Different times of oestradiol treatment combined with the progesterone in the Heatsynch protocols at the presence or absence of corpus luteum in dairy cows

M. R. AHMADI1*, A. MIRZAEI1, M. ALIPOUR2

1 Department of Clinical Sciences, School of Veterinary Medicine, Shiraz University, Shiraz, Iran
2 Pegah Fars milk company, Shiraz, Iran

*Corresponding author: rahmadi@shirazu.ac.ir

ABSTRACT

Estrous detection rates are often low (< 50%) in dairy herds. Several protocols have been used in recent years for synchronization of cows based on timed insemination. Synchronization of estrus and ovulation are two of the methods for improvement of the estrous detection rate in dairy farms. The objectives of the study were to evaluate the time effect of oestradiol benzoate (OB) injection on the pregnancy rate in the Heatsynch (HS1; 24h and HS2; 48h after prostaglandin injection) and Intravaginal progesterone-releasing devices -Heatsynch (CIDRHS) protocols and to determine the efficiency of these protocols in the cows with and without corpus luteum (CL) in first and second parities. There was no significant difference between conception rate of cows that received estradiol injection at different time (37.9 vs 34.2 % for 24 and 48h after prostaglandin treatment, respectively; P = 0.15). Cows inseminated in winter had a higher conception rate compared to those inseminated in other (fall, spring and summer) seasons (P < 0.05). Conception rate of HS2 group was significantly greater than cows in CIDRHS1 group of primiparous cows; while greater conception rate of CIDRHS1 cows compared to HS2 group was found in cows with parity two (P < 0.05). In parity one, the result showed greater conception rate in HS2 group compared to cows in other groups (P < 0.05). Tendency to significant differences were observed in conception rate of cows in HS1 compared to that of cows in CIDRHS1 (35.4 vs 43.1%; P = 0.06). The results showed that time of oestradiol did not affect conception rates irrespective of parity. In conclusion, the present study showed that OB injection 24h after prostaglandin in cows with or without CL combined with CIDR can be responsible for acceptable pregnancy rate in cows with second parity. While, OB injection 48h after prostaglandin can be improved pregnancy rate in cows had first parity.

Keywords: Heatsynch; CIDR; Synchronization; conception rate; dairy cows

RESUME

Effet de différents temps de traitements combinés avec oestradiol et progestéron sur la synchronisation des chaleurs chez les vaches laitières en présence ou en l'absence de corps jaune

Les taux de détection œstraux sont souvent faibles (<50%) dans les troupeaux laitiers. Plusieurs protocoles ont été utilisés ces dernières années pour la synchronisation des vaches en vue de leur insémination. La synchronisation de l'oestrus et de l’ovulation sont deux méthodes d’amélioration du taux de gestation dans les fermes laitières. Les objectifs de l’étude étaient d’évaluer l’impact du moment de l’injection de benzoate d’oestradiol (OB) sur le taux de gestation dans le protocole Heatsynch (HS1 et HS2; 24h et 48h après l’injection de prostaglandine) et la durée d’application d’un dispositif intravaginal de libération de progestérone -Heatsynch protocole (CIDRHS)- chez les vaches laitières avec et sans corps jaune (CL) en première et deuxième gestation. Les résultats obtenus ont montré que le moment d’injection de l’estradiol n’a pas d’incidence sur les taux de conception, quel que soit de parité. L’injection d’oestradiol 24h après la prostaglandine combinés avec CIDER recommandée chez les vaches avec ou sans corps jaune au cours de la deuxième gestation alors que l’injection d’oestradiol 48 heures après les prostaglandine peut améliorer le taux de grossesse chez vaches lors de la première gestation.

Mots-clés : Synchronisation, chaleurs, oestrus, gestation, oestradiol, progestérone, vaches laitières, corps jaunes

Introduction

Reproductive performance lessens with increasing milk yield (10; 32). It has been reported that pregnancy rate of dairy cows reduced from 66 % in 1951 to about 40% in 1997 (24). Estrous detection rates are often low (< 50%) in dairy herds (27). Several protocols have been used in recent years for synchronization of cows based on timed insemination (26). Synchronization of estrus and ovulation are two of the methods for improvement of the estrous detection rate in dairy farms (36). Different hormonal protocols have been used in order for estrous or ovulation synchronization; Specifically Ovsynch, which has been recommended for improving reproductive performance in dairy cows (26). Synchronization protocols of estrus and ovulation combining TAI have been used in order to progress toward improving reproductive efficiency in dairy cows. These protocols can be a useful alternative for reproductive management of dairy herds, especially with suboptimal heat detection rate (26).

Ovsynch (GnRH-7d- PGF2α-2d-GnRH) is a program developed to synchronize ovulation for timed breeding. Pregnancy rate of dairy cows had been reported between 30 – 45 % in previous studies (12; 25) and 20 % (7) after synchronization with Ovsynch.
The Heatsynch protocol is achieved by replacing the second GnRH in the Ovsynch with estradiol that is administered 24 h after the PGF2α and followed by fixed timed insemination 48 h after estradiol injection (18; 23). In Heatsynch protocol, estradiol is used in order to increase the number of animals that show estrous signs, synchronize ovulation, and improve pregnancy rate (20). Substituting estrogen for the second GnRH injection increased the proportion of cows displaying normal estrual characteristics such as mucous secretion, uterine tone, and resulting sexual behavior, and cows detected in estrus had increased pregnancy and lower cost. Treatment simulates a natural estrus when estrogen is given at the proper time after PGF2α (15; 30). Injection of estradiol could be responsible for increasing serum E2 when administered at the time of the second dose of GnRH in the Ovsynch protocol (28). Success of the Heatsynch/Ovsynch protocols could be altered by administration of other hormones such as progesterone (37).

Intravaginal progesterone-releasing devices (CIDR) are used in cycling and anestrous cows for improving ovulation synchronization and increasing pregnancy rate (4; 22). Inclusion of progesterone (P4) supplementation at the onset of Ovsynch increased fertility (54%) similar to estrus (14). Other studies reported improvement of approximately 5 to 9% in pregnancy rate after using a vaginal supplementation of P4 during Ovsynch protocol (6; 9; 29). Some reports showed that progesterone does not affect pregnancy rate (31).

Adding exogenous progesterone, estradiol or hCG can improve the effect of Ovsynch protocol on the fertility rate of dairy cows (12; 13; 29). Although numerous studies have been done using these protocols, there is still substantial need for research to improve the synchronization, efficacy, simplicity, and practical application of these protocols (33).

The objectives of the study were to evaluate the time effect of oestradiol injection on the pregnancy rate in the Heatsynch and CIDR-Heatsynch protocols and to determine the efficiency of these protocols cows with and without corpus luteum (CL) at the time of Heatsynch onset in first and second parities.

Materials and Methods

ANIMAL, HOUSING AND MANAGEMENT

All the cows (n = 1680) assigned into the study were in their first (n = 916) and second (n = 764) parity, on 70 to 300 days postpartum. The study was carried out in a large commercial dairy farm near Shiraz, Fars province, in central Iran (29°58'34''N, 52°40'45''E). The farm milked 1900 Holstein cows three times daily. The cows were housed in free stall barns with mat bedding for primiparous and sand bedding for multiparous cows. Cows calved throughout the year, and the herd had annual average milk yields of 9000 liters per cow. Cows received corn silage, alfalfa hay and concentrates (containing corn meal, soybean meal, vitamins and minerals). They were maintained in close-up dry group for 3 weeks before calving. The cows calved in an open-shed barn. Fresh cows were kept in a transition group for 1 month.

STUDY DESIGN

The present study was carried out from spring of 2011 to summer of 2012. All the cows were examined by ultrasonography and ovarian structures determined by presence or absence of corpus luteum. All cows without CL received CIDR (InterAg, Hamilton, New Zealand) in induction protocols. The following protocols were used for studying different groups (Figure 1 and 2).

Group HS1: Day 0: administration of 100 μg of a synthetic analogue of gonadotropin-releasing hormone (Gonabreed, Gonadorelin (as acetate), 100μg/ml, Parnell lab, New Zealand; Intramuscular injection (im.)). Day 7: administration of 500μg of a synthetic analogue of prostaglandin F2α (Estron, Cloprostenol 0.25mg/ml; Bioveta, Czech Republic; im.). Day 8: administration of 1mg of Oestradiol Benzoate (OB; Vetasterol, 2mg/ml, Aburaihan, Iran; im.).

Group HS2: All used drugs were similar to HS1 except the time of OB injection which was day 9: administration of 1mg of OB (Vetasterol, 2mg/ml, Aburaihan, Iran; im.).

Group CDRHS1: administration of 100μg of a synthetic analogue of gonadotropin-releasing hormone (Gonabreed, Gonadorelin (as acetate) 100μg/ml, Parnell lab, New Zealand; Intramuscular injection (im.)). Day 7: administration of 500μg of a synthetic analogue of prostaglandin F2α (Estron, Cloprostenol 0.25mg/ml; Bioveta, Czech Republic; im.). Day 8: administration of 1mg of Oestradiol Benzoate (OB; Vetasterol, 2mg/ml, Aburaihan, Iran; im.) and insertion of a controlled internal drug

![Figure 2: Conception rate of cows with and without corpus luteum in first and second parities.](image)

a,b Different letters on columns represent significant differences (P < 0.05).
release (CIDR). Day 7: administration of 500μg of a synthetic analogue of prostaglandin F2α (Estron, Cloprostenol 0.25mg/ml; Bioveta, Czech Republic; im.) and withdrawal of the CIDR. Day 8: administration of 1mg of OB (Vetasterol, 2mg/ml, Aburaihan, Iran; im.).

Group CIDRHS2: All used drugs were similar to HS1 except the time of OB injection which was day 9: administration of 1mg of OB (Vetasterol, 2mg/ml, Aburaihan, Iran; im.).

Estrous detection was performed 3 times a day for a period of 20min each at 16-18, 20-24, and 4-6h. Cows that showed standing heat were artificially inseminated using frozen semen of proven fertility 12 h later.

All cows not considered to be in heat underwent fixed time artificial insemination (FTAI) on Day 10. Pregnancy was diagnosed by transrectal ultrasonography (5MHz rectal linear probe, BCF Company, UK) from 31 to 35 days for cows inseminated.

STATISTICAL ANALYSIS

Conception rate of studied groups in first or second parity were statistically analyzed with the Chi-square test using SPSS (SPSS for Windows, version 11.5, SPSS Inc, Chicago, Illinois). Moreover, the effect of CL at the time of Heatsynch onset on fertility was investigated using Chi-square test. Comparison of cows that showed standing heat between different groups of cow in second parity was also done by Chi-square test. Data are presented as the percentage (number) and probability values of P ≤0.05 were considered statistically significant.

### Results

There were 412 and 504 cows with and without CL in the first and 486 and 278 in the second parities, respectively. Cows without CL were higher in the first parity compared to that of the cows in the second parity (55.1% vs. 36.4%; P < 0.001). One hundred and forty-six cows (16%) in the first and one hundred and seventy-seven (26.3%) in the second parity were not inseminated because of various causes such as abnormal discharges at the time of AI and no signs of estrus, sickness of cows. Two hundred and fifty-three (32.9%) and two hundred and thirty-four (39.9%) cows in the first and second parities were pregnant, respectively.

In second parity, the percentage of cows that showed estrous signs was higher in CIDRSH1 (86.2 %) compared to that of cows in HS1 (74.7 %; P = 0.001) and HS2 (63.8 %; P < 0.0001) groups. There were also significant differences in percentage of cows with estrous signs in CIDRHS2 (83.8 %) compared with HS2 (63.8 %; P = 0.003). In addition, cows showed estrous signs were higher in HS1 (74.7 %) than HS2 group (63.8%) (P = 0.01). There was no significant difference between conception rate of cows that received estradiol injection at different time (37.9 vs 34.2 % for 24 and 48h after prostaglandin treatment, respectively; P = 0.15). Conception rate of HS2 group was significantly greater than cows in CIDRHS1 group of primiparous cows; while greater conception rate of CIDRHS1 cows compared to HS2 group was found in cows with parity two (P < 0.05).

In parity one, the result showed greater conception rate in HS2 group compared to cows in other groups (P < 0.05; Table 1). Tendency to significant differences were observed in conception rate of cows in HS1 compared to that of cows in CIDRHS1 (35.4 vs 43.1%; P = 0.06; Table I).

### Table I: Comparison of conception rate (pregnant cows/total inseminated cows) between different groups of cows

<table>
<thead>
<tr>
<th>Lactation number</th>
<th>Treatment groups % (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HS1</td>
</tr>
<tr>
<td>First parity (n=770)</td>
<td>29.2 (42/144)a</td>
</tr>
<tr>
<td>Second parity (n=587)</td>
<td>38.7 (103/266)</td>
</tr>
<tr>
<td>Total (n=1357)</td>
<td>35.4 (145/410)</td>
</tr>
</tbody>
</table>

a,b Different superscript letters indicate significant difference in the same rows (P<0.05)

### Table II: Comparison of conception rate (pregnant cows/total inseminated cows) between studied cows that received estradiol injection at 24 and 48h after prostaglandin treatment in different seasons

<table>
<thead>
<tr>
<th>Season</th>
<th>24h Treatment groups % (N)</th>
<th>48h Treatment groups % (N)</th>
<th>Total Treatment groups % (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spring (n=487)</td>
<td>37 (95)c</td>
<td>29.6 (68)d</td>
<td>33.5 (163)e</td>
</tr>
<tr>
<td>Summer (n=469)</td>
<td>36.4 (59)</td>
<td>30.9 (95)</td>
<td>32.8 (154)z</td>
</tr>
<tr>
<td>Fall (n=128)</td>
<td>8.3 (2)a</td>
<td>41.3 (43)b</td>
<td>35.2 (45)f</td>
</tr>
<tr>
<td>Winter (n=273)</td>
<td>45 (77)</td>
<td>47.1 (48)</td>
<td>45.8 (125)h</td>
</tr>
<tr>
<td>Total (n=1357)</td>
<td>37.9 (233)</td>
<td>34.2 (254)</td>
<td>35.9 (487)i</td>
</tr>
</tbody>
</table>

a,b Different superscript letters indicate significant difference in the same rows (P<0.05); c, d, e, f indicate significant difference in the total column.
The conception rate in cows without CL (45 %) was significantly higher than those cows had CL (36.4 %) in second parity (P=0.03); while there was no significant difference in the first lactation (Figure 2). Significant difference in conception rate of cows without CL between first (30.7 %) and second parities (45 %; P < 0.001) was observed. There was no significant difference in conception rate of cows with CL between first and second parities (P > 0.05).

Cows inseminated in winter had a higher conception rate compared to those inseminated in other seasons (P < 0.05; Table II).

Discussion

In the present study, pregnancy rate (PR) of cows in the Heatsynch 1 and 2 groups was 35.4 and 37% and in the CIDRHS1 and 2 groups was 43.1 and 32.6%, respectively.

In this study, the PR for Heatsynch 1 (35.4 %) and 2 (37 %) groups was higher than those reported by previous studies (25.8% in the study of Bartolome, et al, 2005; 35.1% in Florida and 29% in Texas in the study of Pancarci et al., 2002); but lower than results reported by (80 %, Cevik, et al., 2010) (3; 8; 23). In their studies, estradiol cypionate was injected on day 8 (24 h after PGF2α injection). In Heatsynch, estradiol administration induces estrus, ovulation followed by corpus luteum development in dairy heifers and cows (16; 23; 30). Pancarci et al., (2002) reported that the optimal inseminated time is 48 h after estradiol injection. In the present study, cows that received insemination 24 and 48 h after estradiol injection have higher pregnancy rate in the HS and CIDRHS groups, respectively. Pregnancy rate of cows treated by Heatsynch (an injection of 2 mg estradiol benzoate, 48h after the PGF2α) was 45 % in the study of Karimi, et al. (2007), though, 80% in the Cevik, et al. (2010) study in cows treated by Heatsynch (an injection of 1 mg estradiol cypionate, 24 h after the PGF2α) protocol (8; 17).

Pregnancy rates in the present study were similar to those in previous studies (4; 21; 22) indicating that the use of CIDR can improve fertility in anestrous non-cyclic cows. Previous studies reported that pregnancy rate per AI is observed between 30% and 45% after using Ovsynch protocol in dairy cows (12; 25). Kasimanickam et al. (2008) reported that the overall PR was 36 and 28% after Ovsynch and PRID protocols and this difference was not significant (19). Pregnancy rate of cows that received CIDR-based protocol and PGF2α was 53.3% in the Cevik et al., (2010) study (8). But in this study, PR for CIDRHS 1 (43.1 %) and 2 (32.6 %) groups was lower than that of Cevik et al. (2010). Ahmadi and Ghaisari (2007) reported that the conception rate was 57.6 and 47.4 % in synchronized cows with CIDR-based protocol + E2 (48 h after PGF2α injection) and CIDR + Ovsynch, respectively (1; 8). First service conception rate was reported 23.8% in cows synchronized with Ovsynch-CIDR by Fallahrad and Ajam in 2010 (11).

Pregnancy rate of cows treated by Heatsynch-progesterone (an injectable and intravaginal device formed with an injection of 1 mg of estradiol benzoate or estradiol 17β, 24h after the PGF2α) was 24.9% (2). A recent study was done by Andringa et al. (2013) to compare an injectable progesterone with intravaginal device, and natural estradiol-17β with synthetic estradiol in a synchronization Heatsynch protocol was used in four groups. They showed that estrous behaviour, number of cows in estrus, follicular diameter and pregnancy rate were similar among cows in different groups (2). Anovular cows at Ovsynch initiation had low fertility rate after the Ovsynch protocol (5; 34). An increase in fertility was found when P4 is elevated during preovulatory follicle growth (5; 35). It seems use of the intravaginal P4 device during the synchronization protocols in this type of dairy cows is likely to improve fertility (33). Therefore, pregnancy rate was in an acceptable range in the studied cows of the present investigation. Pregnancy rate of synchronized cows without CL by CIDR-HS1 and 2 was comparable with PR in other groups. Our data indicate that using of CIDR in cows without CL and OB injection 24h after prostaglandin treatment in cows with CL improves the estrous signs. The results showed that time of oestradiol did not affect conception rates irrespective of parity. So, it can recommend that OB injection 24h or 48h after prostaglandin treatment were used based on the manager programs in dairy herds. In conclusion, the present study showed that OB injection 24h after prostaglandin in cows with or without CL combined with CIDR can be responsible for acceptable pregnancy rate in cows had second parity. While, OB injection 48h after prostaglandin can be improved pregnancy rate in cows had first parity. In addition, the different management of estrous detection and artificial insemination programs of the herds maybe influence on the efficiency of injected time of oestradiol benzoate.

Acknowledgments

The authors thank the owners and staff of the Pegah Fars milk company for their cooperation.

References

TIMES OF OESTRADIOL TREATMENT IN THE HEATSYNCH PROTOCOLS


26. RABIEE A.R., LEAN I.J., STEVENSON M.A.: Efficacy of

Revue Méd. Vét., 2016, 167, 3-4, 59-64


