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Effect of Post Insemination Progesterone Supplement on Pregnancy Rates of Repeat Breeder Friesian Cows

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ABSTRACT : Fifty repeat breeder (RB) Friesian cows were allocated to five groups of 10 cows each, to determine the effect of progesterone (P4) supplement on P4 concentrations and pregnancy rates during the periods of corpus luteum (CL) formation and development between days 2-7 and 7-12 following a spontaneous or $PGF_{2\alpha}$-induced estrus. Cows were artificially inseminated during $PGF_{2\alpha}$-induced (PGF-P4-d2 and PGF-P4-d7 groups) or spontaneous (S-P4-d2, S-P4-d7, and control groups) estrus. Progesterone-releasing intravaginal device (PRID) devoid of estrogen capsule were inserted either on d 2 (PGF-P4-d2 and S-P4-d2 groups) or d 7 (PGF-P4-d7 and S-P4-d7 groups) post-insemination and left in place for 5 days. Control cows did not receive any treatment. Blood samples were collected for progesterone analysis from all cows once daily for 4 days starting on the day of estrus (d 0) and once every 3 days thereafter until d 22. Progesterone treatment by day interaction accounted for higher plasma P4 in treated than non-treated control cows. Progesterone concentrations differed significantly ($p<0.05$) during metestrus (d 2 to d 7) but not during diestrus (d 7 to d 12). $PGF_{2\alpha}$ treatment, lactation number, service number or their interactions did not affect progesterone concentrations and pregnancy rates. Therefore, cows were grouped according to the day of P4 supplement irrespective of the $PGF_{2\alpha}$ treatment. Progesterone supplement on d 7 but not d 2 significantly increased ($p<0.03$) pregnancy rates in repeat breeding cows with four or more previous services but not in cows in their third service. In conclusion, post-insemination P4 supplement to repeat breeding cows with four or more previous services improved pregnancy rates and should be advocated when no specific reason for infertility is diagnosed. Further studies with larger numbers of repeat breeding cows under field conditions are needed to ascertain the findings of this study. (**Key Words** : Repeat Breeder Cow, Progesterone Supplement, PRID, Pregnancy)

INTRODUCTION

Early embryonic death is the main cause of pregnancy failure in cows. The incidence of repeat breeding, a major factor involved in reduced fertility, varies, ranging from 18 to 24% (Stevenson et al., 1990). About 40% of the repeat-breeding (RB) cows experience early embryonic losses (Thatcher et al., 1994). Several factors have been suggested to be responsible for reduced conception rate including management practices, genetics, diseases, physiological disturbances, anatomical defects, feeding practices, estrus detection errors, embryonic mortality (Dekruif, 1978; Roussel et al., 1988; Wathes, 1992; Jainudeen and Hafez, 1993; Heuwieser et al., 1997), and heat stress (Badinga et al., 1985). The involvement of progesterone (P4) in the maintenance of pregnancy and regulation of uterine $PGF_{2\alpha}$ secretion is well documented (Royal et al., 2000; Lucy, 2001). Progesterone stimulates endometrial gland growth and prepares the uterus to receive and nourish a new embryo. Insufficient luteal P4 has been implicated as a cause for abnormal embryonic development and early embryonic loss (Lamming et al., 1989; Mann et al., 1995; Ahmad et al., 1996). Kimura et al. (1987) reported that RB cows have lower P4 concentrations than cows with normal fertility during the first week after ovulation. Furthermore, Mann and Lamming (1995) found that cows with low P4 concentrations during the luteal phase of the estrous cycle had an enhanced surge of $PGF_{2\alpha}$ after oxytocin treatment on days 15 and 16 of the cycle. During that period, embryonic growth and differentiation are highly dependent on uterine secretions. Thus, delayed embryonic development in RB

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cows maybe related to insufficient P4 secretion during the first week post insemination (PI). Delayed embryo development may impair maternal recognition of pregnancy since the embryo will not be able to produce sufficient quantities of interferon τ to prevent $PGF_{2\alpha}$ secretion and luteolysis (Mann and Lamming, 1995; Mann et al., 1995; 1998).

Attempts to improve pregnancy rates using GnRH or gonadotropin to stimulate endogenous P4 secretion of the corpus luteum (CL) have been inconsistent. Instead, P4 supplementation has been advocated as an alternative strategy to improve pregnancy rate. Providing exogenous P4 at various regimens after breeding produced variable results, which were affected by the day of supplement and length of treatment. Excellent reviews on the subject have been published (Royal et al., 2000; Thatcher et al., 2001). Mann et al. (2001) reported that meta-analysis of 17 published P4 supplementation studies showed that pregnancy rates were increased when P4 treatment was initiated during the first week PI but not during the second or third weeks. However, most of these studies were carried out on dairy cattle without discrimination between normal and RB cows. Therefore, our objective was to determine the effect of P4 supplement during the periods of CL formation and development between days 2-7 and 7-12 following a spontaneous or $PGF_{2\alpha}$ induced estrus on P4 concentrations and pregnancy rates in RB cows.

MATERIALS AND METHODS

This study was conducted during the winter months (November 1, 2002 to January 31, 2003) in a private Friesian dairy farm in the Northeast part of Jordan (32°30'N, 35°51'E). Repeat breeders (RB) are usually defined as those cows that were inseminated at least three times and did not become pregnant. Cows within this farm were experiencing high rates of repeat breeding (24% with four or more inseminations and first service conception rate between 30 to 40%). Average daily milk production for this herd was 28 kg per cow. Body condition was scored using a five-point scale, where 1 = thin and 5 = obese (Edmondson et al., 1989). In this experiment, cows were in good body condition (BCS = 2.5-3), calved 120-240 d prior to the experiment, had at least three unsuccessful inseminations, and were in their first to seventh lactation. Cows were housed in free-stall barns provided with shade and were fed mixed ration (TMR) of 40% forage (corn silage and alfalfa hay) and 60% concentrate (whole cottonseed, barely, wheat bran, soybean meal, and commercial concentrate for lactation with trace minerals and vitamins) containing 7.118 MJ of NE_L/kg and 17.8% CP (percentage of DM) according to NRC recommendations (NRC, 1989). Cows had *ad libitum* access to fresh water.

All cows were rectally palpated to ensure normal reproductive tract and ovarian structures according to the procedure described by Mortimer et al. (1997). Estrus detection was performed by visual observation throughout the day. Cows were submitted for AI based mainly on standing estrus or on combination of two or more of the following indicators of estrus: 1) mounting activity, 2) redness and edema of external genitalia with clear vaginal mucus discharge, 3) nervousness and excessive vocalization, 4) rubbed tailhead with veterinary recommendation based on ovarian palpation per rectum. On the start of the experiment, routine biweekly veterinary visits were used to identify non pregnant cows 45 days after the last service. Cows fits the criteria described above (ie: at least 3 previous inseminations, calved 120-240 days, 1st to 7th lactation and had normal reproductive tract) were matched for lactation number and number of previous services after each veterinary visit. Once cows were stratified, they were assigned to one of five groups of ten cows each. Cows in group 1 (PGF-P4-d2; n = 10) and group 2 (PGF-P4-d7; n = 10) were administered i.m. with 25 mg of $PGF_{2\alpha}$ (Lutalyse, Phamacia & Upjohn S.A. Puurs- Belgium) when a mature corpus luteum (CL, more than 2 cm in diameter; Mortimer et al., 1997) was present and allocated into two induced-estrus groups. Cows in group 3 (S-P4-d2; n = 10), group 4 (S-P4-d7; n = 10) and group 5 (Control) were submitted to AI after spontaneous estrus. All cows in the five groups were inseminated artificially 12 h after detected estrus. Progesterone intravaginal releasing device impregnated with 1.55 g P4 without the estradiol capsule (PRID, CEVA, Sanofi, Sante Animale) were inserted either on d 2 in the PGF-P4-d2 and S-P4-d2 cows or d 7 post insemination in PGF-P4-d7 and S-P4-d7 cows and left in place for 5 days. Cows in group five served as a control with no P4 supplementation.

Blood samples were collected from all cows once daily for 4 days starting on the day of estrus and once every 3 days thereafter until d 22. Immediately after collection, blood samples were centrifuged at 3,000 g for 15 min and plasma was harvested and stored at -20°C until analyzed for P4. Plasma P4 concentrations were determined by a solid-phase radioimmunoassay (Immunotech a Coulter Company, Marseille, France) in one assay. The intra-assay coefficient of variation was 5.5%.

Pregnancy was determined based upon sustained P4 concentrations (P4>1 ng/ml) on d 19 and 22, because concentrations of P4 equal or exceeding 1 ng/ml were considered indicative of a functional CL and reconfirmed by transrectal palpation of the uterus on d 40. Pregnancy loss was considered to have occurred when cows diagnosed pregnant based upon P4 concentrations on d 19 and d 22 but were found not pregnant by rectal palpation on d 40.

Data were analyzed using statistical procedures of the

Table 1. Reproductive performance of cows treated with PRID on day 2 (PGF-P4-d2) or day 7 (PGF-P4-d7) after $PGF_{2\alpha}$ induced-estrus or cows treated with PRID on day 2 (S-P4-d2) or day 7 (S-P4-d7) and untreated control after spontaneous estrus

Treatment	Parameter					
	Service number	Lactation number	BCS[1]	Pregnancy rates based on		Pregnancy loss
	Mean±SEM	Mean±SEM	Mean±SEM	P4[2] %(n)	RP[3] % (n)	% (n)
PGF-P4-d2	3.8±0.32	3.5±0.40	2.73±0.04	60 (6/10)	50 (5/10)	16.6 (1/6)
PGF-P4-d7	3.8±0.32	3.9±0.38	2.69±0.03	80 (8/10)	50 (5/10)	37.5 (3/8)
S-P4-d2	3.7±0.33	3.6±0.43	2.67±0.04	30 (3/10)	20 (2/10)	33.3 (1/3)
S-P4-d7	4±0.33	3.7±0.37	2.71±0.03	60 (6/10)	50 (5/10)	16.6 (1/6)
Control	3.8±0.25	3.2±0.29	2.66±0.04	40 (4/10)	20 (2/10)	50 (2/40)

[1] Body condition score. [2] Based on serum P4 concentrations (>1 ng/ml) on d 19 and 22 post AI.
[3] RP: Rectal palpations at 40 d post AI.

Table 2. Pregnancy rates based upon P4 concentrations and rectal palpation of RB cows after data grouping according to lactation number, service number, nature of estrus, and day of P4 supplement

Grouping parameter		Pregnancy rate based on	
		P4[1] % (n)	Rectal palpation[2] % (n)
Lactation number	<3	60 (12/20)	45 (9/20)
	≥3	50 (15/30)	33.3 (10/30)
Service number	3	62.5 (15/24)	50 (12/24)
	≥4 (4.56±0.15)	46.1 (12/26)	26.9 (7/26)
Nature of estrus	$PGF_{2\alpha}$ induced estrus	70 (14/20)	50 (10/20)
	Spontaneous estrus	43.3 (13/30)	30 (9/30)
Progesterone	Day 2 supplement	45 (9/20)	35 (7/20)
	Day 7 Supplement	70 (14/20)	50 (10/20)
	No supplement	40 (4/10)	20 (2/10)

[1] Based on serum P4 concentrations (>1 ng/ml) on d 19 and 22 post AI.
[2] Rectal palpations at 40 d post AI.

SAS program (SAS/STAT. 1996). Data are presented as means±SEM in tables and figure. Categorical data including pregnancy rates depending on P4 levels and rectal palpation and pregnancy loss were analyzed using chi-square test. Effect of treatments on P4 concentrations over time was analyzed using repeated measures analysis of variance of the GLM. The model included treatments (PRID and $PGF_{2\alpha}$), lactation number, and number of previous services, pregnancy status, and their interactions.

RESULTS

Reproductive performance among groups is summarized in Table 1. Service number and lactation number did not differ significantly among groups. Proportion of cows that became pregnant based upon P4 concentrations and rectal palpation and rates of pregnancy loss were not different ($p>0.1$) among the treatment groups (Table 1). Proportions of cows that did not become pregnant based upon P4 concentrations were 40, 20, 70, 40, and 60% for groups PGF-P4-d2, PGF-P4-d7, S-P4-d2, S-P4-d7, and control, respectively. These cows returned to estrus after a regular interval of 20-22 days with the exception of 2 cows from S-P4-d2 group which exhibited estrus earlier than expected (<17 days).

For purpose of analysis, cows were grouped according to lactation number into young cows with one or two calving and older cows with three or more calving. Pregnancy rates were not different between the two lactation number groups (Table 2). Cows were additionally classified according to the number of their previous inseminations into cows with three previous services and cows with four or more previous services. In this regard, cows with three previous services tended ($p = 0.09$) to have higher in pregnancy rates than those with four or more previous services (Table 2).

Pregnancy rates were not influenced ($p>0.1$) by the nature of estrus ($PGF_{2\alpha}$-induced vs. spontaneous estrus). Therefore, cows were further grouped according to the day of P4 supplement into three groups: d 2 P4 supplement, d 7 P4 supplement and control groups. Pregnancy rates based upon P4 concentrations and rectal palpation were not different among the three groups (Table 2). Early pregnancy loss was not different among the three treatment groups.

Progesterone supplement by lactation number interactions did not affect pregnancy rates ($p>0.1$). On the other hand, number of previous inseminations influenced the effect of P4 supplement on pregnancy rates ($p = 0.03$; Table 3). No P4 effect was observed in cows with three previous services but a significant effect was observed in cows with four or more previous services. Unlike day 2, P4 supplement on day 7 improved pregnancy rates in cows

Table 3. Pregnancy rates based upon P4 concentrations and rectal palpation of RB cows after stratifying animals according to service number and interaction with day of P4 supplement

Progesterone treatment	Service number (Mean±SEM)	Pregnancy rate based on	
		P4[1] % (n)	Rectal palpation[2] % (n)
Day 2 supplement	3	54.5 (6/11)	54.5 (6/11)
	≥4 (4.67±0.29)	33.3 (3/9)	9.1 (1/9)
Day 7 Supplement	3	77.7 (7/9)	44.4 (4/9)
	≥4 (4.64±0.24)	63.6 (7/11)	54.5 (6/11)
Control	3	50 (2/4)	25 (1/4)
	≥4 (4.33±0.21)	33.3 (2/6)	16.6 (1/6)

[1] Based on serum P4 concentrations (>1 ng/ml) on d 19 and 22 post AI. [2] Rectal palpations at 40 d post AI.

Table 4. Mean±SEM of progesterone concentration in progesterone treated and control groups

Day / Treat	0	1	2	3	7	10	13	16	19	22
PGF-P4-d2	0.01±0.01^{a}	0.04±0.02^{b}	0.96±0.40^{abc}	3.18±0.31^{a}	3.03±0.42^{ab}	3.62±0.45^{a}	4.15±0.52^{a}	3.97±0.45^{a}	4.26±0.50^{ac}	4.21±0.75^{ab}
PGF-P4-d7	0.14±0.09^{a}	0.16±0.10^{ab}	1.28±0.13^{bc}	1.74±0.16^{c}	3.11±0.39^{a}	3.79±0.67^{a}	4.05±0.78^{a}	5.49±1.08^{a}	5.61±0.62^{c}	6.36±1.11^{b}
S-P4-d2	0.07±0.05^{a}	0.28±0.12^{ab}	0.43±0.13^{a}	2.98±0.18^{a}	3.22±0.36^{a}	3.40±0.24^{a}	4.75±0.27^{a}	3.59±0.75^{a}	2.60±0.78^{a}	2.20±0.83^{a}
S-P4-d7	0.02±0.01^{a}	0.38±0.15^{a}	1.52±0.40^{b}	2.15±0.31^{c}	2.89±0.30^{ab}	3.48±0.58^{a}	5.18±0.24^{a}	5.11±0.66^{a}	3.89±0.79^{ac}	5.37±1.01^{b}
Control	0.03±0.01^{a}	0.20±0.09^{ab}	0.50±0.11^{a}	1.28±0.17^{c}	2.12±0.13^{b}	3.53±0.78^{a}	3.98±0.47^{a}	5.06±0.58^{a}	3.17±0.65^{a}	2.41±0.83^{a}

Within columns, values with similar scripts are not different; $^{a, b}$ $p<0.05$; $^{a, c}$ $p<0.01$; $^{b, c}$ $p<0.05$.

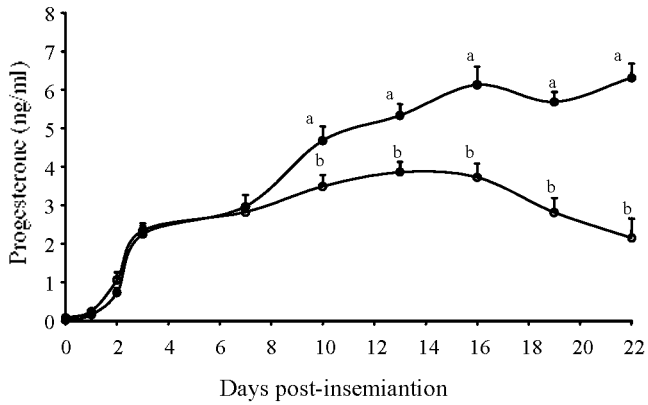

Figure 1. Mean plasma progesterone concentration±SEM in cows confirmed pregnant (●; n = 19) and non-pregnant (○; n = 31) based on rectal palpation. Values at the same day with different scripts are significantly different: $^{a, b}$ $p<0.05$.

with four or more previous services ($p = 0.03$) but not in cows with three previous services ($p = 0.12$).

Progesterone concentrations increased after estrus in all groups (Table 4) and were not affected by $PGF_{2\alpha}$ treatment, lactation number, service number or their interactions. During the treatment periods, P4 concentrations were generally higher in the P4-treated groups than the control. Progesterone concentrations were significantly higher in the P4 supplemented groups between days 2 and 7 but not between days 7 and 12 than control group (Table 4).

Data were grouped retrospectively to compare reproductive parameters between pregnant and non-pregnant cows. With the exception of P4 concentrations between d 10 and d 22 (Figure 1), parameters including parity and lactation stage were found to be similar.

DISCUSSION

The importance of P4 during early pregnancy is well documented (Lucy, 2001). This was supported by the higher P4 levels in cows confirmed pregnant by rectal palpation than nonpregnant cows starting on d 10 PI and thereafter until the end of the sampling period (Figure 1). Progesterone levels in PRID-treated cows during the treatment periods were generally higher than the control cows. Progesterone supplement led to a significant increase in P4 concentration during the period of CL formation (d 2-7) but not during the period of mature CL (d 7-12). These findings are in agreement with previous reports by Robinson et al. (1989) and Lynch et al. (1999). Such a low increase in P4 concentration induced by intravaginal P4 devices is typical of high-yielding dairy cows. A low increase (Stevenson and Mee, 1991; Chenault et al., 2003), moderate increase (1 ng/ml) above that in the control cows, has been reported previously in high milk producing cows treated with CIDR (Shaham-Albalancy et al., 2001), whereas a high increase (4.2 ng/ml; and 2.4 ng/ml) in plasma P4 concentrations was achieved by CIDR treatment in low producing ovariectomized cows (Stevenson and Mee, 1991; Van Cleeff et al., 1991). The insignificant increase in P4 concentrations is probably the results of the high individual variation and the possible suppressive effect of P4 supplement on endogenous P4 production.

Pregnancy rates were generally improved in P4 supplemented groups. However, this improvement was not significant, and might be related to the small sample size used in this experiment. Previous reports have met with mixed results. The reasons why P4 supplement did not have

consistent effects on pregnancy rates in cattle maybe related to animal selection criteria, and day and length of P4 supplement. In agreement with Thuemmel et al. (1992), the findings of the present study indicate that low fertility in RB cows may benefit from exogenous P4 supplementation on d 7 PI. In the current study, P4 supplement on d 7 PI significantly improved pregnancy rates in cows with four or more services. Therefore, this significant improvement was found in cows selected for poor conception rate and therefore the magnitude of differences in pregnancy rates between d 7 treated group and control was largest in the group of cows with the lowest fertility rates. In addition, the insignificant improvement in cows with three previous services maybe related to the relatively low but probably acceptable pregnancy rates (25%) achieved in the control group. However, this rate is lower than 30 to 40% conception rates recorded in first service cows found in this farm during summer (Alnimer and Lubbadeh, 2003) and winter seasons (Alnimer, 2005). Thus, failure of previous studies to demonstrate beneficial effect of P4 supplement maybe related to the experimental animal selection criteria and day of treatment. Treatment of cows on their third service, therefore animals not requiring treatment, may mask the improvement achieved in cows with luteal insufficiency. The results of the current study are in agreement with recent report on larger number of cows with improved pregnancy rates in cows with advanced lactation (Villarroel et al., 2004) and insignificant improvement in cows in their first (Stevenson and Mee, 1991), or second service after P4 supplement (Mann et al., 2001). Similar findings were reported with GnRH injection. While GnRH injections failed to increase pregnancy rates in normal heifers, GnRH improved pregnancy rates in repeat breeder cows (Lee et al., 1983).

Pregnancy losses before and after maternal recognition of pregnancy are believed to be a significant cause of RB. The improved pregnancy rates, although not significant, in PRID treated groups might be the results of rescuing weak embryos during the period of maternal recognition of pregnancy. Studies in cattle showed that P4 administration during the first week PI resulted in increased conceptus length 10-fold on d 14 (Garrett et al., 1988). Moreover, interferon τ production on d 16 is positively related to the circulating P4 levels (Mann et al., 1998). Mann and Lamming (1995) demonstrated that low plasma P4 concentration results in the development of stronger luteolytic signal and provides an explanation for the fact that cows with lower plasma concentrations of P4 are more likely to experience early embryonic loss. Therefore, P4 supplement might have enhanced embryonic growth and interferon secretion and weakened the luteolytic signal and hence accounts for the improved pregnancy rates.

Pregnancy rates based on P4 concentration were generally higher than pregnancy rates by palpation. The sustained increase in P4 levels on d 22 PI might represent established pregnancies that were lost before pregnancy diagnosis on d 40 PI. The number of non-pregnant cows with extended luteal phase was not different between the groups. Thus, the beneficial effect of P4 supplement was not extended beyond the period of maternal recognition of pregnancy and embryos were not capable of sustained development and maybe lost during the period of placentation. Progesterone supplemented groups were not at additional risk of pregnancy loss over control. However, because pregnancy loss after maternal recognition of pregnancy is a significant cause of RB, RB cows should be monitored closely for signs of heat until pregnancy can be confirmed by rectal palpation or ultrasonography.

In agreement with Lynch et al. (1999), PRID insertion on d 2 PI did not improve pregnancy rates. The early increase of P4 in the d 2 PRID supplemented estrous cycles might have activated mechanisms leading to precocious secretion of $PGF_{2\alpha}$ and therefore shortened estrous cycle. Shortened estrous cycle has been associated with early metestrus P4 supplement and therefore lower conception rates. Shortened estrous cycle was found only in two cows in the d 2 PRID group. Higher percentage of cows treated on d 2 with shorter inter-estrus interval was reported by Lynch et al. (1999) and accounted for the lower pregnancy rates in their study. The low percentage of cows with shortened estrous cycle reported in this study confirms the previously reported effect of P4 administrations during metestrus (Macmillan and Peterson, 1993; Burke et al., 1994). Limiting the period of treatment to 5 days could prevent the effect of P4 metestrus supplementation on length of the estrous cycle. However, P4 supplementation on d 2 cannot be advocated as a treatment for RB cows as only one of the cows with 4 or more previous services (1/9 cows) in the d 2 supplemented groups was pregnant on d 40 PI. This negative effect of P4 supplement on d 2 PI was not extended to cows with 3 previous inseminations (pregnancy = 6/11). The reasons for this difference between early and advanced lactation are not known. In lactating cows, luteal function and fertility can be affected by nutrition and metabolic factors. Metabolism and progesterone clearance has been found to vary according to the metabolic rates of cows (Butler, 2000). However, perusal of the available databases failed to yield any studies concerning any direct effect of stage of lactation on progesterone metabolism. The existence of a threshold in P4 concentration for embryo survival to occur is controversial. If such a threshold exists, P4 concentrations at the uterine levels and not that of the peripheral circulation should be measured. Reaching this threshold in a critical period could provide an explanation

for the improved pregnancy rates in the absences of significant increase in P4 concentrations found in this study.

In conclusion, post insemination short period progesterone supplement to repeat breeding cows with four or more services improved pregnancy rates and should be advocated when no specific reason for infertility is diagnosed. Further studies with larger numbers of repeat breeding cows under field conditions are needed to ascertain the findings of this study.

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REFERENCES

Ahmad, N., S. W. Beam, W. R. Butler, D. R. Deaver, R. T. Duby, E. Elder, J. E. Fortune, L. C. Griel, L. S. Jones, R. A. Milvae, L. Pate, I. Revah, D. T. Scheriber, D. H. Townson, P. C. W. Tasnag and E. K. Inskeep. 1996. Relationships of fertility to patterns of ovarian follicular development and associated hormonal profiles in dairy cows and heifers. J. Anim. Sci. 74:1943-1952.

Alnimer, M. A. 2005. Comparison of an oestrus synchronization protocol with Oestradiol Benzoate and PGF2α and insemination at detected oestrus to a timed insemination protocol (Ovsynch) on reproductive performance of lactating dairy cows. Reprod. Nutr. Dev. 45:699-708.

Alnimer, M. A. and W. Lubbadeh. 2003. Effect of Using Progesterone Releasing Intravaginal Device with Ovsynch Program on Reproduction in Dairy Cattle during Summer Season. Asian-Aust. J. Anim. Sci 16:1268-1273.

Badinga, L., R. J. Collier, W. W. Thatcher and J. Wilcox. 1985. Effects of climatic and management factors on conception rate in subtropical environment. J. Dairy Sci. 68:78-85.

Burke, C. R., M. Mihm, K. L. Macmillan and J. F. Roche. 1994. Some effects of prematurely elevated concentrations of progesterone on luteal and follicular characteristics during the oestrus cycle in heifers. Anim. Reprod. Sci. 35:27-39.

Butler, W. R. 2000. Nutritional interactions with reproductive performance in dairy cattle. Anim. Reprod. Sci. 60-61:449-457.

Chenault, J. R., R. E. Hornish, Y. C. Anderson, L. F. Krabill, J. F. Boucher and M. J. Prough. 2003. Concentrations of progesterone in milk of cows administered an intravaginal progesterone insert. J. Dairy Sci. 86:2050-2060.

Dekruif, A. 1978. Factors influencing the fertility of a cattle population. J. Reprod. Fertil. 54:507-518.

Edmonson, A. J., I. J. Lean, L. D. Weaver, T. Farver and G. Webster. 1989.Abody condition scoring chart for Holstein dairy cows. J. Dairy Sci. 72:68-78.

Garrett J. E., R. D. Geisert, M. T. Zavy and G. L. Morgan. 1988. Evidence for maternal recognition of early conceptus growth and development in beef cattle. J. Reprod. Fertil. 84:437-446.

Heuwieser, W., P. A. Oltenacu, A. J. Lednor and R. H. Foote. 1997. Evaluation of different protocols for prostaglandin synchronization to improve reproductive performance in dairy herds with low estrus detection efficiency. J. Dairy Sci. 80:2766-2774.

Jainudeen, M. R. and E. S. E. Hafez. 1993. Reproductive failure in females. In: E. S. E. Hafez, Reproduction in farm animals. 6th Ed., Lea and Febiger, Pheladelphia, 261-311.

Kimura, M. T., T. Nakao, M. Moriyoshi and K. Kawata. 1987. Luteal phase deficiency as a possible cause of repeat breeding in dairy cows. Br. Vet. J. 143:560.

Lamming, G. E., A. O. Darwash and H. L. Back. 1989. Corpus luteum function in dairy cows and embryo mortality. J. Reprod. Fertil. Suppl. 37:245-252.

Lee, C. N., E. Maurice, R. L. Ax, J. A. Ennington, W. F. Aoffman and M. D. Rown. 1983. Efficacy of gonadotropin – releasing hormone administered at the time of artificial insemination of heifers and postpartum and repeat breeder dairy cows. Am. J. Vet. Res. 44:2160-2163.

Lucy, M. C. 2001. Reproductive loss in high producing dairy cattle: where will it end? J. Dairy Sci. 84:1277-1293.

Lynch, P. R., K. L. Macmillan and V. K. Taufa. 1999. Treating cattle with progesterone as well as a GnRH analogue affects estrous cycle length and fertility. Anim. Reprod. Sci. 56:189-200.

Macmillan, K. L. and A. J. Peterson. 1993. A new intravaginal progesterone releasing device for cattle (CIDR-B) for oestrus synchronisation, increasing pregnancy rates and the treatment of postpartum anoestrus. Anim. Reprod. Sci. 33:1-25.

Mann, G. E. and G. E. Lamming. 1995. Progesterone inhibition of the development of the luteolytic signal in cows. J. Reprod. Fertil. 104:1-5.

Mann, G. E., G. E. Lamming and M. D. Fray. 1995. Plasma oestradiol and progesterone during early pregnancy in the cow and the effect of treatment with buserelin. Anim. Reprod. Sci. 37:121-131.

Mann, G. E., G. E. Lamming, R. S. Robinson and D. C. Wathes. 1998. The regulation of interferon τ production and uterine hormone receptors during early pregnancy. J. Reprod. Fertil. Suppl. 54:317-328.

Mann, G. E., P. Merson, M. D. Fray and G. E. Lamming. 2001. Conception rate following progesterone supplementation after sejk5cond insemination in dairy cows. Vet. J. 162:161-162.

Mortimer, R. G, P. W. Farin and R. D. Stevens. 1997. Reproductive examination of the non- pregnant cow (Ed. R. S. Youngquist). Current Therapy in Large Animal Theriogenology. Published By W.B Saunders Co., Philadelphia, USA. pp.268-275.

National Research Council. 1989. Nutrient Requirements of Dairy Cattle. 5th Ed. National Academy Sci. Washington, DC.

Robinson, N. A., K. E. Leslie and J. S. Walton. 1989. Effect of treatment with progesterone on pregnancy rate and plasma concentrations of progesterone in Holstein cows. J. Dairy Sci. 72:202-207.

Roussel, J. D., J. F. Beatty and K. Koonce. 1988. Gonadotropin releasing hormone therapy in functional infertility of dairy cattle. Theriogenology. 30:1115-1119.

Royal, M.D., G.E. Mann and A.P.F. Flint. 2000. Strategies for reversing the trend towards subfertility in dairy cattle. Vet. J.

160:53-60.

SAS Institute Inc. 1996. SAS/STAT User's Guide: Version 7 Edition. SAS. Institute Inc. Cary NC.

Shaham- Albalancy, A., Y. Folman, M. Kaim, M. Rosenberg and D. Wolfenson. 2001. Delayed effect of low progesterone concentrations on bovine uterine PGF2alpha secretion in the subsequent oestrus cycle. Reprod. 122:643-648.

Stevenson, J. S. and M. O. Mee. 1991. Pregnancy rates of Holstein cows after postinsemination treatment with a progesterone-releasing intravaginal device. J. Dairy Sci. 74:3849-3856.

Thatcher, W. W., F. Moreira, J. E. P. Santos, R. C. Matoyos, F. L. Lopes, S. M. Pancarci and C.A. Risco. 2001. Effects of hormonal treatments on reproductive performance and embryo production. Theriogenol. 55:75-89.

Thatcher, M. M., C. R. Staples, G. Desnoyers, D. Oldick and E. P. Shmitt. 1994. Embryo health and mortility in sheep and cattle. J. Anim. Sci. 72(suppl.3):16.

Thuemmel, A. E., F. C. Gwazdauskas, W. D. Whittier and M. L. McGilliard. 1992. Effect of progesterone supplementation in repeat breeder cattle on conception and plasma progesterone. J. Endocrinol. Invest. 15:393-396.

Van Cleeff, K. L., M. Drost and W. W. Thatcher. 1991. Effects of post insemination progesterone supplementation on fertility and subsequent estrous responses of dairy heifers. Theriogenol. 36:795-807.

Villarroel, A., A. Martino, R. H. Don Durant, F. Deleting and W. M. Sischo. 2004. Effect of post insemination supplementation with PRID on pregnancy in repeat- breeder Holstein cows. Theriogenol. 61:1513-1520.

Wathes, D. C. 1992. Embryonic mortality and the uterine environment. J. Endocr. 134:321-325.