ( IM )</td>
<td>-</td>
</tr>
<tr>
<td>Day 9</td>
<td>10 µg busereline ( IM )</td>
<td>10 µg busereline ( IM ) +</td>
<td>-</td>
</tr>
<tr>
<td>AI 12 hours after busereline injection</td>
<td>AI 12 hours after busereline injection</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Day 11</td>
<td>-</td>
<td>-</td>
<td>0.150 mg d-cloprostenol ( IM )</td>
</tr>
<tr>
<td>Day 13</td>
<td>-</td>
<td>10 µg busereline ( IM )</td>
<td>+</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>AI 12 hours after busereline injection</td>
<td></td>
</tr>
</tbody>
</table>

Table I. — Treatment protocol for each group.
Collection of blood samples

Blood samples were daily collected from jugular vein of the cows starting from the first injections (Day 0, hCG, GnRH and first PGF₂α injections) for 13 days in the groups I and II and for 15 days in the group III. Plasma progesterone levels were determined by RIA technique.

Ultrasonographic examinations

The cycle of each cow was followed through the transrectal ultrasonography to determine follicular and luteal developments. Corpus luteum and follicle diameters of cows were daily measured through until the 13th day in the groups I and II and 16th day in the group III.

Estrous detection and mating

After injections of PGF₂α in the groups I and II and injection of the second PGF₂α in the group III oestrus of each cows was followed through teasing (standing heat, vaginal discharge) and transrectal ultrasonography. Cows were inseminated at fixed time 12 hours after the GnRH injection. Follicles were monitored every 6 hours with ultrasonography until the ovulation occurred.

The time interval between the end of treatments (after the injection of PGF₂α in the groups I and II and injection of the second PGF₂α in group III) and the onset of behavioral estrus signs (standing heat, vaginal discharge) was recorded as injection-estrous interval. The time interval between injections of PGF₂α and ovulation was recorded as ovulation time in all groups.

Pregnancy diagnosis was determined by ultrasonography on day 35 after insemination.

Statistical analysis

Data were analyzed as the percentage of estrous and pregnancy with chi-square analysis to compare estrous and pregnancy rates between groups, and t-test was performed to compare average injection-estrous interval (hr), injection-ovulation interval (hr), corpus luteum diameter (cm) at the moment of the PGF₂α injections (in second PGF₂α injection in the group III), follicles diameter (cm), insemination time and plasma progesterone levels (ng/ml) in PGF₂α injections (in the second PGF₂α injection in the group III).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>GnRH+PGF₂α* (I, n=10)</td>
<td>hCG+PGF₂α** (II, n=10)</td>
<td>PGF₂α+PGF₂α*** (III, n=10)</td>
</tr>
<tr>
<td>Injection-estrous interval (hr)</td>
<td>52.2±1.69a</td>
<td>48.4±1.34a</td>
<td>68.2±1.31b</td>
</tr>
<tr>
<td>(n=10)</td>
<td>(n=10)</td>
<td>(n=8)*</td>
<td></td>
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<tr>
<td>Injection-ovulation interval (hr)</td>
<td>70.4±1.17a</td>
<td>67.3±1.36a</td>
<td>90.6±2.15b</td>
</tr>
<tr>
<td>(n=10)</td>
<td>(n=10)</td>
<td>(n=8)*</td>
<td></td>
</tr>
<tr>
<td>Pregnancy rate (%)</td>
<td>60 (6/10)*</td>
<td>60 (6/10)*</td>
<td>30 (3/10)*</td>
</tr>
<tr>
<td>Synchronization rate (%) (with observation, standing heat and vaginal discharge)</td>
<td>80 (8/10)*</td>
<td>70 (7/10)*</td>
<td>50 (5/10)*</td>
</tr>
<tr>
<td>Synchronization rate (%) (with plasma progesterone level)</td>
<td>100 (10/10)*</td>
<td>100 (10/10)*</td>
<td>80* (8/10)*</td>
</tr>
</tbody>
</table>

*a,p<0.05; b,p<0.01

*: two cows had a low progesterone level during the second injection of PGF₂α

**: hCG (3000 IU) was injected intravenously to the cows in this group. Seven days after hCG injection (Day 0 = injection of hCG), 0.150 mg d-cloprostenol was injected to the same group of cows intramuscularly. GnRH (10 µg) was injected to the cows 48 hours after the injection of PGF₂α. The cows have been inseminated 12 hours after the GnRH injection.

***: GnRH (20 µg) was injected intramuscularly to the cows in this group. Seven days after GnRH injection (Day 0 = injection of GnRH), 0.150 mg d-cloprostenol was injected to the same group of cows intramuscularly. GnRH (10 µg) was injected to the cows 48 hours after the injection of PGF₂α. The cows have been inseminated 12 hours after the GnRH injection.

****: D-cloprostenol (0.150 mg), an analogue of PGF₂α, was intramuscularly injected two times (At day 0 and day 11) at 11-day interval to the cows of this group. GnRH (10 µg) was injected to the cows 48 hours after the second PGF₂α injection. The cows have been inseminated 12 hours after the GnRH injection.

Table II: Injection-estrous, injection-ovulation interval (hr) (mean ± SEM) estrous and pregnancy rates (%) in three groups of synchronized cows.
Results

Injection-estrous (hr), injection-ovulation time (hr) (mean ± SEM), estrous and pregnancy rates were summarized in Table 2. Plasma progesterone levels (mean(SEM) on the day 0 and 7 in the groups I and II, and 0, 7 and 11 in the group III and on the insemination day in all groups were showed in Table 3. Corpus luteum diameter (cm) at the time of presumed luteolysis (on the day of 7 in the groups I and II and 11 in the group III) and follicles diameter (cm) on the insemination time were presented in Table 4.

The mean interval injections-estrus, injections-ovulation in the groups I, II and III were 52.2 ± 1.69, 48.4 ± 1.34 and 68.2 ± 1.31 hours; 70.4 ± 1.17, 67.3 ± 1.36 and 90.6 ± 2.15 hours, respectively. There were significant differences (p < 0.01) between the groups I and III and II and III.

The synchronization rates were higher in the groups I and II (100 %) than in the group III (80 %). Pregnancy rates of the groups I, II and III were 60 %, 60 % and 30 %, respectively. There were not significant differences among the groups.

Plasma progesterone levels on day 0 and on insemination day were similar (p > 0.05) among the groups. On the day 7 (groups I and II), and the day of 11 (group III), plasma progesterone levels were significantly higher (p < 0.01) in the groups I and II than in the group III.

Corpus luteum diameters (cm) were 2.4 ± 0.17, 2.6 ± 0.18 and 2.1 ± 0.26 on the day of synchronization (PGF 2α injections in the groups I and II, and the second PGF 2α injection in the group III) in the groups I and II, and III, respectively. The differences among the groups were not significant (p > 0.05) for corpus luteum diameter.

Follicles diameters (cm) during the insemination time (on the day of estrous) were 1.9 ± 0.17, 1.8 ± 0.05 and 1.4 ± 0.13 in the groups I, II and III, respectively. There was a significant difference (p < 0.05) between the groups I and III, and II and III.

Discussion and conclusion

Fixed-timed artificial insemination regimens often require two inseminations after 2 injections of PGF 2α 11 days apart, because of variation in timing of oestrous which add further cost to an already expensive programme. Furthermore, variances in ovulation times following oestrus also are cost-effective by altering fertility at induced oestrus. Therefore, it would be desirable to control ovulation through a single fixed time insemination at 60 hours to yield sufficient fertility.

The protocol used in this study has been termed the ‘Ovsynch’ regimen and has been shown to give acceptable fertility with a single fixed-time insemination [21]. This treatment provides an alternative approach for inducing oestrus and ovulation.

In our study, both GnRH and HCG treatments associated with PGF 2α were found to be highly effective in the control of oestrus, this result is in agreement with the reports of Kaya et al. [9] who reported that GnRH treatment resulted in a high degree of synchronization of oestrus and ovulation rate.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Day 0</th>
<th>Day 7</th>
<th>Insemination Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>GnRH + PGF 2α (I)</td>
<td>1.1±0.58°</td>
<td>2.7±0.42°</td>
<td>0.17±0.02°</td>
</tr>
<tr>
<td>(n:10)</td>
<td>(n:10)</td>
<td>(n:10)</td>
<td></td>
</tr>
<tr>
<td>HCG + PGF 2α (II)</td>
<td>2.7±0.77°</td>
<td>3.7±0.88°</td>
<td>0.18±0.04°</td>
</tr>
<tr>
<td>(n:10)</td>
<td>(n:10)</td>
<td>(n:10)</td>
<td></td>
</tr>
<tr>
<td>PGF 2α + PGF 2α (III)</td>
<td>2.2±1.02°</td>
<td>1.5±0.34°(*)</td>
<td>0.19±0.02°</td>
</tr>
<tr>
<td>(n:10)</td>
<td>(n:10)</td>
<td>(n:8)°</td>
<td></td>
</tr>
</tbody>
</table>

Different superscripts on the same column indicate significant differences between values (p < 0.05).

(*) Represents the progesterone level with on the day of second PGF 2α injection in group III.

# Blood samples were daily collected from jugular vein of the cows starting from the first injection (Day 0, hCG, GnRH and first PGF 2α injections) for 13 days in groups I and II and for 15 days in group III.

TABLE III. — Plasma progesterone levels (mean ± SEM) on day 0, 7 in groups I (n:10), II (n:10) and III (n:10) on day 11 in groups III, and insemination day (#).
Injection-estrous interval and injection-ovulation interval were shorter in the hCG and GnRH treated groups than in the PGF$_{2\alpha}$ treated group. Similar results were reported by others [3] and [23]. However, several researchers [24, 27, 31] stated that injections of PGF$_{2\alpha}$ given between the day 5 and 8 of the estrous cycle resulted in mean intervals injection-oestrus < 50 hours. Injections given at midcycle (day 8-11) or later in the luteal phase (day 12-15) resulted in mean intervals of 70 and 62 hour to estrous, respectively. Induction of corpus luteum regression by injection of PGF$_{2\alpha}$ early in the estrous cycle probably induced luteal regression and eventually ovulation of the first wave dominant follicle [12]. Longer intervals to estrous probably were associated with different stages of dominant follicle maturation at the time of luteolysis [11, 24]. GnRH stimulates the synthesis and secretion of the gonadotrophic hormones, follicle stimulating hormone (FSH) and luteinizing hormone (LH). On the other hand, hCG has primarily LH effect with very little FSH effect [4]. GnRH + PGF$_{2\alpha}$ and hCG + PGF$_{2\alpha}$ combinations may enable the development of graffian follicles earlier than the double PGF$_{2\alpha}$ regimen.

Administration of GnRH causes an alteration of follicular distribution in the ovary by increasing the number of medium-sized follicles and decreasing the number of large follicles by inducing luteinization and/or atresia [26, 29, 30]. Consequently, a GnRH agonist could be used to inhibit recurring estrous and ovulation when used in late diestrus [14,15]. Similar luteinization and atresia of follicles were also observed after GnRH and hCG injections on day 0 in our study.

In our study, for the groups I and II all the cows had high progesterone concentrations at the time of luteolysis. On the other hand, 2 cows had a low progesterone concentration on the day 11 in the group III. This suggests that formation of corpus luteum was delayed during that time. The plasma progesterone levels tend to be higher in the groups I and II than in the group III. This may be resulted from GnRH and hCG administration, causing ovulation and luteinization of dominant follicle with the resultant effect of formation of corpus luteum and luteotropic effect of LH. In addition, corpus luteum diameters tend to be higher in the groups I and II than in the group III. Greater follicle diameters in the groups I and II corroborate the shortness of injection-ovulation interval.

After the second PGF$_{2\alpha}$ injection, there were no signs of the estrous behavior and follicular development in some of the cows in the group III. This may be due to late luteolysis or formation of corpus luteum after the first PGF$_{2\alpha}$ injection. All the cows ovulated at 22, 20 and 42 hours after GnRH injection in groups I, II and III, respectively. Delayed ovulation was probably due to prolongation of luteolysis or follicle maturation in the group III. Ovulations were synchronized 100% in the groups I and II based on the protocol used in this study. Using the same protocol, identical results (80 - 100%) were reported by Pursley et al [20] and Twarigumangul et al [29].

Peters et al [19] have reported that higher proportion of cows was observed in the responsive luteal phase when PGF$_{2\alpha}$ was administered suggesting that injection of the first GnRH may have been effective in inducing new corpora lutea or exerting an luteotropic effect in some cows already in the luteal phase. hCG has also similar effects [26]. These obviously caused the extension of the luteal phase with

<table>
<thead>
<tr>
<th>Corpus luteum diameter at PGF$_{2\alpha}$ injections (cm)</th>
<th>Diameter of graffian follicle at insemination day (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GnRH + PGF$_{2\alpha}$ (I) 2.4±0.17*</td>
<td>1.9±0.17*</td>
</tr>
<tr>
<td>(n:10)</td>
<td>(n:10)</td>
</tr>
<tr>
<td>HCG + PGF$_{2\alpha}$ (II) 2.6±0.18*</td>
<td>1.8±0.05*</td>
</tr>
<tr>
<td>(n:10)</td>
<td>(n:10)</td>
</tr>
<tr>
<td>PGF$<em>{2\alpha}$ + PGF$</em>{2\alpha}$ (III) 2.1±0.26*</td>
<td>1.4±0.13*</td>
</tr>
<tr>
<td>(n:8)*</td>
<td>(n:8)*</td>
</tr>
</tbody>
</table>

Significance: a : p>0.05 a : p<0.05

Different superscripts on the same column indicate significant differences (p<0.05).

*: Corpus luteum diameter (cm) on the day of 7 in groups I and II and 11 in group III during PGF$_{2\alpha}$ injection.

(+) : Two cows had a low progesterone level with no palpable corpus luteum during the second injection of PGF$_{2\alpha}$.

*: The cycle of each cow was followed through transrectal ultrasonography to determine follicular and luteal developments. Corpus luteum and follicle diameters of cows were daily measured until the 13th day in groups I and II and 16th day in group III.

Table IV. — Corpus luteum diameter (cm) at the time of presumed luteolysis (on the day of 7 in groups I and II and 11 in group III) and follicles diameter (cm) (mean ± SEM) on the day of insemination (+).
increased responsiveness to PGF$_{2\alpha}$. We have found similar results with regard to the injection of GnRH and HCG on day 0, prostaglandin on day 7, and GnRH on day 9.

Pregnancy rates of the groups I, II and III were 60%, 60% and 30%, respectively, and are higher in groups I and II compared with this of group III. These findings are inaccordance with reports of Kaya et al [9]. However, pregnancy rates were higher in synchronized animals with higher progesterone levels than those of with lower progesterone levels at the time of luteolysis [7] as in this study. Lower pregnancy rate in the group II was 100% and 30%, respectively and were higher in groups I and II compared to the double PGF$_{2\alpha}$ regimen is similar with the reports of Morrell et al [17].

In conclusion, the use of GnRH and HCG on day 0- PGF$_{2\alpha}$ on day 7- and GnRH on day 9, has been found effective to produce acceptable fertility with a single fixed time insemination. These protocols could be used as an alternative for both synchronization and induction of estrus and ovulation, because the induced estrus with the prostaglandins F$_{2\alpha}$ treatments alone is too variable to use for fixed time insemination in cows.

In addition, the advantages of the two protocols used in this study are as follows:

- Application of only one AI in GnRH + PGF$_{2\alpha}$ and hCG + PGF$_{2\alpha}$ protocols compared to two AI at the 72 and 96 hours in double injections of PGF$_{2\alpha}$ with 11 days apart.
- The time interval until the AI is shorter (9 days) in the two protocols, compare with the double PGF$_{2\alpha}$ treatment (13 days).
- GnRH or hCG combined with PGF$_{2\alpha}$ is cheaper compared to double PGF$_{2\alpha}$ treatment because while the prior treatment requires one AI at 60 hours, the later requires 2 Al at 72 and 96 hours.

References