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Risk Factors Influencing Conception Rate in Holstein Heifers before Artificial Insemination or Embryo Transfer

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ABSTRACT

The objective of this study was to show the risk factors affecting the conception rate in Holstein heifers after synchronization of estrus. A total of 275 Holstein heifers housed in a free barn were used for the experiment. The herd was visited regularly at four week intervals for synchronization of estrus using Heatsynch and CIDR-Heatsynch protocols. A group of four to 14 animals, depending on the availability, were referred to the experiment at each visit. Estrus induction rates in the two protocols were 93.9% and 94.9%, respectively. There was no difference in the conception rate between the two protocols. Conception rate after artificial insemination (AI) or embryo transfer (ET) were 46.3% and 51.4%, respectively. The risk factors affecting conception rate in heifers were daily weight gain (odds ratio [OR]= 4.673; P= 0.036) and body condition score (BCS) (OR= 3.642; P= 0.018). Furthermore, estrus synchronization protocol (OR= 1.774; P= 0.083) and the absence of corpus luteum (CL) at the initiation of treatment (OR= 0.512; P= 0.061) had a tendency to affect the conception rate, while age (OR= 0.715; P= 0.008) was a protective factor to conception rate. In conclusion, positive daily weight gain before AI or ET, higher BCS, younger age, and the presence of CL at the initiation of estrus synchronization in dairy heifers increased the likelihood to conceive.

Key words: risk factors, conception rate, Holstein Heifers, induced estrus

ABSTRAK

Tujuan penelitian ini adalah untuk menganalisis faktor risiko yang mempengaruhi angka konsepsi pada ternak sapi dara setelah perlakuan sinkronisasi berahi. Sebanyak 275 ternak sapi perah dara Holstein yang dilepaskan di dalam kandang digunakan pada penelitian ini. Pelaksanaan sinkronisasi berahi dilakukan secara reguler dengan interval 4 minggu dengan menggunakan metode Heatsynch dan CIDR-Heatsynch. Sebanyak 4 sampai 14 ekor ternak digunakan dalam penelitian ini pada setiap kunjungan, bergantung pada persediaan. Angka induksi berahi pada kedua metode sinkronisasi berahi adalah masing-masing 93,9% dan 94,9%. Tidak terdapat perbedaan angka konsepsi pada kedua metode sinkronisasi berahi. Angka konsepsi setelah pelaksanaan inseminasi buatan (IB) dan *embryo transfer* (ET) masing-masing 46,3% dan 51,4%. Faktor risiko yang mempengaruhi angka konsepsi pada ternak sapi dara adalah pertambahan bobot badan harian (*odds ratio* [OR]= 4,673; P= 0,036) dan kondisi tubuh (OR= 3,642; P= 0,018). Lebih lanjut, metode sinkronisasi berahi (OR=1,774; P=0,083) dan tidak terdapatnya *corpus luteum* (CL) pada awal perlakuan sinkronisasi berahi (OR=0,512; P=0,061) cenderung mempengaruhi angka konsepsi, sedangkan umur (OR= 0,715; P= 0,008) merupakan faktor yang memicu kebuntingan. Dapat disimpulkan bahwa pertambahan bobot badan sebelum IB atau ET, kondisi tubuh yang lebih tinggi, dan keberadaan CL pada awal perlakuan sinkronisasi berahi pada ternak sapi perah dara meningkatkan kemungkinan terjadinya konsepsi.

Kata kunci: faktor risiko, angka konsepsi, sapi perah dara Holstein, induksi berahi

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INTRODUCTION

One of the reasons in declining fertility in dairy cows is the increased capability for milk production (Butler, 2003). The detrimental effects of negative energy balance (NEB) in early lactation appear to be manifested as the problem during the breeding period, resulting in a low fertility. In dairy heifers, a better fertility than in lactating dairy cows has been reported (Pryce *et al*., 2004). This difference may be due to the difference in the metabolic and physiological status between cows and heifers (Royal *et al*., 2000). The more dairy cows lose body condition score (BCS) during the postpartum period, the lower is their conception rate. Cows losing one unit or more BCS (five-point scale) during early lactation are at a greater risk for low fertility. In cows, nutritional requirements increase rapidly with milk production following parturition, and the resulting NEB extends for 10-12 weeks, that is strongly associated with the length of the postpartum unovulatory period (Butler, 2003).

A number of factors influence conception rate (CR) in heifers after artificial insemination (AI). Change in body weight and/or BCS before breeding could be one of the important factors affecting CR in heifers. Brickell *et al*. (2009) reported that heifers with faster growth rates had a lower age at first breeding as well as a lower age at first calving. It has also been believed that heifers gaining body weight show a higher CR after embryo transfer (ET) than those losing weight. We assumed that heifers gaining weight before AI or ET after induction of estrus have a higher conception rate than those not gaining weight.

This study aimed to show the risk factors affecting the conception rate in Holstein heifers after synchronization of estrus.

MATERIALS AND METHODS

Animals and Housing

Holstein-Friesian heifers which were being reared at Yamaguchi Prefectural Heifer Raising/Breeding Farm, Yamaguchi, the south-western region of Japan, were used for the experiments. The calves at the age of 1 to 3 months were brought from a number of commercial dairy farms in Yamaguchi Prefecture to the farm and kept there until they became pregnant after AI or ET under the contract. Those pregnant heifers were returned to each farm a few months before due date.

The experimental heifers were housed in a free barn where the concrete floor was covered with sawdust. They were fed a diet formulated in line with NRC recommendation.

Reproductive Management

Estrus was detected by visual observation as a routine twice a day (09:00-10:00 and 15:00-16:00 h) with the help of tail paint (ALL-WEATHER® PAINSTICK®, LA-CO Industries Inc., Elk Grove Village, IL, USA). Heifers which were in estrus at 09:00-10:00 or 15:00-16:00 were inseminated artificially by veterinarians within one hour after the confirmation of estrus. Embryo transfer (ET) was conducted 7 days after estrus by the veterinary staff.

The heifers were bred either by AI or ET according to the request of the owners. The owners generally preferred for their heifers to be transferred with embryos from Japanese beef cattle for the first breeding. In case the heifers did not conceive after ET, the owners requested the farm to do AI for subsequent breeding. Pregnancy was diagnosed by palpation per rectum at 35 d or more after AI or 28 d after ET or later.

Those heifers which had not shown signs of estrus for more than one month were referred to the experiment. In addition, the heifers which had been subjected to ET 7 d after estrus, but were not suitable for ET due mainly to the formation of poor CL were also used for the study.

Data Collection for Factors which may Affect Conception Rate after AI or ET

The effects of eight factors on conception rate in heifers were investigated in this study; protocol for synchronization of estrus, breeding methods, stage of estrus cycle at the initiation of treatment, body condition score (BCS), body weight, daily weight gain, changes of body weight, and age (Table 1).

Protocols for Synchronization of Estrus

The herd was visited regularly at 4 week intervals for synchronization of estrus. A group of 4 to 14 animals, depending on the availability, were referred to the experiment at each visit. During a period from August 2005 to August 2008, the animals were allocated into 2 treated groups (Heatsynch and CIDR-Heatsynch groups) without regarding to the stages of the estrus cycle at the beginning of treatment (Yusuf *et al*., 2010; Bridges & Lake, 2011). During the following period from September 2008 to November 2009 all animals were referred to only CIDR-Heatsynch protocol.

In Heatsynch group, 99 animals were injected 100 µg of GnRH-analogue (fertirelin acetate, Conceral®, Schering-Plough Animal Health, Tokyo, Japan) IM at the beginning of the treatment (day-0), 500 µg $PGF_{2\alpha}$-analogue (Cloprostenol; Resipron®-C, ASKA Pharmaceutical Co., Ltd, Tokyo, Japan) IM on day-7, and 1.0 mg estradiol benzoate (EB) (Kawasaki-Mitaka Pharmaceuticals Co., Ltd, Tokyo, Japan) IM on day-8. In CIDR-Heatsynch group (n= 176), heifers were injected with GnRH, $PGF_{2\alpha}$, and EB, similar to heifers in Heatsynch group. However, in addition, a CIDR (EAZI-BREED CIDR® (Livestock Improvement Association of Japan, Tokyo, Japan) was inserted into the vagina at the time of GnRH injection and was removed on the day of $PGF_{2\alpha}$ injection (Yusuf *et al*., 2010).

All heifers were applied with heat detection devices; KAMAR® heat mount detector (HMD) or ALL-WEATHER® PAINTSTIK® (tail paint). The changes of the heat detection devices were monitored twice daily. The animals showing positive response of the devices, wearing off the tail paint of 50% or more, and the reac-

Table 1. Risk factors assessed for possible effects on the conception rate in Holstein heifers after induced estrus

Risk factor	*N* classes	Class description (*N* per class)	Mean±SD (range)
Protocol	2	Heatsynch (99) CIDR-Heatsynch (176)	
Breeding methods	2	AI (133) ET (142)	
Presence of CL at the initiation of treatment	2	Present (200) Absent (75)	
Body condition score	7	2.50 (1), 2.75 (18), 3.00 (87), 3.25 (56), 3.50 (85), 3.75 (26), 4.00 (2)	
Body weight (kg)	Continuous	(275)	384.8±35.5 (306-514)
Daily weight gain (kg)	Continuous	(275)	0.65±0.53 (-1.21-2.4)
	Categorical (3)[a]	Increased (241) Stable (13) Decreased (17)	
Age (month)	Continuous	(275)	14.7±1.5 (12.1-19.9)

Note: [a] Increased >0.05 kg/day; stable = -0.10 – 0.05 kg/day; decreased <-0.10 kg/day

tion of HMD with red color were palpated per rectum. Heifers showing clear uterine contraction were confirmed to have been in estrus (Yusuf *et al*., 2010).

AI or ET

Heifers assigned to AI were inseminated artificially with frozen/thawed semen from proven sires within 1 h after confirming estrus. For ET, a fresh embryo of grade A or B or grade A of frozen/thawed embryo was non-surgically transferred into the uterine horn ipsilateral to the corpus luteum 7 d after the estrus was detected.

The Presence of CL at the Initiation of Treatment

Synchronization of estrus in both protocols was initiated at random stages of the estrus cycle. Before administration of GnRH, all heifers were palpated per rectum to examine the ovarian structures. Heifers with inactive ovaries were excluded from the study. The animals were classified into those with corpus luteum (CL) and the others without CL based on palpation per rectum as well as plasma progesterone determination. Blood samples were collected via coccygeal venipuncture into evacuated heparinized vacuum tubes (VENOJECT® II TERUMO, Terumo Corporation, Tokyo, Japan) for determination of plasma progesterone concentrations at the initiation of protocol. The samples were centrifuged at 1500 x *g* for 15 min immediately after collection. The plasma was collected and stored frozen at -20°C until the analysis of progesterone concentrations by enzyme immunoassay (EIA) validated by Isobe & Nakao (2003). Intra- and inter-assay coefficients of variation were 5.7% and 6.6% in the plasma with 11.8 ng/mL, 19.1% and 19.3% in the plasma with 0.5 ng/mL, respectively.

All heifers with CL showed 1.0 ng/mL or higher plasma progesterone concentrations. Among the heifers with CL or dominant follicle (DF) which had not been classified by palpation per rectum, those with progesterone concentrations of 1.0 ng/mL or higher were grouped to have CL, while those with the progesterone concentrationss less than 1.0 ng/mL were grouped to have DF.

Body Condition Score

Heifers' body condition score was determined at the beginning of the experiment by the same person throughout the experiment using a-5 point system (1= thin to 5= fat) in increments of 0.25 (Edmonson *et al*., 1989).

Body Weight

Body weight of heifers was measured routinely by a weight measurement scale at one month interval to monitor their growth. Heifers reaching body weight of 350 kg and age of 12 months were submitted to the first breeding. Daily weight gain in the present study was calculated by the change in body weight during a period of one month before AI or ET including a period of synchronization treatment divided by the days.

Statistical Analyses

Statistical package SPSS 12.0 for windows (SPSS Inc., Chicago, Illinois, USA) was used in the study. AI submission rate and ET submission rate were calculated as the number of heifers inseminated or transferred with an embryo divided by the number of heifers to be inseminated or to be transferred with an embryo. Conception rate was calculated as the number of heifers conceiving divided by the total number of heifers inseminated or embryo-transferred. The pregnancy rate was defined as the number of heifers conceiving divided by the total number of heifers to be inseminated or to receive an embryo transfer. The percentages of heifers coming into estrus before $PGF_{2\alpha}$ treatment between Heatsynch and CIDR-Heatsynch groups were compared using Fisher's exact test, whereas the percentages of heifers coming into estrus within 5 d after $PGF_{2\alpha}$ treatment between the 2 groups were compared using

Chi-square test. Chi-square was also used to compare the combined percentages of heifers coming into estrus before and within 5 d after $PGF_{2\alpha}$ treatment, AI or ET submission rate, conception rate, and pregnancy rate after AI or ET between Heatsynch and CIDR-Heatsynch groups.

The binary logistic regression model was used to analyze the effects of body weight change before AI or ET and the other possible factors affecting the reduced likelihood of heifers to conceive. All calculations were categorized having significant effect if the P-value is less than 0.05.

RESULTS

Reproductive Performance in Heifers after Induced Estrus

Reproductive performance in dairy heifers after induced estrus using Heatsynch and CIDR-Heatsynch is shown in Table 2. The overall estrus induction rate was 94.5%. In Heatsynch group, 17 heifers (17.2%) came into estrus before $PGF_{2\alpha}$ treatment, but none in CIDR-Heatsynch group.

The overall conception rate in the present study, including those with different treatment protocols and those with different breeding methods was 48.2%. AI and ET submission rates, conception rate, and pregnancy rate did not differ between Heatsynch and CIDR-Heatsynch groups (Table 2). The conception rate at different variables and classes are shown in Table 3.

Effect of Daily Weight Gain before Treatment and other Factors on Conception Rate after AI or ET

Table 4 presents the binary logistic regression model for the factors causing the failure of heifers to conceive. Daily weight gain and BCS were the factors which contributed to the failure of heifers to conceive. Protocol and the presence of CL at the initiation of treatment had a tendency to affect the conception rate. As the BCS of the heifers increased by 0.25 unit, the likelihood of heifers to conceive increased by a factor of 3.624. Similarly, for the increased daily gain, the chance for heifers to become pregnant increased by a factor of 4.673. Age was a protective factor to conception rate, the increased age of animals affected the probability to conceive decreased by a factor of 0.715.

DISCUSSION

In this study, we analyzed the effects of daily weight gain before AI or ET and other factors on conception rate in heifers. The factors include the protocol for synchronization of estrus, breeding method, the presence of CL at initiation of treatment, BCS, body weight, and age. The lower BCS of the heifers at the initiation of treatment, negative daily weight gain, and the increased age of animals were the risk factors to reduce the probability of the heifers to become pregnant. Heifers with CL at the initiation of treatment had more chance to conceive than those without CL.

The occurrence of estrus before $PGF_{2\alpha}$ treatment in Heatsynch group might be caused by the different stages of the estrus cycle in the initiation of protocol,

Table 2. Reproductive performance of Holstein heifers after induction of estrus at different protocols of estrous synchronization

Variables	Estrous synchronization protocol		Total
	Heatsynch	CIDR-Heatsynch	
Total number of heifers treated	99	176	275
No. of heifers coming into estrus before $PGF2\alpha$ treatment (%) (a)	17 (17.2)[a]	0 (0)[b]	17 (6.2)
No. of heifers coming into estrus within 5 d after $PGF2\alpha$ treatment (%) (b)	76 (76.8)[a]	167 (94.9)[b]	243 (88.4)
a + b	93 (93.9)	167 (94.9)	260 (94.5)
No. of heifers to be inseminated	49	84	133
Artificial insemination (AI) submission rate (%)[1]	91.8 (45/49)	92.9 (78/84)	92.5 (123/133)
Conception rate (%)[2]	42.2 (19/45)	48.7 (38/78)	46.3 (57/123)
Pregnancy rate (%)[3]	38.8 (19/49)	45.2 (38/84)	42.9 (57/133)
No. of heifers to receive an embryo via embryo transfer (ET)	50	92	142
Embryo transfer (ET) submission rate (%)[4]	46.0 (23/50)	53.3 (49/92)	50.7 (72/142)
Conception rate (%)[5]	43.5 (10/23)	55.1 (27/49)	51.4 (37/72)
Pregnancy rate (%)[6]	20.0 (10/50)	29.3 (27/92)	26.1 (37/142)

Note: [a,b] Means in the same row with different superscripts differ significantly (P<0.01).
[1]Number of heifers inseminated/number of heifers to be inseminated.
[2]Number of heifers pregnant/number of heifers inseminated.
[3]Number of heifers pregnant/number of heifers to be inseminated.
[4]Number of heifers received an embryo via ET/number of heifers yet to receive an embryo.
[5]Number of heifers pregnant/number of heifers received an embryo.
[6]Number of heifers pregnant/number of heifers yet to receive an embryo.

Table 3. Conception rate at different variables in Holstein heifers after induction of estrus

Variables	Class	CR (%)
Protocol	Heatsynch	42.6 (29/68)
	CIDR-Heatsynch	51.2 (65/127)
Breeding methods	AI	46.3 (57/123)
	ET	51.4 (37/72)
The presence of corpus luteum (CL) at the initiation of treatment	Present	51.7 (75/145)
	Absent	38.0 (19/50)
Body condition score (BCS)	2.50	0.0 (0/1)
	2.75	40.0 (6/15)
	3.00	42.6 (23/54)
	3.25	42.9 (15/35)
	3.50	54.3 (38/70)
	3.75	55.6 (10/18)
	4.00	100.0 (2/2)
Body weight (kg) [a]	Moderate	50.9 (22/55)
	Thin	47.5 (18/38)
	Heavy	47.5 (48/101)
Daily weight gain [b]	Increased	47.7 (82/172)
	Stable	66.7 (6/9)
	Decreased	46.2 (6/13)
Age (month)	<14 months	54.5 (30/55)
	14–16 months	49.0 (50/102)
	>16 months	36.8 (14/38)

Note:
[a] Moderate = 340-365 kg; thin <340 kg; heavy >365 kg (Chebel *et al*., 2007)
[b] Increased >0.05 kg/day; stable = -0.10–0.05 kg/day; decreased <-0.10 kg/day.

resulting in different responses to initial GnRH treatment at random stages of the estrus cycle (Yusuf *et al*., 2010). Although the estrus induction rate within 5 d after $PGF_{2\alpha}$ treatment was lower (P<0.01) in Heatsynch group than in CIDR-Heatsynch group (76.8% vs 94.9%), the overall estrus induction rate within 12 d after initiation of the treatment did not differ between the two groups (93.9% vs 94.9%). Higher estrus response using Heatsynch and CIDR-Heatsynch protocols to synchronize estrus in dairy heifers that were achieved in this study had similar results to the study of Leitman *et al.* (2009) and Bridges & Lake (2011) using long-term CIDR protocols in beef heifers. The involvement of CIDR in Heatsynch protocol in the present study prevented the occurrence of premature estrus before $PGF_{2\alpha}$ treatment as was shown in the Heatsynch group. This result was in line with the study of Bridges & Lake (2011) and Thomas *et al*. (2016) using beef heifers. This result is related to the formation of CL with normal life-span following CIDR removal (Wheaton & Lamb 2007), the suppressed estrus during CIDR insertion that subsequently allow a high rate of submission for AI (Rabaglino *et al*., 2010; Palomares *et al*., 2015; Thomas *et al*., 2016).

The conception rate after AI at inducing estrus or ET 7 d after the estrus obtained in the present study was compared with the results of previous studies using some combinations of hormonal treatment; GnRH, $PGF_{2\alpha}$, EB, estradiol cypionate (ECP), and intravaginal device (CIDR) (Cavalieri *et al*., 2007; Silva *et* al., 2015; Pereira *et al*., 2016; Say *et al*., 2016).

Estrus detection rate within 5 d after $PGF_{2\alpha}$ treatment in Heatsynch group was 76.8%. When the heifers detected in estrus before $PGF_{2\alpha}$ injection were included, the total estrus detection rate within 12 d after the initiation of the protocol was 93.9%, equivalent to the estrus detection rate in the animals treated with CIDR-Heatsynch. The high estrus induction rate achieved in the present study might be due to the use of heat detection devices, HMD or TP, which were effective to improve the estrus detection rate. Although the estrus induction rate was high in the present study, the pregnancy rate was lower than the rate at spontaneous estrus as reported in previous studies (Cavalieri *et al*., 2007). Likewise, the study of Dorsey *et al*. (2011) showed that pregnancy rates to AI were significantly affected by the time of insemination relative to the onset of estrus.

The higher BCS of the heifers in the present study resulted in the higher conception rate after AI or ET. Similar result was obtained in beef cattle using fixed time AI protocols (Richardson *et al*., 2016). Low conception rate in heifers with low BCS might not be due to anovular conditions found in lactating dairy cows (Chebel *et al*., 2006), since all the heifers were confirmed to have ovulated one day after estrus.

Table 4. Odds ratio and 95% confidence interval of the variables included in the binary logistic regression model for Holstein heifers that failed to conceive

Variables	Odds ratio	95% confidence interval	P-value
Protocol	1.774	0.927–3.395	0.083
Breeding methods	0.739	0.352–1.554	0.425
The presence of corpus luteum (CL) at the initiation of treatment	0.512	0.253–1.032	0.061
Body condition score (BCS)	3.624	1.245–10.554	0.018
Body weight (kg)	1.000	0.991–1.010	0.925
Daily weight gain (kg) (continuous)	4.673	1.106–19.736	0.036
Daily weight change (categorical)	1.623	0.856–3.078	0.138
Age (month)	0.715	0.556–0.918	0.008

In this study, we emphasized how daily weight gain affects conception rate in heifers in comparison to the other factors. Out of eight factors included in the present study, daily weight gain was the third factor affecting conception rate in heifers after age and BCS (Table 4) and followed by the presence of CL at the initiation of treatment and estrus synchronization protocol. Heifers with moderate daily weight gain at around the initiation of the study had higher conception rate than thin or heavy heifers. This result is consistent with the study of Chebel *et al*. (2007). The body weight of heifers did not affect conception rate after AI or ET (Table 3). Changes in body weight before treatment as was shown by the daily weight gain had an influence on conception rate (Table 3). Heifers gaining more body weight had more chance to conceive than those heifers gaining less body weight. This results was consistent with the study of Brickell *et al*. (2009). Their study showed that poor growth with low circulating concentrations of IGF-1 during the rearing period was the main factor associated with the delayed first breeding and first calving.

The age of the heifers was found to affect the conception rate in the current study. This result was similar to that reported in the study of Kuhn *et al*. (2006). They demonstrated that the age heifer was the second most important main effect in terms of magnitude of impact on the conception rate. Furthermore, they reported that breeding heifers at the age of 15 to 16 months maximized the conception rate, and the conception rate was lower either for breeding at the age of <15 months or for breeding at the age of >16 months. In the present study, heifers bred at the age of less than 14 months showed a relatively higher conception rate than those bred at the age of greater than 14 months. This implied that heifers bred at the age of greater than 14 months required more inseminations to conceive.

The addition of CIDR in the protocol prevented the occurrence of premature estrus and resulted in a higher synchrony of estrus after treatment and formation of a CL with normal life-span (Wheaton & Lamb, 2007). Similar results were reported by Bishop *et al*. (2016) using the 14-d CIDR- $PGF_{2\alpha}$ protocol. Their study used pubertal and prepubertal or peripubertal heifers in comparison to lactating cows. This result indicated that inclusion of CIDR in a protocol suppressed estrus during CIDR insertion. Likewise, the use of long-term CIDR insert-based protocol (Leitman *et al*., 2009) using beef heifers resulted in a high rate of estrus response.

Stage of estrus cycle at the initiation of the treatment affected the conception rate. The conception rate in heifers with CL at the initiation of protocol tended to be higher than those in heifers without CL. Contrary, low peripheral progesterone concentrations during follicular development may lead to the decreased fertility (Atanasov *et al*., 2015). The presence of CL at the initiation of estrus synchronization increased the likelihood of heifers to conceive. This might probably due to fact that heifers that were in luteal phase at the beginning of protocol showed clear estrus signs than heifers that were in the follicular phase. The presence of CL at the initiation of estrus synchronization coincident with the administration of GnRH, most probably affected the occurrence of ovulation (Imron *et al*., 2015), the emergence of a new follicular wave that subsequently affect the expression of estrus (Sahu *et al*., 2014). The expression of estrus in cattle after synchronized estrus is associated with the increased fertility (Pereira *et al*., 2016).

CONCLUSION

Positive daily weight gain before AI or ET, higher BCS, younger age, and the presence of CL at the initiation of estrus synchronization in dairy heifers increased the likelihood to conceive.

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REFERENCES

Atanasov, B., J. De Koster, L. Bommelé, T. Dovenski, & G. Opsomer. 2015. Pathways of the dominant follicle after exposure to sub-luteal circulating progesterone concentrations are different in lactating dairy cows versus non-lactating heifers. Anim. Reprod. Sci. 154: 8-15. http://dx.doi.org/10.1016/j.anireprosci.2015.01.002

Bishop, B. E., J. M. Thomas, J. M. Abel, S. E. Poock, M. R. Ellersieck, M. F. Smith, & D. J. Patterson. 2016. Split-time artificial insemination in beef cattle: I–Using estrous response to determine the optimal time(s) at which to administer GnRH in beef heifers and postpartum cows. Theriogenology 86:1102-1110. http://dx.doi.org/10.1016/j.theriogenology.2016.03.043

Brickell, J. S., N. Bourne, M. M. McGowan, & D. C. Wathes. 2009. Effect of growth and development during the rearing period on the subsequent fertility of nulliparous Holstein-Friesian heifers. Theriogenology 72:408-416. http://dx.doi.org/10.1016/j.theriogenology.2009.03.015

Bridges, G. A. & S. L. Lake. 2011. Comparison of the CIDR Select and 5-day Select Synch + CIDR protocols that included limited estrus detection and timed insemination for synchronizing estrus in beef heifers. The Professional Animal Scientist 27: 141-146. http://dx.doi.org/10.15232/S1080-7446(15)30461-7

Butler, W. R. 2003. Energy balance relationships with follicular development, ovulation and fertility in postpartum dairy cows. Livest. Prod. Sci. 83:211-218. http://dx.doi.org/10.1016/S0301-6226(03)00112-X

Cavalieri, J., G. Hepworth, V. M. Smart, M. Ryan, & K. L. Macmillan. 2007. Reproductive performance of lactating dairy cows and heifers resynchronized for a second insemination with an intravaginal progesterone-releasing device for 7 or 8 d with estradiol benzoate injected at the time of device insertion and 24 h after removal. Theriogenology 67:824-834. http://dx.doi.org/10.1016/j.theriogenology.2006.10.016

Chebel, R. C., F.A. Fernando, & J. C. Dalton. 2007. Factors affecting reproductive performance of Holstein heifers.

Anim. Reprod. Sci. 101:208-224. http://dx.doi.org/10.1016/j.anireprosci.2006.09.010

Chebel, R. C., J. E. P. Santos, R. L. A. Cerri, H. M. Rutigliano, & R. G. S. Bruno. 2006. Reproduction in dairy cows following progesterone insert presynchronization and resynchronization protocols. J. Dairy Sci. 89:4205-4219. http://dx.doi.org/10.3168/jds.S0022-0302(06)72466-3

Dorsey, B. R., R. Kasimanickam, W. D. Whittier, R. L. Nebel, M. L. Wahlberg, & J. B. Hall. 2011. Effect of time from estrus to AI on pregnancy rates in estrous synchronized beef heifers. Anim. Reprod. Sci. 127:1-6. http://dx.doi.org/10.1016/j.anireprosci.2011.07.014

Edmonson, A. J., I. J. Lean, L. D. Weaver, T. Farver, & G. Webster. 1989. A body condition scoring chart for Holstein dairy cows. J. Dairy Sci. 72:68-78. http://dx.doi.org/10.3168/jds.S0022-0302(89)79081-0

Imron, M., I. Supriatna, Amrozi, & M. A. Setiadi. 2015. Ovarian dynamic in ongole grade cattle after GnRH injection in ovsynch protocol based on progesterone device. Med. Pet. 38:82-88. http://dx.doi.org/10.5398/medpet.2015.38.2.82

Isobe, N. & T. Nakao. 2003. Direct enzyme immunoassay of progesterone in bovine plasma. Anim. Sci. J. 74:369-373. http://dx.doi.org/10.1046/j.1344-3941.2003.00128.x

Kuhn, M. T., J. L. Hutchison, & G. R. Wiggans. 2006. Characterization of Holstein heifer fertility in the United States. J. Dairy Sci. 89:4907-4920. http://dx.doi.org/10.3168/jds.S0022-0302(06)72541-3

Leitman, N. R., D. C. Busch, D. A. Mallory, D. J. Wilson, M. R. Ellersieck, M. F. Smith, & D. J. Patterson. 2009. Comparison of long-term CIDR-based protocols to synchronize estrus in beef heifers. Anim. Reprod. Sci. 114:345-355. http://dx.doi.org/10.1016/j.anireprosci.2008.10.014

Palomares, R. A., H. J. Fishman, A. L. Jones, M. S. Ferrer, M. Jenerette, & A. Vaughn. 2015. Comparison of 4- versus 5-day Co-Synch + controlled internal drug release (CIDR) + timed artificial insemination protocols in dairy heifers. Theriogenology 84: 868-874. http://dx.doi.org/10.1016/j.theriogenology.2015.05.021

Pereira, M. H. C., M. C. Wiltbank, & J. L. M. Vasconcelos. 2016. Expression of estrus improves fertility and decreases pregnancy losses in lactating dairy cows that receive artificial insemination or embryo transfer. J. Dairy Sci. 99 2237-2247. http://dx.doi.org/10.3168/jds.2015-9903

Pryce, J. E., M. D. Royal, P. C. Garnsworthy, & I. L. Mao. 2004. Fertility in the high-producing dairy cow. Livest. Prod. Sci. 86:125-135. http://dx.doi.org/10.1016/S0301-6226(03)00145-3

Rabaglino, M. B., C. A. Risco, M. J. Thatcher, I. H. Kim, J. E. P. Santos, & W. W. Thatcher. 2010. Application of one injection of prostaglandin F2α in the five-day Co-Synch + CIDR protocol for estrous synchronization and resynchronization of dairy heifers. J. Dairy Sci. 93:1050-1058. http://dx.doi.org/10.3168/jds.2009-2675

Richardson, B. N., S. L. Hill, J. S. Stevenson, G. D. Djira, & G. A. Perry. 2016. Expression of estrus before fixed-time AI affects conception rates and factors that impact expression of estrus and the repeatability of expression of estrus in sequential breeding seasons. Anim. Reprod. Sci. 166:133-140. http://dx.doi.org/10.1016/j.anireprosci.2016.01.013

Royal, M. D., A. O. Darwash, A. P. F. Flint, R. Webb, J. A. Woolliams, & J. E. Lamming. 2000. Declining fertility in dairy cattle: changes in traditional and endocrine parameters of fertility. Anim. Sci. 70:487-501. http://dx.doi.org/10.1017/S1357729800051845

Sahu, S. K., J. F., Cockrem, T. J. Parkinson, & R. A. Laven. 2014. The effects of exclusion of progesterone or Day 0 GnRH from a GnRH, prostaglandin, GnRH + progesterone program on synchronization of ovulation in pasture-based dairy heifers. Theriogenology 82:643-651. http://dx.doi.org/10.1016/j.theriogenology.2014.06.004

Say, E., S. Çoban, Y. Nak, D. Nak, U. Kara, S. White, V. Kasimanickam, & R. Kasimanickam. 2016. Fertility of Holstein heifers after two doses of PGF2α in 5-day CO-Synch progesterone-based synchronization protocol. Theriogenology 86:988-993. http://dx.doi.org/10.1016/j.theriogenology.2016.03.026

Silva, T. V., F. S. Lima, W. W. Thatcher, & J. E. P. Santos. 2015. Synchronized ovulation for first insemination improves reproductive performance and reduces cost per pregnancy in dairy heifers. J. Dairy Sci. 98:7810-7822. http://dx.doi.org/10.3168/jds.2015-9704

Thomas, J. M., B. E. Bishop, J. M. Abel., M. R. Ellersieck, M. F. Smith, & D. J. Patterson. 2016. The 9-day CIDR-PG protocol: Incorporation of PGF2α pretreatment into a long-term progestin-based estrus synchronization protocol for postpartum beef cows. Theriogenology 85:1555-1561. http://dx.doi.org/10.1016/j.theriogenology.2016.01.010

Wheaton, J. E. & G. C. Lamb. 2007. Induction of cyclicity in postpartum anestrous beef cows using progesterone, GnRH and estradiol cypionate (ECP). Anim. Reprod. Sci. 102:208-216. http://dx.doi.org/10.1016/j.anireprosci.2006.11.006

Yusuf, M., T. Nakao, C. Yoshida, S. T. Long, S. Fujita, Y. Inayoshi, & Y. Furuya. 2010. Comparison in effect of Heatsynch with heat detection aids and CIDR-Heatsynch in dairy heifers. Reprod. Dom. Anim. 45:500-504. http://dx.doi.org/10.1111/j.1439-0531.2008.01277.x