The effect of body condition loss and milk yield on the efficiency of Ovsynch in cycling Holstein dairy cows

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SUMMARY

Reproductive efficiency of dairy cows is low mainly due to reduction in conception and estrous detection. Ovsynch protocol (synchronization of ovulation) has been developed to improve the pregnancy rate in dairy farms. Therefore, the present study was performed to compare pregnancy rate using the Ovsynch protocol in lactating dairy cows with different body condition score (BCS), and to evaluate the effect of postpartum BCS loss on the pregnancy rate. One hundred and fifty five cows (primiparous, n=58 and multiparous, n=97) were assigned randomly and divided into three groups: BCS<3, BCS=3, and BCS>3 at the first GnRH injection. Cows received Ovsynch protocol (GnRH-7d-PGF2α-2d-GnRH) at diestrous stage of the estrous cycle. Cows were artificially inseminated based on the estrous detection or fixed-time insemination at 18-24 hours after second GnRH. All cows were examined by ultrasonography between 30 and 35 days after insemination for pregnancy diagnosis. Pregnancy rate was higher (P < 0.05) in multiparous cows with BCS >3 (53.8%) than that of the cows of the other BCS groups (32.4 and 16.7% in cows with BCS=3 and <3, respectively). Mean (±SD) of postpartum BCS loss from calving to first GnRH injection was lower (P<0.05) in multiparous and primiparous cows with BCS >3 (0.32 ± 0.42 and 0.33 ± 0.10) in comparison with those of the cows with BCS=3 (0.52 ± 0.39 and 0.62 ± 0.10) and cows with BCS<3 (0.78± 0.34 and 0.98 ± 0.20). The mean milk yield was significantly (P < 0.05) greater for cows with BCS > or = 3 in comparison with that of the cows with BCS < 3. In conclusion, the postpartum body condition loss is an important factor for achieving acceptable pregnancy rate after Ovsynch protocol in Holstein lactating dairy cows.

Keywords: Pregnancy rate; Ovsynch; Body condition score; Milk yield; Dairy cows

RÉSUMÉ

Effet de l'engraissement et de la production laitière sur l'efficacité de la synchronisation chez les vaches laitière Holstein

Le protocole de synchronisation d'œstrus (Ovsynch) a été développé pour améliorer le taux de gestation dans les troupeaux laitiers. Cette étude vise à mesurer l'effet de la note d'engraissement corporel (BCS) sur le taux de gestation après synchronisation utilisant le protocole Ovsynch chez les vaches laitières en lactation. Cent cinquante-cinq vaches primipares, (n = 58) et multipares, (n = 97) ont été réparties en trois groupes lors de la première injection de GnRH en fonction de leur note d'état corporel: BCS <3, BCS = 3, et BCS> 3. Les vaches ont été traitées selon le protocole Ovsynch (GnRH-7d-PGF2a-2d-GnRH) au stade de dioestrus du cycle œstral. Les vaches ont été inséminées artificiellement après détection de l'œstrus ou à date fixe 18-24 heures après la deuxième injection de GnRH. Toutes les vaches ont été examinées par échographie entre 30 et 35 jours après insémination pour le diagnostic de gestation. Le taux de grossesse était plus élevé (P <0,05) chez les vaches multipares du groupe avec une BCS> 3 (53,8%) que celui des autres groupes (respectivement 32,4 et 16,7% chez les vaches avec BCS = 3 et <3). La perte moyenne de BCS entre vêlage et la première injection de GnRH était inférieure (P <0,05) chez les vaches multipares et primipares avec une BCS> 3 (respectivement 0,32± 0,42 et 0,33 ± 0,10) par rapport aux vaches avec une BCS = 3 (0,52 ± 0,39 et 0,62 ± 0,10) et aux vaches avec une BCS <3 (0,78 ± 0,34 et 0,98 ± 0,20). La production laitière moyenne était significativement (P <0,05) plus élevée pour les vaches avec une BCS> ou = 3 par rapport à celle des vaches avec BCS <3. En conclusion l'état corporel post-partum est un facteur important de réussite lors de la synchronisation des chaleurs chez la vache laitière Holstein en lactation.

Mots-clés: synchronisation; œstrus ; note d'état corporel; engraissement ; lait; vache laitière

Introduction

Body condition score (BCS) of cow is an indicator to estimate subcutaneous fat [5] ranging from 1 (thin) to 5 (obese) with different decimal increments [6, 22]. The risk of conception in synchronized cows with Ovsynch (GnRH-7d-PGF2a-2d-GnRH) vary based on the body condition score [11]. It has been reported that low BCS at parturition can affect the onset of cyclicity and result in the poor reproductive performance [9]. Mirzaei et al. [10] reported that high producing dairy cows that lost greater than 0.5 units BCS within 70 days postpartum had longer calving to first detected estrus interval. Furthermore, Kafi and Mirzaei [8] reported that loss of BCS ≥0.75 points during 49 days after calving increased the risk of delayed first ovulation after calving. Similarly, Shrestha et al. [16] reported a high occurrence of first delayed ovulation in dairy cows losing more than 1 point BCS during 49 days after calving. A decrease in BCS of ≥1 unit by the 8th week after calving significantly increased the occurrence of delayed commencement of the luteal activity [18]. Postpartum ovarian cyclicity is a very important factor affecting conception rate and Ovsynch result [4, 21].

Pregnancy rate was positively associated with body condition score after synchronized insemination within a short fixed post partum period [3]. Synchronization of estrus and ovulation are two methods used to improve the estrous detection rate in dairy farms [23]. Ovsynch is a program developed to synchronize ovulation for timed breeding. A positive association between body condition score and fertility to the Ovsynch/timed artificial insemination (TAI) protocol has been established [11].

Synchronization protocols, such as Ovsynch, have been developed and used to mange reproduction more efficiently [15]. MOREIRA et al. [11] compared pregnancy rates with the Ovsynch/TAI protocol in cows with BCS < 2.5 versus BCS 2 2.5. They didn't investigate the effect of postpartum BCS loss on the efficiency of Ovsynch protocol. They used cows which were in different stages of oestrous cycle. In order to gain some results which we thought were important and have not been achieved in previous studies, we performed the present study to compare pregnancy rate using the Ovsynch protocol in dairy cows with different body condition score (BCS) and had corpus luteum at first GnRH injection, and to evaluate the effect of postpartum BCS loss and milk yield at first GnRH injection on the efficiency of Ovsynch protocol in cycling Holstein dairy cows.

Materials and Methods

ANIMALS

Holstein cows (n = 155) with annual average milk yield of 10500 liters per cow were examined. They were registered primiparous (n = 58) and multiparous (n = 97) Iranian Holstein cattle at the farm of Farzis Milk and Meat Producing Complex in Shiraz, Fars province, south of Iran. Shiraz is located at a latitude of 29° 38′ N and longitude 52° 36′ E. Its altitude is 1296 m above sea level. This study began in January and lasted until April, 2014 (cold months in Iran). All cows were housed in open shed barns and artificially inseminated. All of the cows were kept under the same weather and management conditions in a similar manner. Cows were fed standard rations (total mixed ration) including mainly alfalfa, corn silage, beet pulp, cotton seed, soybean, corn and barley. The cows were milked three times daily with the use of a pipeline milking machine. Transrectal palpation and ultrasonography were used to inspect genital tract.

DESIGN OF STUDY

The cows were examined by ultrasonography and ovarian structures determined the presence or absence of corpus luteum (CL). The date of the first genital examination (cleat test) was 35-40 postparum days. Days in milk (mean ± SD) at the first GnRH injection of total studied cows were 72.8 ± 34.1 days after parturition. All cows with CL and normal uterus were selected after ultrasonography examination and received Ovsynch protocol. Day 0: The first administration of 100 μg of a synthetic analogue of gonadotropin-releasing hormone (i.m., Cinnarelin, Luli berin, CinnaGen, Iran). Day 7: administration of 200 μg of a synthetic analogue of prostaglandin F2α (Cloprostenol, Gestavet prost, Laboratorious Hipra, Spain). Day 9: The second administration of 100 μg of a synthetic analogue of gonadotropin-releasing hormone. Body condition score (BCS; scale 1–5 with 0.25 increments) of the cows was taken at calving and the first GnRH injection (Ferguson et al. 1994). The cows were randomly divided into three groups based on the BCS at first GnRH injection including BCS < 3 (n = 36), BCS = 3 (n = 55), and BCS > 3 (n = 64). Postpartum body condition score loss on 72.8 ± 34.1 days after calving was calculated by the following formula:

BCS loss = BCS at Calving - BCS at the first GnRH injection

Oestrous detection was performed 4 times a day for a period of 20 min for each time. Cows that showed standing heat were artificially inseminated using frozen semen of proved fertility. All cows not considered to be in heat underwent fixed time artificially insemination (FTAI) at 18-24 h later. Pregnancy was diagnosed by transrectal ultrasonography (SIUI, CTS-900V, China) from 30 to 35 days after artificial insemination. Blood samples (5 ml) were randomly collected via caudal venipuncture into tubes on day 0 (the first GnRH injection) from 27 animals for measuring serum progesterone in order to confirm the luteal activity. Serum was separated by centrifugation at 1500 × g for 15 minutes and stored at - 21°C until progesterone was assayed. Progesterone concentrations were determined using ELISA kit (Monobind Inc. 100 North Pointe Drive, Lake Forest, CA 92630 USA), which detects the concentrations as low as 0.105 ng/mL and has coefficients of variation of 3.1 and 7 % for intra- and inter-assays, respectively. Serum progesterone concentration ³ 1 ng/mL was considered indicative of luteal activity.

STATISTICAL ANALYSIS

The results were statistically analyzed using the SPSS statistical software (Version 15.0, SPSS Inc, Chicago, Illinois). Percentage of pregnancy rate of different BCS groups was analyzed using the Chi-squared test. Mean (± SD) of BCS loss, milk production and days in milk on day 0 (the first GnRH injection) in each treated groups were compared between different BCS groups using One-way ANOVA (LSD, Post Hoc Multiple comparisons). Data are presented as the percentage (number) and values of $P \leq 0.05$ were considered as significant data.

Results

Overall pregnancy rate of total studied cows was 40.6% (63/155) included 46.6% (27/58) and 37.1% (36/97) in primiparous and multiparous cows respectively. There was no significant difference in the Mean (± SD) of days in milk of cows in different BCS groups at the first GnRH injection (Table I). The pregnancy rate of treated multiparous cows with BCS > 3 (53.8%) was higher in comparison with those with BCS < or = 3 (16.7 and 32.4%; $P < 0.05$, Table II). There was no significant difference in the pregnancy rate of treated primiparous cows with different BCS at the first GnRH injection ($P > 0.05$); however, the pregnancy rates were 33.3, 42.9 and 56 % in cows with BCS less, equal and more than 3 respectively.

A comparison of the BCS loss from parturition to the first GnRH in the studied groups was shown in Table III. The

results showed that the mean (±SD) of postpartum BCS loss was lower (P<0.05) in multiparous and primiparous cows with BCS >3 (0.32 ± 0.42 and 0.33 ± 0.10) in comparison with that of cows with BCS=3 (0.52 ± 0.39 and 0.62 ± 0.10) and that of cows with BCS<3 (0.78± 0.34 and 0.98 ± 0.20). The lowest BCS loss was found in the cows with BCS > 3 at the first GnRH injection in primiparous and multiparous studied cows (Table III).

The mean milk yield was significantly (P < 0.05) greater for cows with BCS > or = 3 compared to those with BCS < 3 (Table IV). The mean (±SD) milk yield in multiparous cows with BCS<3 (35.9 ± 8.8 kg) was significantly lower than that of the other studied BCS groups at the first GnRH injection (42.2 ± 7.1 and 40.9 ± 9.1 kg in cows with BCS =3 and >3, respectively; P<0.05). Primiparous cows with BCS<3 had significantly lower milk yield than those with BCS =3 at the first GnRH injection (31.3 ± 7.7 versus 36.4 ± 5.8 kg; P<0.05). The mean (±SD) of progesterone concentration of randomly sampled cows was 6.7 ± 5.5 ng/mL which confirmed the luteal activity.

Discussion

The pregnancy rate of treated multiparous cows with BCS > 3 was higher in comparison with those with BCS < or = 3. There was no significant difference in the pregnancy rate of treated primiparous cows with different BCS at first

	BCS Groups		
Studied cows	< 3	= 3	> 3
Primiparous	80.0 ± 26.7 (n = 12)	75.4 ± 29.7 (n = 21)	76.1 ± 44.1 (n = 25)
Multiparous	70.4 ± 34.1 (n = 24)	64.4 ± 16.3 (n = 34)	76.1 ± 42.1 (n = 39)
Total	73.6 ± 31.7	68.6 ± 22.8	76.1 ± 42.5

Table I: The Mean (± SD) of days in milk of cows in different BCS groups at the first GnRH injection

	BCS Groups		
Studied cows	< 3	= 3	> 3
Primiparous	33.3 (4/12)	42.9 (9/21)	56 (14/25)
Multiparous	16.7 (4/24) [a]	32.4 (11/34) [a]	53.8 (21/39) [b]
Total	22.2 (8/36) [a]	36.4 (20/55) [a]	54.7 (35/64) [b]

[a,b] Different superscripts in rows indicate significant differences ($P < 0.05$)

Table II: Pregnancy rate of studied primiparous and multiparous cows in different BCS groups at the first GnRH injection

	BCS Groups		
Studied cows	< 3	= 3	> 3
Primiparous	0.98 ± 0.20 [a] (n = 12)	0.62 ± 0.10 [b] (n = 21)	0.33 ± 0.10 [c] (n = 25)
Multiparous	0.78 ± 0.34 [a] (n = 24)	0.52 ± 0.39 [b] (n = 34)	0.32 ± 0.42 [c] (n = 39)
Total	0.85 ± 0.32 [a]	0.56 ± 0.30 [b]	0.33 ± 0.30 [c]

[a,b] Different superscripts in rows indicate significant differences ($P < 0.05$)

Table III: The Mean (± SD) of BCS loss of cows in different BCS groups at the first GnRH injection

	BCS Groups		
Time	< 3 (n = 36)	= 3 (n = 55)	> 3 (n = 64)
At first GnRH injection	34.3 ± 8.6 [a]	40.1 ± 7.2 [b]	38.2 ± 8.8 [b]
30 days after first GnRH injection	34.2 ± 8.2 [a]	38.8 ± 7.1 [b]	38.1 ± 7.3 [b]

[a,b] Different superscripts in rows indicate significant differences ($P < 0.05$)

Table IV: The Mean (± SD) of milk yield of studied cows in different BCS groups at first GnRH injection

GnRH injection (Table II). The results showed that the mean of postpartum BCS loss was lower in multiparous and primiparous cows with BCS >3 in comparison with that of cows with BCS=3 and <3. The lowest BCS loss was found in the cows with BCS > 3 in primiparous and multiparous studied cows. Therefore, BCS at the first GnRH injection and postpartum BCS loss could be responsible for the efficiency of the Ovsynch protocol in lactating dairy cows.

Our findings are consistent with earlier observations [11, 12]. Low BCS cows had reduced pregnancy rates. It has been reported that a higher percentage of cows in the low BCS group had low plasma progesterone concentrations at the first injection of GnRH compared to cows in the control group [11]. So, pregnancy rates of cows submitted to the Ovsynch/TAI protocol were influenced by BCS [11]. Pregnancy rates increased in multiparous cows had BCS>3, and this increase occurred at a BCS of 3.25 and 3.50 for Heatsynch and Ovsynch cows, respectively [12]. Pancarci et al. [12] reported that neither milk weight at the month of TAI nor 120-d milk yield (kg) affected pregnancy rates. Tenhagen et al. [20] reported that the stage of lactation had a significant impact on first service conception rate (FSCR). They found that cows inseminated later in lactation stage had higher FSCR than cows inseminated earlier. Resumption of normal cyclicity has been occurred higher later in lactation, and influences of negative energy balance were diminished with the increase of postpartum interval [20]. In this study, there was no significant difference in the mean of days in milk of cows in different BCS groups at the first GnRH injection and we used cows at the end of early lactation and at the beginning of mid lactation stage in different BCS groups. Therefore, we considered cows with the same postpartum days in milk. So, milk production level had no significant impact on conception rates after TAI in cows synchronized at the same stage of lactation [20]. A higher conception rate has been reported in cows with above average milk production [13]. In the present study, we found that the cows with BCS > or = 3 had more milk yield in comparison with those with BCS < 3 at the first GnRH injection, and a higher pregnancy rate of cows with BCS > 3 was found in this study. Similar findings were reported by Peters and Pursley [13] and Pancarci, et al. [12].

The results of this study showed that the postpartum BCS loss was lower in studied cows with BCS >3 in comparison with that of cows with BCS=3 and <3 and the lowest BCS loss was found in the cows with BCS > 3 at first GnRH injection. Changes in body condition correlate with cumulative negative energy balance (NEB) and reflect the total negative energy deficit. It has been reported that NEB may adversely affect ovarian activity and subsequent fertility [8]. In the present study, we found that the cows with BCS < or = 3 had more postpartum BCS loss in comparison with those with BCS > 3 at the first GnRH injection and a lower pregnancy rate of cows with BCS=3 and <3 was found in this study. So, higher postpartum BCS loss due to the NEB may be responsible for the lower pregnancy rate in these cows.

Pregnancy rate of dairy cow had been reported between 30 – 45 % in the previous studies [1, 2, 7] after synchronization with Ovsynch protocol. It has been reported that primiparous cows had higher conception rates after Ovsynch protocol [4, 13, 17, 19]. In this study, overall pregnancy rate of total studied cows was 40.6% included 46.6% and 37.1% in primiparous and multiparous cows, respectively. Pregnancy rate was higher in studied primiparous than in multiparous cows in different BCS groups. The result of this study is similar to that of the earlier mentioned study; consequently, primiparous cows were more likely to have conceived after Ovsynch than cows in second or greater lactation.

Non-cycling cows had poor fertility in comparison with cycling cows after using Ovsynch protocol [4]. In addition, higher conception rate was reported in cows that had more estrous cycles before artificial insemination [21]. In this study, all cows with corpus luteum and normal uterus at the first GnRH injection were selected; the mean of progesterone concentration of randomly sampled cows indicated that the cows had luteal activity at the first GnRH injection.

Synchronization protocols such as Ovsynch protocol can be a useful alternative method for reproductive management of dairy herds especially with suboptimal heat detection rate; so, it has been used in order to progress toward improving reproductive efficiency in dairy cows [14].

We can conclude that the postpartum body condition loss is an important factor for achieving acceptable pregnancy rate after Ovsynch protocol. Thus, the results of this study suggest considering postpartum body condition loss and score at the first GnRH injection is very important when selecting cows as a candidate to inseminated after the Ovsynch/TAI protocol.

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References

1. - ALNIMER M., De ROSA G., GRASSO F., NAPOLITANO F., BORDI A.: Effect of climate on the response to three oestrous synchronization techniques in lactating dairy cows. *Anim. Reprod. Sci.,* 2002, **71**, 157-168.
2. - BELLO N.M., STEIBEL J.P., PURSLEY J.R.: Optimizing ovulation to first GnRH improved outcomes to each hormonal injection of Ovsynch in lactating dairy cows. *J. Dairy Sci.,* 2006, **89**, 3413-3424.
3. - BURKE J.M., DE LA SOTA R.L., RISCO C.A., STAPLES C.R., SCHMITT E.J.P., THATCHER W.W.: Evaluation of timed insemination using a gonadotropin-releasing

hormone agonist in lactating dairy cows. *J. Dairy Sci.,* 1996, **79**, 1385-1393.

4. - CARTMILL J.A., EL-ZARKOUNY S.Z., HENSLEY B.A., LAMB G.C., STEVENSON J.S.: Stage of cycle, incidence, and timing of ovulation, and pregnancy rates in dairy cattle after three timed breeding protocols. *J. Dairy Sci.,* 2001, 84, 1051–1059.
5. - EDMONSON A.J., LEAN I.J., WEAVER L.D., FARVER T., WEBSTER G.: A body condition scoring chart of Holstein dairy cows. *J. Dairy Sci.,* 1989, **72,** 68-78.
6. - FERGUSON J.D., GALLIGAN D.T., THOMSEN N.: Principal descriptors of body condition score in Holstein cows. *J. Dairy Sci.,* 1994, **77**, 2695-2703.
7. - GALVAO K.N., SANTOS J.E.P.: Factors affecting synchronization and conception rate after the Ovsynch protocol in lactating Holstein dairy cows. *Reprod. Domest. Anim.,* 2010, **45**, 439-446.
8. - KAFI M., MIRZAEI A.: Effects of first postpartum progesterone rise, metabolites, milk yield, and body condition score on the subsequent ovarian activity and fertility in lactating Holstein dairy cows. *Trop. Anim. Health. Prod.,* 2010, **42**, 761-767.
9. - MARKUSFELD O., GALON N., EZRA E.: Body condition score, health, yield and fertility in dairy cows. *Vet. Rec.* 1997, **141**, 67-72.
10. - MIRZAEI A.**,** KAFI M., GHAVAMI M., MOHRI M., GHEISARI H.: Ovarian activity in high and average producing Holstein cows under heat stress conditions. *Comp. Clin. Path.,* 2007, **16**, 235-241.
11. - MOREIRA F., RISCO C., PIRES M.F.A., AMBROSE J.D., DROST M., DELORENZO M., THATCHER W.W.: Effect of body condition on reproductive efficiency of lactating dairy cows receiving a time insemination. *Theriogenology,* 2000, **53**, 1305–1319.
12. - PANCARCI S.M., JORDAN E.R., RISCO C.A., SCHOUTEN M.J., LOPES F.L., MOREIRA F., THATCHER W.W.: Use of estradiol cypionate in a presynchronized timed artificial insemination program for lactating dairy cattle. *J. Dairy Sci.,* 2002*,* **85**, 122-131.
13. - PETERS M.W., PURSLEY J.R.: Fertility of lactating dairy cows treated with Ovsynch after pre-synchronization injections of PGF2α and GnRH. *J. Dairy Sci.,* 2002, **85**, 2403–2406.
14. - RABIEE A.R., LEAN I.J. STEVENSON M.A.: Effects of Ovsych and Other Synchrony Programs in Lactating Dairy Cows: A Meta-analysis. Proceedings of the 11th International Symposium on Veterinary Epidemiology and Economics. 2006, *www.sciquest.org.nz*.
15. - RABIEE A.R., LEAN I.J., STEVENSON M.A.: Efficacy of ovsynch program on reproductive performance in dairy cattle: A Meta analysis. *J. Dairy Sci.,* 2005, **88**, 2754-2770.
16. - SHRESTHA H.K., NAKAO T., SUZUKI T., AKITA M., HIGAKI T.: Relationships between body condition score, body weight, and some nutritional parameters in plasma and resumption of ovarian cyclicity postpartum during pre-service period in high-producing dairy cows in a subtropical region in Japan. *Theriogenology,* 2005, **64**, 855–866.
17. - STEVENSON J.S., KOBAYASHI Y., SHIPKA M., RAUCHHOLZ K.C.: Altering conception of dairy cattle by Gonadotropin releasing hormone preceding luteolysis induced by prostaglandin F2α. *J. Dairy Sci.,* 1996, **79**, 402–410.
18. - TAMADON A., KAFI M., SAEB M., GHAVAMI M.: Association of milk yield and body condition score indices with the commencement of luteal activity after parturition in high producing dairy cows. *Iran. J. Vet. Res.,* 2011, **12**, 184-191.
19. - TENHAGEN B.A., DRILLICH M., HEUWIESER W.: Analysis of cow factors influencing conception rates after two timed breeding protocols. *Theriogenology*, 2001, **56**, 831–838.
20. - TENHAGEN, B.A., VOGEL, C., DRILLICH M., THIELE G., HEUWIESER W.: Influence of stage of lactation and milk production on conception rates after timed artificial insemination following Ovsynch. *Theriogenology*, 2003, **60**, 1527-1537.
21. - THATCHER W.W., WILCOX C.J.: Postpartum estrus as an indicator of reproductive status in the dairy cow. *J. Dairy Sci.,* 1973, **56**, 608–615.
22. - WILDMAN E.E., JONES G.M., WAGNER P.E., BOWMAN R.L., TROUTT H.F., LESCH T.N.: A dairy cows body condition scoring system and its relationship to selected production characteristics. *J. Dairy Sci.,* 1982, **65**, 495-501.
23. - YAMADA K.: Development of ovulation synchronization and fixed time artificial insemination in dairy cows. *J. Reprod. Dev.,* 2005, **51**, 177-186.