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Conception Rate in Lactating Dairy Cows using Ovsynch after Presynchronization with Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) or Gonadotropin Releasing Hormone (GnRH)

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Abstract

This study compared the pregnancy rate in cows inseminated after two different pre-synchronization protocols based on clinical features of the genital tract and treatment with either $PGF_{2\alpha}$ or GnRH. A total of 340 reproductively normal lactating dairy cows was divided in two groups. Cows in Group 1 (n=216) had a corpus luteum when palpated *per rectum* on day 0. These cows were administered 25 mg of $PGF_{2\alpha}$ on day 0, 100 υg of GnRH on day 14, 25 mg of $PGF_{2\alpha}$ on day 21, 100 υg of GnRH on day 23, and time-inseminated 16-h later. Cows detected in estrus from day 0 to day 14 were inseminated 10 to 12-h after signs of estrus.

Cows in Group 2 (n=124) either had a large ovarian follicle and uterine tone, or a corpus hemorragicum when palpated *per rectum* on day 0. These cows received 100 υg of GnRH on day 0, 100 υg of GnRH on day 8, 25 mg of $PGF_{2\alpha}$ on day 15, 100 υg of GnRH on day 17, and time-inseminated 16-h later. Cows detected in estrus before the end of the treatment protocol were inseminated 10 to 12-h after signs of estrus and removed from the study. Data collected were pregnancy rate for cows time-inseminated in Group 1, cows inseminated at detected estrus in Group 1, and cows time-inseminated in Group 2. Pregnancy rates were compared using logistic regression adjusted for number of previous services, parity, season and days in milk. There was no difference (p=0.83) in pregnancy rate between cows time-inseminated in Group 1 (35.9%) and Group 2 (32.8%). In Group 1, there was no difference (p=0.21) in pregnancy rate between cows time-inseminated (35.9%) and cows inseminated at detected estrus within 14-d after treatment with $PGF_{2\alpha}$ (43.5%). Results of this study suggest that cows in different stages of the estrous cycle, as determined by rectal palpation of the genital tract, had similar pregnancy rates when they were selectively pre-synchronized with either $PGF_{2\alpha}$ or GnRH.

Résumé

Cette étude comparait le taux de gestation chez des vaches inséminées suite à deux protocoles de pré-synchronisation basés sur des caractéristiques cliniques du tractus génital et un traitement avec soit $PGF_{2\alpha}$ ou soit GnRH. Un total de 340 vaches laitières en lactation et normales ont été réparties en deux groupes. Les vaches du groupe 1 (n = 216) avaient un *corpus luteum* lors de la palpation rectale au jour 0. Ces vaches reçurent 25 mg de $PGF_{2\alpha}$ au jour 0, 100 µg de GnRH au jour 14, 25 mg de $PGF_{2\alpha}$ au jour 21, 100 µg de GnRH au jour 23 et furent inséminées sur rendez-vous 16 heures plus tard. Les vaches qui étaient en chaleur entre les jours 0 et 14 furent inséminées de 10 à 12 heures suivant les signes d'estrus.

Les vaches du groupe 2 (n = 124) avaient soit un gros follicule ovarien et un tonus utérin ou soit un *corpus hemorragicum* lors de la palpation rectale au jour 0. Ces vaches reçurent 100 µg de GnRH au jour 0, 100 µg de GnRH au jour 8, 25 mg de $PGF_{2\alpha}$ au jour 15, 100 µg de GnRH au jour 17 et furent inséminées sur rendez-vous 16 heures plus tard. Les vaches qui étaient en chaleur avant la fin du traitement ont été inséminées de 10 à 12 heures suivant la détection de chaleur et retirées de l'étude. Les données se rapportaient au taux de gestation des vaches inséminées sur rendez-vous dans le groupe 1, des vaches inséminées après la détection

de chaleur dans le groupe 1 et finalement des vaches inséminées sur rendez-vous dans le groupe 2. Le taux de gestation était comparé à l'aide de la régression logistique en ajustant pour le nombre d'inséminations, la parité, la saison et le nombre de jours en lactation. Il n'y avait pas de différence (p = 0.83) dans le taux de gestation des vaches inséminées sur rendez-vous dans le groupe 1 (35.9%) et dans le groupe 2 (32.8%). Dans le groupe 1, il n'y avait pas de différence (p = 0.21) dans le taux de gestation des vaches inséminées sur rendez-vous (35.9%) et des vaches inséminées à la détection de chaleur pendant la période de 14 jours suivant le traitement avec la $PGF_{2\alpha}$ (43.5%). Les résultats de cette étude suggèrent que les vaches qui sont à différentes étapes du cycle d'ovulation, tel que déterminé par la palpation rectale du tractus génital, ont des taux de gestation similaires selon qu'elles soient pré-synchronisées de façon sélective avec la $PGF_{2\alpha}$ ou le GnRH.

Introduction

Reproductive efficiency has a significant impact on the gross income of dairy farms. Pregnancy rate, the product of the conception rate and the estrus detection rate, is an important measure of reproductive success.[6] Breeding programs based on insemination at detected estrus achieve acceptable conception rates, but pregnancy rates are low due to shortfalls of estrus detection. With timed-insemination protocols, estrus detection is eliminated, all cows are inseminated, and the conception rate is equivalent to the pregnancy rate. By modeling reproductive efficiency using different conception and estrus detection rates, it was concluded that timed-insemination programs are economically beneficial in dairy herds when estrus detection is compromised.[8]

In lactating dairy cows, the Ovsynch protocol for timed-insemination has been developed.[12] With this protocol, GnRH administered on day 0 synchronizes the onset of a new follicular wave, and administration of $PGF_{2\alpha}$ on day 7 regresses the corpus luteum (CL) and synchronizes estrus.[18,12] A second GnRH injection on day 9 synchronizes ovulation 24 to 32-h later, and timed-insemination is done 16-h after the second injection of GnRH.[12] Numerous studies report that using Ovsynch results in acceptable pregnancy rates in normal lactating dairy cows[5,9,13,14] and lactating dairy cows with ovarian cysts.[3]

Although Ovsynch improves pregnancy rates by eliminating estrus detection, conception rates tend to be higher when cows are inseminated after detection of estrus.[2,4,5,15,16,17] Recent research has shown that lower pregnancy rates occur with the Ovsynch protocol when GnRH is first administered between day 1 and day 4 of the estrous cycle because a follicle has been already recruited, therefore the follicular wave cannot be synchronized. If GnRH is first administered on day 13 to day 15 of the estrous cycle, cows with two follicular waves may not have a follicle ready to ovulate and form a CL. In these cows, the spontaneous release of $PGF_{2\alpha}$ between day 16 and day 17 of the estrous cycle will regress the CL, and early ovulation will occur.[19] Therefore, the pregnancy rate increases when cows are subjected to the Ovsynch protocol between day 5 and day 10 of the estrous cycle.[10,11,19,21] Integration of timed-insemination protocols into veterinary herd health programs is a useful and economical tool for reproductive management.[8]

The rationale for the protocols used in this study was that cows with a CL and treated with $PGF_{2\alpha}$ would have the opportunity to express estrus within 2 to 7 days. However, cows not detected in estrus by day 14 will be between days 7 and 12 of the estrous cycle. In addition, cows with either a palpable ovarian follicle and uterine tone or a corpus hemorragicum which are treated with GnRH 8 days later, will also be between days 7 and 12 of the estrous cycle. Therefore, both groups of cows could be subjected to the Ovsynch protocol at the right stage of the estrous cycle.

Our hypothesis was that using Ovsynch at different stages of the estrous cycle will result in similar pregnancy rates if they are selectively pre-synchronized with either $PGF_{2\alpha}$ or GnRH based upon clinical features of the uterus and ovaries obtained by rectal palpation. The objective of this study was to compare the pregnancy rate in cows inseminated after pre-synchronization using two different protocols based on clinical features of the genital tract and treatment with either $PGF_{2\alpha}$ or GnRH.

Materials and Methods

The study was conducted between September 2000 and May 2001 in a large dairy herd (3,000 milking cows) in northcentral Florida. At 60 to 65 days post-partum, all cows in the herd were treated with 500 mg of bovine somatotropin (bST)[a] every 14-d until milk production decreased to a level established by the herd managers.

Cows were milked three times daily and were kept in shaded lots between milkings. The rolling herd average for milk production was 22,557 lb (10,253 kg). The herd was enrolled in a reproductive herd health program and was visited bi-weekly by the attending veterinarian. All reproductive, health and management records were maintained using a cow-side computer program.

A total of 340 reproductively normal, lactating, non-pregnant dairy cows was used in this study. Cows were selected during bi-weekly reproductive visits and were presented for pregnancy diagnosis or for estrus synchronized and timed insemination. Cows with ovarian cysts, granulosa cell tumor, endometritis, pyometra, uterine adhesions and abnormal vaginal discharge were ex-

cluded. Cows in Group 1 (n=216) which had a CL on day 0, determined by rectal palpation of the genital tract, received 25 mg of $PGF_{2\alpha}$[b] on day 0, 100 υg of GnRH[c] on day 14, 25 mg of $PGF_{2\alpha}$ on day 21, 100 υg of GnRH on day 23 and time-inseminated 16-h later ($PGF_{2\alpha}$ + Ovsynch). Cows detected in estrus from day 0 to day 14 were inseminated 10 to 12-h after first signs of estrus. Cows detected in estrus between day 14 and the end of the treatment were inseminated 10 to 12-h after first signs of estrus, but were not included in the study. Cows in Group 2 (n=124) had either a palpable ovarian follicle and uterine tone, or a corpus hemorragicum when palpated *per rectum* on day 0. They received 100 υg of GnRH on day 0, 100 υg of GnRH on day 8, 25 mg of $PGF_{2\alpha}$ on day 15, 100 υg of GnRH on day 17 and time-inseminated 16-h later (GnRH + Ovsynch). Cows detected in estrus between day 0 and the end of the treatment were inseminated 10 to 12-h after first signs of estrus, but were not included in the study. Both $PGF_{2\alpha}$ and GnRH were administered by intramuscular injection.

Pregnancy was determined by rectal palpation of the uterus approximately 45-d after artificial insemination, as previously described.[23] When cows were enrolled in the study (day 0), number of previous services, parity, season, and days-in-milk (DIM) were recorded. Categorization of DIM was based in quartiles to address the effect of different stages of lactation on fertility. In addition, season was categorized based on previous research in northcentral Florida indicating that conception rate is higher during October to December.[9] The distribution of cows and baseline comparison for number of previous services, parity, season and DIM for both groups are shown in Table 1.

Since there was a significant difference in parity between groups ($p<0.01$), the effect of group on conception rate was evaluated using an adjusted logistic regression model, which included parity. Data collected included pregnancy rate for cows time-inseminated in Group 1, cows inseminated at detected estrus in Group 1, and cows time-inseminated in Group 2. Pregnancy rates between cows time-inseminated in Groups 1 and 2, and between cows time-inseminated in Group 1 and inseminated at detected estrus in Group 1 were compared using logistic regression adjusted for number of previous services, parity, season and DIM.[1]

Results

In Group 1, 85 of 216 (39.3%) cows were inseminated at estrus within 14-d after administration of $PGF_{2\alpha}$, and 131 of 216 (60.7%) cows were time-inseminated. In Group 2, 119 of 124 (96%) cows were time-inseminated. Pregnancy rate for cows time-inseminated was 35.9% (47/131) for cows in Group 1, and 32.8% (39/119) for cows in Group 2. In Group 1, the pregnancy rate for cows that showed estrus within 14-d after $PGF_{2\alpha}$ injection on day 0 was 43.5% (37/85).

Table 1. Distribution of cows and baseline comparison for number of previous services, parity, season and DIM for both groups.

	Group 1 N (%)	Group 2 N (%)	*p*-value
Previous services			0.95
0	122 (56.5)	72 (58.0)	
1	39 (18.0)	22 (17.7)	
2	22 (10.2)	11 (8.9)	
3+	33 (15.3)	19 (15.3)	
Parity			
1	78 (36.1)	63 (50.8)	0.01
2	59 (27.3)	35 (28.2)	
3+	79 (36.6)	26 (20.9)	
Season			0.29
Sep-Dec	114 (52.8)	70 (58.8)	
Jan-May	102 (47.2)	49 (41.2)	
DIM			0.81
61-120	54 (25.0)	36 (29.0)	
121-150	56 (25.9)	30 (24.2)	
151-206	51 (23.6)	31 (25.0)	
207-631	55 (25.5)	27 (21.8)	

There was no difference in pregnancy rate (OR=1, 95% CI=0.6-1.8, $p=0.83$) between cows time-inseminated in Groups 1 and 2 (Table 2). In Group 1, there was no significant difference in pregnancy rate (OR=0.7, 95% CI=0.4-1.2, $p=0.21$) between cows inseminated at detected estrus within 14-d of $PGF_{2\alpha}$ injection and those cows time-inseminated.

Discussion

A valid criticism of this study is the exclusion of cows at different stages of the estrous cycle. However, this was a conscious decision since pregnancy rates are reduced when Ovsynch is started at stages of the estrous cycle other than early diestrus.[11,21]

The objective of this study was to compare the pregnancy rates of cows time-inseminated after pre-synchronization using clinical features of the genital tract, and treatment with either $PGF_{2\alpha}$ or GnRH. There was no difference in pregnancy rate between cows in Group 1 and cows in Group 2. In Group 1, there was no significant difference in pregnancy rate between cows inseminated at detected estrus within 14-d of treatment with $PGF_{2\alpha}$ and cows that were time-inseminated.

Timed-insemination protocols may improve pregnancy rate by reducing the need for estrus detection.[5,9,13,14,15] However, pregnancy rate still tends to be

Table 2. Number of cows, pregnancy rate, adjusted odds ratio (OR), 95% confidence interval (CI), and level of significance for probability of pregnancy of cows time-inseminated in Groups 1 and 2.

Variable	Pregnancy N	Pregnancy %	OR	95% CI	*p*-value
Group					
Group 2	39/119	32.8	-	Referent	NA
Group 1	47/131	35.9	1.0	0.6-1.8	0.83
Previous services					
3+	10/36	27.8	-	Referent	NA
0	56/146	38.6	1.8	0.5-7.2	0.38
1	16/45	35.6	1.3	0.4-4.5	0.61
2	4/23	17.4	0.5	0.1-1.9	0.32
Parity					
3+	29/73	39.7	-	Referent	NA
1	31/114	27.2	0.6	0.3-1.2	0.15
2	26/63	41.3	1.0	0.5-2.0	0.93
Season					
Sep-Dec	45/115	39.1	-	Referent	NA
Jan-May	41/135	30.4	0.5	0.3-0.9	0.04
DIM					
207-631	15/59	25.4	-	Referent	NA
61-120	25/59	42.4	1.3	0.3-4.9	0.68
121-150	25/65	38.5	1.4	0.4-4.7	0.60
151-206	21/67	31.3	1.7	0.4-3.7	0.71

NA=not applicable

higher when cows are inseminated at detected estrus compared with cows that are time-inseminated.[2,4,5,16,17]

Overall, synchronization of ovulation using Ovsynch is approximately 85%.[5,21] However, ovulation rates following the first and second injection of GnRH are higher when Ovsynch is started between day 5 and day 9 of the estrous cycle.[8,21] In addition, when using Ovsynch, the conception rate was higher (10% increase) when the first GnRH injection was given between day 5 and day 9[21] or between day 5 and day 10[11] of the estrous cycle.

The advantage of the protocols used in this study was that cows were subjected to Ovsynch at the optimum time of the estrous cycle. In addition, cows treated with $PGF_{2\alpha}$ on day 0 (Group 1) could be inseminated at detected estrus within 14 d. However, if not detected in estrus, they could be time-inseminated following Ovsynch. There was no difference in the pregnancy rate between cows time-inseminated (35.9%), and cows inseminated 10 to 12-h after first signs of estrus (43.5%) in Group 1. These results are in agreement with a previous study that reported no difference in pregnancy rate between cows inseminated at detected estrus and cows subjected to timed-insemination during the luteal phase of the estrous cycle as determined by rectal palpation.[9] The pregnancy rate of cows in both Group 1 and Group 2 may have been affected by the accuracy of detection of a corpus luteum and/or a corpus hemorrhagicum when the ovaries were palpated. Previous reports have shown that the positive predictive value of determining the presence of a functional CL by rectal palpation of the ovaries is between 85 to 90 percent.[2,7,20]

Conclusions

This study suggests that cows in different stages of the estrous cycle, as determined by rectal palpation of the genital tract, had similar pregnancy rates when they were selectively pre-synchronized with either $PGF_{2\alpha}$ or GnRH. The use of these protocols could be beneficial to both the practitioner and the client.

With these protocols, the practitioner has a tool to manage nonpregnant cows at various stages of the estrous cycle in order to achieve acceptable pregnancy rates. It is recommended that the $PGF_{2\alpha}$ + Ovsynch protocol be used for cows in diestrus, and that the GnRH + Ovsynch protocol be used for cows at other stages of the estrous cycle.

A benefit to the client is that these protocols could be less labor-intensive, since a certain percentage of diestrual cows will exhibit estrus following the use of $PGF_{2\alpha}$. Therefore, a reduced number of cows would need

to be inseminated at any one time, which could result in better efficiency of insemination. In addition, diestrual cows not detected in estrus, and cows at other stages of the estrous cycle could be subjected to the Ovsynch protocol at the optimum stage of the estrous cycle. Future research is aimed at evaluating the economic benefit of these protocols compared to that obtained by the Ovsynch protocol.

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Footnotes

[a]Posilac®, Monsanto Co, St Louis, MO
[b]Lutalyse®, Pharmacia Animal Health, Kalamazoo, MI
[c]Cystorelin®, Rhone Merieux Inc, Athens, GA

References

1. Agresti A: *An Introduction to Categorical Data Analysis.* New York: John Wiley & Sons, Inc., 1996, pp 103-132.
2. Archbald LF, Tran T, Massey R, Klapstein E: Conception rate in dairy cows after timed-insemination and simultaneous treatment with gonadotrophin releasing hormone and/or prostaglandin F2 alpha. *Theriogenology* 37: 723-731, 1992.
3. Bartolome JA, Archbald LF, Morresey P, *et al*: Comparison of synchronization of ovulation and induction of estrus as therapeutic strategies for bovine ovarian cysts in the dairy cow. *Theriogenology* 53:815-825, 2000.
4. Burke JM, de la Sota RL, Risco CA, *et al*: Evaluation of timed insemination using a gonadotropin-releasing hormone agonist in lactating dairy cows. *J Dairy Sci* 79: 1385-1393, 1996.
5. de la Sota RL, Burke JM, Risco CA, *et al*: Evaluation of timed insemination during summer heat stress in lactating dairy cows. *Theriogenology* 49: 761-770, 1998.
6. Ferguson JD: Diet, production and reproduction in dairy cows. *Anim Fed Tech* 59: 173-184, 1996.
7. Kelton DF, Leslie KE, Etherington WG, *et al*: Accuracy of rectal palpation and of a rapid milk progesterone enzymeimmunoassay for determining the presence of a functional corpus luteum in subestrus dairy cows. *Can Vet J* 32: 286-291, 1991.
8. Leblanc S: The ovsynch breeding program for dairy cows- a review and economic perspective. *Bov Pract* 35: 13-22, 2001.
9. Momcilovic D, Archbald LF, Walters A, *et al*: Reproductive performance of lactating dairy cows treated with gonadotrophin-releasing hormone (GnRH) and/or prostaglandin F2a (PGF2a) for synchronization of estrus and ovulation. *Theriogenology* 50: 1131-1139, 1998.
10. Moreira F, de la Sota RL, Diaz T, Thatcher WW: Effect of day of the estrous cycle at the initiation of a timed artificial insemination protocol on reproductive responses in dairy heifers. *J Anim Sci* 78: 1568-1576, 2000.
11. Moreira F, Orlandi C, Risco CA, *et al*: Effects of presynchronization and bovine somatotropin on pregnancy rates to a timed artificial insemination protocol in lactating dairy cows. *J Dairy Sci* 84: 1646-1659, 2001.
12. Pursley JR, Mee MO, Wiltbank MC: Synchronization of ovulation in dairy cows using PGF2a and GnRH. *Theriogenology* 44: 915-923, 1995.
13. Pursley JR, Wiltbank MC, Stevenson JS, *et al*: Pregnancy rates in cows and heifers inseminated at a synchronized ovulation or synchronized estrus. *J Dairy Sci* 80:295-300, 1997.
14. Pursley JR, Kosorok MR, Wiltbank MC: Reproductive management of lactating dairy cows using synchronization of ovulation. *J Dairy Sci* 80: 301-306, 1997.
15. Schmitt EJP, Diaz T, Drost M, Thatcher WW: Use of gonadotropin-releasing hormone agonist or human chorionic gonadotropin for timed insemination in cattle. *J Anim Sci* 74:1084-1091, 1996.
16. Stevenson JS, Kobayashi Y, Shipka MP, Rauchholz KC: Altering conception rates of dairy cattle by gonadotropin-releasing hormone preceding luteolysis induced by prostaglandin $F_{2\alpha}$. *J Dairy Sci* 79: 402-410, 1996.
17. Stevenson JS, Kobayashi Y, Thompson KE: Reproductive performance of dairy cows in various programmed breeding systems including Ovsynch and combinations of gonadotropin-releasing hormone and prostaglandin $F_{2\alpha}$. *J Dairy Sci* 99: 506-515, 1999.
18. Thatcher WW, Macmillan KL, Hansen PJ, Drost M: Concepts for regulation of corpus luteum function by the conceptus and ovarian follicles to improve fertility. *Theriogenology* 1: 149-164, 1989.
19. Thatcher WW, Moreira F, Santos JEP, *et al*: Effects of hormonal treatments on reproductive performance and embryo production. *Theriogenology* 55: 75-89, 2001.
20. Ott RS, Bretzlaff KN, Hixon JE: Comparison of palpable corpora lutea with serum progesterone concentrations in cows. *JAVMA* 188: 1417-1419, 1986.
21. Vasconcelos JLM, Silcox RW, Rosa GJM, *et al*: Synchronization rate, size of the ovulatory follicle, and pregnancy rate after synchronization of ovulation beginning on different days of the estrous cycle in lactating dairy cows. *Theriogenology* 52: 1067-1078, 1999.
22. Wolfenson D, Thatcher WW, Savio JD, *et al*: The effect of a GnRH analogue on the dynamics of follicular development and synchronization of estrus in lactating cyclic dairy cows. *Theriogenology* 42: 633-644, 1994.
23. Zemjanis R: *Diagnostic and Therapeutic Techniques in Animal Reproduction.* Baltimore, Williams & Wilkins, 1962, pp 29-44, 59-60.

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