Anim. Reprod., v.10, n.3, p.206-214, Jul./Sept. 2013

Relationships between growth of the preovulatory follicle and gestation success in lactating dairy cows

J.L.M. Vasconcelos[1,3], M.H.C. Pereira[1], M. Meneghetti[1], C.C. Dias[1], O.G. Sá Filho[1], R.F.G. Peres[1], A.D.P. Rodrigues[1], M.C. Wiltbank[2]

[1]Department of Animal Production, FMVZ-UNESP, Botucatu, SP, Brazil.
[2]Department of Dairy Science, University of Wisconsin, Madison, WI, USA.

Abstract

This report summarizes three studies conducted with lactating dairy cows aiming to increase pregnancy rates to fixed time artificial insemination (TAI) protocols. Experiment 1 was designed to determine if changing the timing of PGF2α treatment during an E2/P4-based program would affect fertility to TAI or fixed-time embryo transfer (TET). In experiment 2, pregnancy rates to AI were compared following synchronized ovulation using two protocols that have been developed to reduce the period between follicular wave emergence and TAI. The Ovsynch-type protocol utilizes GnRH to synchronize the follicular wave by inducing ovulation of a dominant follicle at the beginning of the protocol, and to synchronize ovulation at the end of the protocol allowing TAI. In contrast, E2/P4-based protocols utilize E2 products in the presence of P4 to induce atresia of antral follicles and synchronize emergence of a new follicular wave. At the end of E2/P4-based protocol another E2 treatment in the absence of P4 is used to induce LH release and synchronize ovulation and allow TAI. Experiment 3 was designed to determine whether increasing the length time interval with reduced circulating P4 (proestrus) would increase fertility in a TAI program that utilized E2 and P4 to synchronize ovulation of cycling, lactating dairy cows. The overall conclusions are that circulating concentrations of progesterone and estradiol prior to and circulating concentrations of progesterone following ovulation can affect fertility in cattle. In addition, small increases in P4 concentrations near the time of AI, due to lack of complete CL regression, result in reductions in fertility. Earlier treatment with PGF2α should allow greater time for CL regression, an increase in estradiol and subsequent reductions in circulating P4 that could be critical for fertility. Optimization of follicle size in TAI programs is clearly an intricate balance between oocyte quality, adequate circulating E2 near AI, and adequate circulating P4 after AI.

Keywords: fertility, proestrus lactating dairy cows.

Introduction

Hormonal treatments have been developed to synchronize the time of ovulation in dairy cattle, allowing successful fixed time artificial insemination (TAI) without the need for detection of estrus (Pursley *et al*., 1997).

In dairy cows a variety of methods have been evaluated to increase fertility during synchronization of ovulation programs including: increasing progesterone (P4) concentration during ovulatory follicle development (Bisinotto *et al*., 2010; Martins *et al*., 2011; Wiltbank *et al*., 2011), increasing length of proestrus (Peters and Pursley, 2003; Pereira *et al*., 2013), reducing follicle age (Cerri *et al*., 2009; Santos *et al*., 2010), supplementing estrogen (E2) during proestrus (Cerri *et al*., 2004; Brusveen *et al*., 2009; Souza *et al*., 2011) or increasing P4 after AI (Demetrio *et al*., 2007; Lonergan, 2011).

This report summarizes three studies conducted with lactating dairy cows aiming to increase pregnancy rates to TAI protocols.

Materials and Methods and Results

Experiments

Experiment 1 (Pereira *et al*., 2013) was designed to determine if changing the timing of PGF2α treatment during an E2/P4-based program would affect fertility to TAI or fixed-time embryo transfer (TET). The experiment was conducted on a total of 1,058 lactating Holstein cows at eleven commercial dairy farms in Paraná State, Brazil. Within each farm, cows were randomly assigned to receive one of the following treatments for synchronization of ovulation: 1) an intravaginal P4 device containing 1.9 g of P4 (CIDR®, Zoetis, São Paulo, Brazil) and 2.0 mg (i.m.) estradiol benzoate (Estrogin® - Farmavet, São Paulo, Brazil) on day 0, 25 mg (i.m.) dinoprost tromethamine (Lutalyse®, Zoetis) on day 7 or 8, CIDR removal and 1.0 mg (i.m.) of estradiol cypionate (ECP®, Zoetis) on day 8. On day 8, cows were randomly assigned to receive either TAI

[3]Corresponding author: vasconcelos@fmvz.unesp.br
Received: May 7, 2013
Accepted: July, 29, 2013

on day 10 (48 h after ECP; n = 406) or TET on day 17 (n = 652). All TET cows received 100 µg (i.m.) of gonadorelin (Fertagyl, MSD Animal Health, São Paulo, Brazil) at time of ET (Vasconcelos *et al*., 2011). In a subgroup of cows (n = 444) in both groups, ovaries were evaluated by transrectal ultrasonography (Aloka SSD-500 with a 7.5-MHz linear-array transducer, Aloka, Tokyo, Japan) on day 10 to measure the diameter of the largest follicle present prior to ovulation. Milk production was measured daily between days 10 and 17, and average daily production through this interval was used in the analysis.

Table 1 shows the effect of treatment on pregnancies at days 28 and 60 (P/AI and P/ET). There was a clear effect of breeding technique (AI *vs*. ET) and treatment (PGF2α on day 7 *vs*. day 8) on results of both days 28 and 60 of pregnancy. However, there was no significant interaction between breeding technique and treatment at either pregnancy diagnosis. Treatment with PGF2α on day 7 increased fertility, compared to treatment on day 8, at day 28 of pregnancy. There was no effect of day of PGF2α treatment on pregnancy loss, while pregnancy loss was higher following TET regardless of whether they received PGF2α on day 7 or 8.

Table 1. Pregnancy per AI (TAI) or embryo transfer (TET) on days 28 and 60 and pregnancy losses for lactating dairy cows receiving $PGF_2α$ treatment on days 7 or 8 during an E2/P4-based synchronized ovulation program.

Breeding Technique	Protocol	Pregnancy[1] day 28	Pregnancy[1] day 60	Pregnancy loss[1]
TAI	PGF2α day 7	32.9 (87/238)	30.0 (81/238)	7.7 (6/87)
	PGF2α day 8	20.6 (42/168)	19.2 (40/168)	5.5 (2/42)
TET	PGF2α day 7	47.0 (116/243)	37.9 (95/243)	20.4 (21/116)
	PGF2α day 8	40.7 (100/244)	33.5 (83/244)	19.4 (17/100)
P - values				
Breeding technique (AI *vs*. ET)		0.0006	0.021	0.012
Treatment (day 7 *vs*. day 8)		0.004	0.016	0.676
Breeding technique x Treatment		0.355	0.308	0.872

[1]Each value includes least-squares means % (no./no.).

There was no effect of treatment on the mean P4 concentration at the time of PGF2α injection or on follicle size on day 10 (time of TAI). Figure 1 shows the differences between treatment groups in the distribution of P4 concentration on day 10. Treatment with PGF2α on day 7 increased the percentage of cows with very low P4 concentrations on day 10 (≤0.09 ng/ml). Delaying PGF2α treatment until day 8 increased the percentage of cows with P4 concentrations between 0.1 and 0.21 ng/ml but had no effect on the percentage of cows with P4 > 0.21 ng/ml.

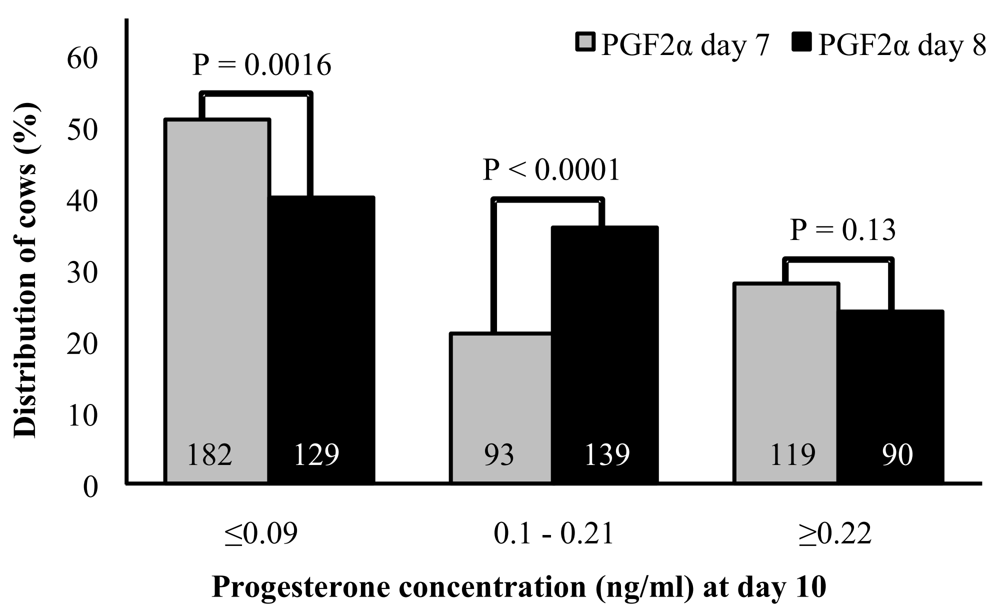

Figure 1. Effect of treatment with $PGF_2α$ on day 7 (n = 394) or day 8 (n = 358) on the distribution of cows with different progesterone concentrations at day 10 (at AI or 7 days before ET).

The fertility of cows based on the 60 day pregnancy diagnosis was influenced by P4 concentration on day 10 as shown in Table 2. At the 28 day pregnancy diagnosis in cows that received TAI, the lowest P4 concentrations on day 10 (≤0.09 ng/ml) resulted in the greatest P/AI (34.1%) with a 13.9% decrease in P/AI for cows with P4 levels of 0.10 to 0.21 ng/ml (68.8% relative difference) and a 12.7% decrease in P/AI for cows with P4 levels ≥0.21 ng/ml (59.4% relative decrease). In contrast, cows that received TET did not show a difference in P/ET when P4 levels of ≤0.09 ng/ml were compared to 0.1 to 0.21 ng/ml. However, there was a decrease of 20.9% in P/ET at day 60 in cows with P4 levels ≥0.21 ng/ml on day 10.

Table 2. Effect of progesterone concentrations on day 10 (at AI or 7 days before ET) on day 60 of pregnancy in lactating dairy cows after fixed timed artificial insemination (TAI) or timed embryo transfer (TET).

Item[1]	Progesterone (ng/ml) on day 10			P - value
	≤0.09	0.10 - 0.21	≥0.22	
TAI P/AI at day 60				
PGF2α day 7	39.4 (36/85)	27.5 (8/26)	24.0 (12/45)	_
PGF2α day 8	23.2 (15/54)	15.1 (8/45)	14.6 (4/22)	_
Combined[2]	34.1 (51/139)[ax]	20.2 (16/71)[b]	21.4 (16/67)[by]	0.05
TET P/ET at day 60				
PGF2α day 7	46.8 (37/77)	44.2 (23/52)	25.3 (12/49)	_
PGF2α day 8	40.0 (24/58)	46.0 (33/73)	20.5 (9/50)	_
Combined[2]	43.8 (61/135)[a]	45.3 (55/125)[a]	22.9 (21/99)[b]	0.0006

[1]Each value includes least-squares means % (no./no.). [2]Combined values of treatments to determine the effect of progesterone at day 10 on P/AI or P/ET. [a,b]Values with different superscripts in the same row differ ($P < 0.05$). [x,y]Values with different superscripts in the same row tended to differ ($P > 0.05$ and $P \leq 0.1$).

In experiment 2 (Pereira *et al*., Department of Animal Production, FMVZ-UNESP, Botucatu, SP, Brazil; unpublished data) pregnancy success to AI were compared following synchronized ovulation using two protocols that have been developed to reduce the period between follicular wave emergence and TAI. The Ovsynch-type protocol utilizes GnRH to synchronize the follicular wave by inducing ovulation of a dominant follicle at the beginning of the protocol, and to synchronize ovulation at the end of the protocol allowing TAI. In contrast, E2/P4-based protocol utilizes E2 products in the presence of P4 (in our study 2.0 mg EB) to induce atresia of antral follicles and synchronize emergence of a new follicular wave. At the end of E2/P4-based protocol another E2 treatment (in our study 1.0 mg ECP) in the absence of P4 is used to induce LH release and synchronize ovulation and allow TAI.

This study used a total of 1,190 lactating Holstein cows. Within each farm (n = 4), cows were blocked by parity (primiparous and multiparous) before randomization. Within each block cows were randomly assigned to receive one of two treatments: 1) The 5-days Cosynch protocol consisting of an intravaginal progesterone device containing 1.9 g of P4 (CIDR), and 100 µg i.m. of gonadorelin (Fertagyl), five days later the CIDR was removed and cows received PGF2α i.m. (Lutalyse), a second i.m. PGF2α was performed 24 h later, the final GnRH treatment was administered and TAI was performed 48 h after the second PGF2α i.e. 72 h after CIDR removal; and 2) The E2/P4 protocol consisted of CIDR insertion and 2.0 mg i.m. of EB (Estrogin), 7 days later 25 mg of i.m. PGF2α (Lutalyse), 24 h later the CIDR was removed and cows received 1.0 mg i.m. of estradiol cypionate (ECP), and 48 h after CIDR removal TAI was performed. Cows were scanned on days -10, -3, and 0. Milk production was measured daily between days 0 and 7, and average daily production through this interval was used in the analysis. Cows were considered to have the estrous cycle synchronized when P4 was ≥1.0 ng/ml on day 7 and luteolysis was considered to have occurred when P4 ≤ 0.4 ng/ml on day 0.

Table 3 shows the effect of treatments on luteolysis, estrus detection, estrous cycle synchronization, P/AI, and pregnancy loss between days 32 and 60. The proportion of cows that had luteolysis did not differ between treatments. The cows in the E2/P4 protocol were more likely to be detected in estrus compared with 5-days Cosynch protocol. A greater percentage of cows in the 5-days Cosynch protocol had their estrous cycles synchronized, compared with cows in the E2/P4 protocol. When all cows were included in the analysis, P/AI on day 32 was not affected by treatment, but there was a tendency (P = 0.07) for cows in the E2/P4 protocol to have greater P/AI on day 60 after AI compared with cows in the 5-days Cosynch protocol. Percentage of cows that had pregnancy loss between days 32 and 60 after AI was lower in the E2/P4 program compared with the 5-days Cosynch program. In cows that had their estrous cycle synchronized, the E2/P4 protocol had greater P/AI on day 60 after AI and lower pregnancy loss between days 32 and 60 compared with cows in the 5-days Cosynch protocol.

Table 3. Luteolysis, estrus detection, estrous cycle synchronization, P/AI at days 32 and 60 and pregnancy loss for lactating dairy cows receiving 5-day Cosynch or E2/P4 protocol.

Item[1]	Treatment		P - value
	5-day Cosynch	E2/P4	
Luteolysis[2]	91.4 (389/424)	90.6 (380/418)	0.67
Estrus detection	43.4 (253/597)	62.8 (375/593)	<0.01
Estrous cycle synchronization[3]	78.2 (287/389)	70.7 (250/380)	0.02
P/AI			
At day 32	20.5 (119/597)	23.2 (135/593)	0.25
At day 60	16.7 (95/597)	20.7 (119/593)	0.07
Pregnancy loss	19.6 (24/119)	11.0 (16/135)	0.05
P/AI[3]			
At day 32	23.0 (66/287)	28.0 (70/250)	0.18
At day 60	17.7 (51/287)	25.6 (64/250)	0.03
Pregnancy loss	21.7 (15/66)	6.7 (6/70)	0.01

[1]Least squares means % (No./No.). [2]Cows that had luteolysis (P4 ≤0.4 ng/ml at day 0). [3]Cows that had luteolysis and their estrous cycle synchronized (P4 ≥1.0 ng/ml at day 7).

On the day of PGF injection, the P4 concentrations were greater for 5-days Cosynch protocol (2.7 ± 0.13 ng/ml) compared with E2/P4 protocol (1.7 ± 0.13 ng/ml). The follicle diameter at AI (day 0) had an effect on estrous cycle synchronization in both treatments, within larger follicles associated with greater estrous cycle synchronization. The data for P/AI by follicle diameter were evaluated only in cows that had their estrous cycle synchronized. In the 5-days Cosynch protocol P/AI at 32 days and day 60 were linearly associated with the follicle diameter while in the E2/P4 program, the effect was curvilinear; there was a decreased P/AI at days 32 and 60 with very small and very large follicles (Fig. 2A and B). The follicle diameter affected pregnancy loss in the 5-days Cosynch protocol, with smaller follicles resulting in greater pregnancy loss and between days 32 and 60, but there was no effect in the E2/P4 protocol (Fig. 3).

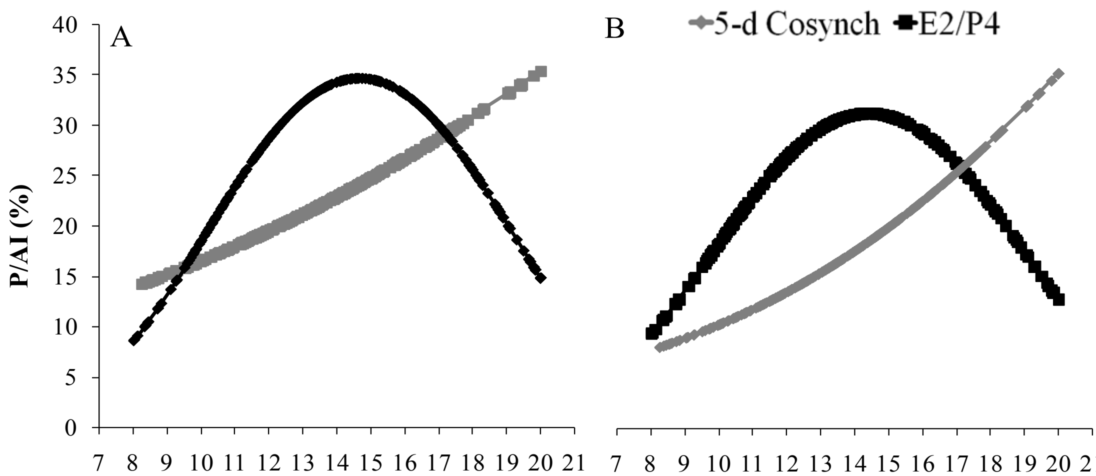

Figure 2. Effect of follicle diameter at AI (day 0) on P/AI at days 32 (Panel A) and 60 (Panel B) in cows that had their estrous cycle synchronized (P4 ≤0.4 ng/ml at day 0 and P4≥ 1.0 ng/ml at day 7) receiving E2/P4 or 5-day Cosynch protocols.

Expression of estrus improved the percentage of cows that had their estrous cycle synchronized (no estrus = 63.8% [220/357], estrus = 83.4% [317/387]) independent of treatment. Expression of estrus was associated with increased P/AI at 32 days (no estrus = 16.2% [89/562], estrus = 26.5% [165/628]) and 60 days (no estrus = 13.3% [71/562], estrus = 23.1% [143/628]) pregnancy diagnosis, independent of treatment. There was a tendency for greater pregnancy loss from 32 to 60 days in cows that did not show estrus (no estrus = 19.7% [18/89] as compared to those that showed estrus = 12.4% [22/165]) independent of treatment. In cows that had their estrous cycle synchronized, expression of estrus was associated with an increase in P/AI at 32 days (no estrus = 20.9% [46/220], estrus = 28.4% [90/317]) and 60 days (no estrus = 17.3% [38/220], estrus = 24.3% [77/317]) pregnancy diagnosis, independent of treatment. No difference was observed for pregnancy loss from 32 to 60 days for cows detected or not in estrus (estrus = 13.2% [13/90]; no estrus = 16.5% [8/46]).

Experiment 3 (Pereira *et al*., Department of Animal Production, FMVZ-UNESP, Botucatu, SP, Brazil; unpublished data) was designed to determine whether increasing the length time interval with reduced circulating P4 (proestrus) would increase fertility in a TAI program that utilized E2 and P4 to synchronize ovulation of cycling, lactating dairy cows. The study used a total of 759 lactating Holstein cows. Within each farm (n = 3), cows were blocked by parity (primiparous and multiparous), all non-pregnant cows that had passed the voluntary waiting period for the farm were utilized and randomized into the study, without regard to whether they had been previously utilized in the study. Within each block, 1,101 cows were scanned to determine the presence of a detectable CL (day -11). Cows with a CL (n = 759) were randomly assigned to receive one of two treatments: 1) an intravaginal progesterone insert containing 1.9 g of P4 (CIDR), and 2.0 mg (i.m.) estradiol benzoate (Estrogin) on day -10, 25 mg (i.m.) dinoprost tromethamine (Lutalyse) on day -3, CIDR withdrawal and 1.0 mg (i.m.) of estradiol cypionate (ECP) on day -2, and TAI on day 0 (treatment 8 days), or 2) CIDR insert + 2.0 mg (i.m.) of EB on day -11, Lutalyse on day -4, CIDR withdrawal + 1.0 mg of ECP on day -2, and TAI on day 0 (treatment 9 days).

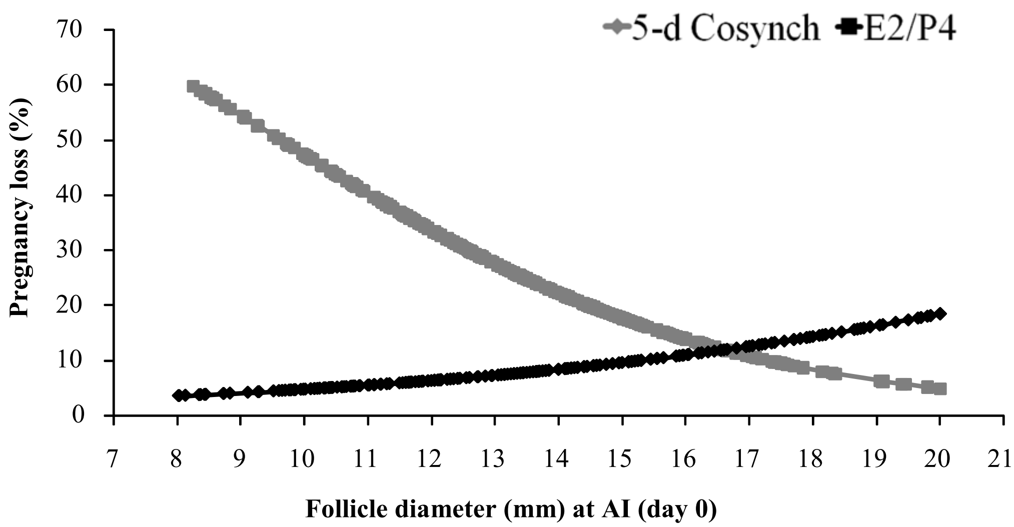

Figure 3. Effect of follicle diameter at AI on pregnancy loss between 32 day and 60 day in cows that had their estrous cycle synchronized (P4 ≤0.4 ng/ml at day 0 and P4 ≥1.0 ng/ml at day 7), receiving E2/P4 or 5-day Cosynch protocols.

The use of only lactating cows with a CL at the beginning of the protocol during the cooler months of the year, resulted in a high percentage of cows that responded to the protocols with ovulation (>90%) and high fertility (>40%). We postulated that increasing the length of time from PGF2a treatment to TAI by 1 day would allow more time for follicle development and thereby increase expression of estrus and follicle diameter at the time of AI. Table 4 shows the effect of treatments on detection of estrus, ovulation to the protocol, P/AI at days 32 and 60, and pregnancy loss for lactating dairy cows receiving protocols of 8 or 9 days duration. The cows in the 9 days protocol were more likely to be detected in estrus compared with the 8 days protocol. However, >90% of cows had their estrous cycles synchronized to either protocol with no difference between groups. In cows with their estrous cycle synchronized, both protocols resulted in a high P/AI at days 32 (~48%) and 60 (>40%) of pregnancy, with no difference between protocols. Nevertheless, increasing the length of the protocol from 8 to 9 days reduced the pregnancy losses that occurred between days 32 and 60 of pregnancy.

Although treatment did not have an effect on average follicle diameter at TAI, expression of estrus (Table 5) increased the percentage of cows with a CL on day 7, increased circulating P4 concentrations on day 7, and increased P/AI at days 32 and 60 of pregnancy in synchronized cows regardless of treatment. There was no interaction detected between expression of estrus and treatment for any of these variables. A greater pregnancy loss from 32 to 60 days was observed in cows that did not show estrus.

Follicle diameter at TAI affected P/AI at the 32 and 60 days pregnancy diagnoses in cows that ovulated to the protocol, independent of whether cows were detected in estrus (Fig. 4). In cows not detected in estrus, the follicle diameter had an effect on pregnancy loss (Fig. 5), however, there was no effect of follicle diameter on pregnancy loss from 32 to 60 days in cows that were detected in estrus.

Table 4. Treatment effects on estrus detection, estrous cycle synchronization, and P/AI at days 32 and 60 after TAI and pregnancy loss in cows receiving an 8 or 9 day synchronization protocol.

Item[1]	Protocol length		P - value
	8 day	9 day	
Estrus detection	63.4 (240/385)	73.0 (269/374)	<0.01
Estrous cycle synchronization[2]	92.8 (352/379)	91.5 (339/370)	0.52
P/AI			
At day 32	45.0 (175/385)	43.9 (166/374)	0.77
At day 60	38.1 (150/385)	40.4 (154/374)	0.52
Pregnancy Loss	14.7 (25/175)	7.6 (12/166)	0.04
P/AI[2]			
At day 32	48.1 (170/352)	47.9 (163/339)	0.96
At day 60	40.5 (145/352)	43.9 (151/339)	0.37
Pregnancy loss	15.2 (25/170)	7.8 (12/163)	0.03

[1]Least squares means % (no./no.). [2]Cows that had their estrous cycle synchronized in response to the protocol (CL at day 7).

Table 5. Treatments effects by estrus detection on distribution, estrous cycle synchronization, P/AI at 32 and 60 day and pregnancy loss from 32 to 60 day in cows that had their estrous cycle synchronized, receiving 8 or 9 day protocol length. Results are reported as least-squares means.

Item[1]	Estrus		P - value
	No	Yes	
Distribution			
8 days	38 (145/385)	62 (240/385)	_
9 days	28 (105/374)	72 (269/374)	_
P - value	<0.01	<0.01	
Ovulatory follicle diameter (mm)	14.6 ± 0.37	14.8 ± 0.33	0.69
Estrous cycle synchronization[1]	81.0 (202/248)	97.4 (489/501)	<0.01
P4 day 7[2]	2.77 ± 0.17	3.22 ± 0.16	<0.01
P/AI[2]			
At day 32	39.4 (81/202)	51.2 (252/489)	<0.01
At day 60	31.1 (66/202)	46.3 (230/489)	<0.01
Pregnancy loss	19.8 (15/81)	9.3 (22/252)	<0.01

[1]Least squares means % (no./no.). [2]Cows that had their estrous cycle synchronized in response to the protocol (CL at day 7).

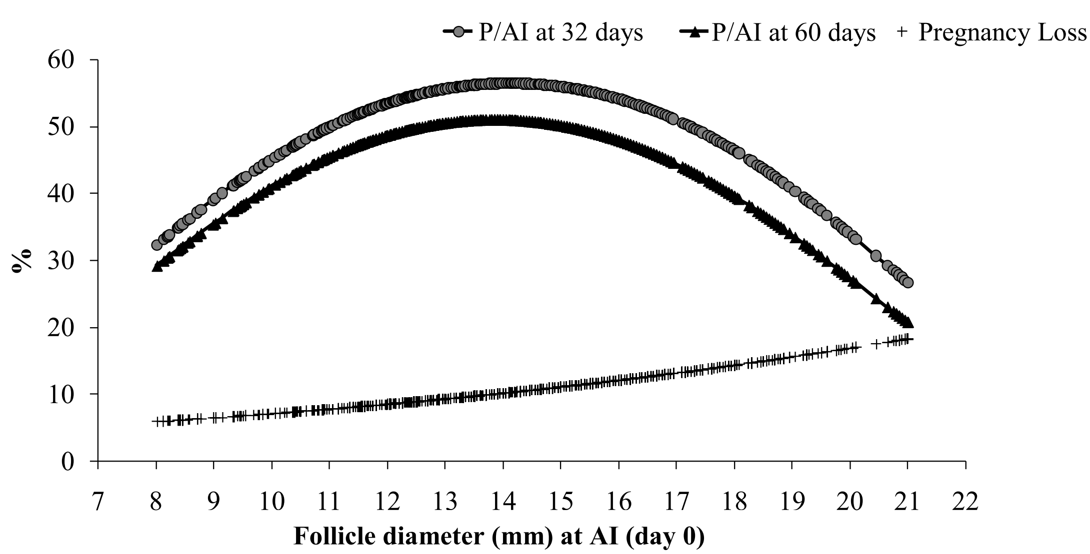

Figure 4. Effect of follicle diameter at day 0 on P/AI at 32 and 60 day and pregnancy loss in dairy cows that had their estrous cycle synchronized in response to the protocol (CL at day 7), receiving 8 or 9 day protocol length. 30 day, $P < 0.01$; 60 day, $P < 0.01$; Pregnancy loss, $P = 0.15$.

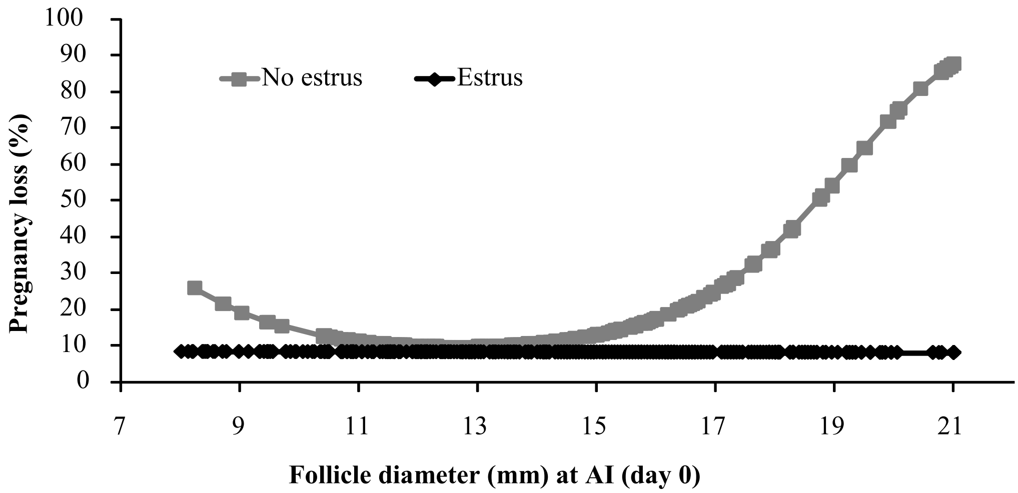

Figure 5. Effect of follicle diameter at AI (day 0) on pregnancy loss between days 32 and 60 in dairy cows that had their estrous cycle synchronized (CL at day 7), receiving an 8 or 9 day synchronization protocol length. No estrus, $P < 0.01$; estrus, $P = 0.97$.

Discussion

In experiment 1, anticipating the timing of the PGF2α treatment in cycling animals bred by TAI, increased pregnancy. Treatment with PGF2α on day 7 also reduced P4 concentrations at the time of P4 device removal/ECP treatment 24 h later. It seems that earlier PGF2α treatment improves fertility to TAI, potentially by increasing period of proestrus and reducing P4 near AI. Some studies have found that increasing the time of proestrus increased fertility. In *Bos taurus* beef cows the premature induction of an LH surge, when follicle diameter reached 10 mm, reduced fertility compared to cows allowed to proceed to spontaneous estrus and ovulation (Mussard *et al*., 2007). Reducing the proestrus period from 2.25 day to 1.25 day resulted in more short luteal phases (35% [40] *vs*. 82% [38], respectively), and reduced fertility (50.0 *vs*. 2.6%, respectively) in another study (Bridges *et al*., 2010). Earlier treatment with PGF2α in an E2/P4-based TAI program for beef cattle resulted in significant improvements in fertility (Meneghetti *et al*., 2009; Peres *et al*., 2009). Cows with reduced circulating P4 concentrations at the time of P4-intravaginal device removal had higher fertility to TAI than cows with higher P4 at the time of P4-intravaginal device removal (Dias *et al*., 2009; Meneghetti *et al*., 2009; Peres *et al*., 2009). In dairy cattle, reducing the proestrus length from 36 to 0 h during an Ovsynch program reduced P/AI (linearly from 8.8% at 0 h; 13.2% at 12 h; 21.4% at 24 h; 28.0% at 36 h) as the proestrus period increased and with increasing size of the ovulatory follicle, and a tendency for reduced short luteal phases (Peters and Pursley, 2003). In grazing lactating dairy cows presynchronized with the Presynch program (n = 1,754), an increase in proestrus period from Cosynch at 56 h to Cosynch at 72 h increased fertility at the 65 day pregnancy diagnosis (54.9 *vs*. 46.5%) but did not alter fertility at the 30 day pregnancy diagnosis and did not alter fertility at either pregnancy diagnosis in cows presynchronized with Double Ovsynch (Ribeiro *et al*., 2012). Most of these data are consistent with our results that increasing the length of proestrus increases fertility in TAI programs. It seems likely that increasing the length of proestrus increased E2 concentrations near the time of AI, reduced short luteal phases and increase P4 concentrations after AI (Vasconcelos *et al*., 2001).

In experiment 2, P/AI were increased with the E2/P4 protocol compared with the 5-day Cosynch protocol. Follicle diameter has been associated with P/AI in a number of studies. Ovulation of small follicles is associated with reduced P/AI, reduced E2 concentration, an increase in the incidence of short luteal phases (Vasconcelos *et al*., 2001), and increased pregnancy loss (Perry *et al*., 2005). A primary reason for the reduced P/AI in the 5-day Cosynch protocol was the high pregnancy loss in this group; pregnancy losses between days 32 to 60 of pregnancy was nearly twice as high as in the E2/P4 protocol. The potential explanation for these results is that circulating E2 concentrations were greater in the cows that had ovulation induced with ECP rather than GnRH. Although we did not measure circulating E2 in this trial, the physiological effects of elevated E2 are clearly manifest with greater expression of estrus in the cows in the E2/P4 protocol compared with the 5-day Cosynch protocol. Thus, we speculate that the improved P/AI and reduced pregnancy loss in the cows in the E2-based protocol resulted from increased circulating E2 concentrations near the time of TAI that may have a positive effect on fertilization, embryonic development, and subsequent pregnancy maintenance.

All of these data are consistent with the concept that greater circulating E2 near AI may reduce pregnancy loss at the later embryonic stages (32 to 60 days after AI). Consistent with this hypothesis, there was an increase in the percentage of cows detected in estrus with the longer compared to the shorter protocol in experiment 3 and detection of estrus was associated with greater synchronization and reduced pregnancy losses between 32 and 60 days. Our main hypothesis was that the longer protocol would result in greater fertility. Although, there was no overall change in P/AI, we observed a decrease in pregnancy loss between 32 and 60 days with the longer protocol. This result highlights the importance of pregnancy loss as a critical reproductive measure in lactating dairy cows, and the association that we detected between pregnancy loss and lack of estrus. Our study does not allow us to determine the mechanism that results in lower fertility and greater pregnancy loss in cows that did not demonstrate estrus. One possibility is that reduced E2 near TAI increases pregnancy loss as demonstrated in a recent study (Roberts *et al*., 2012). Ovariectomized cows that did not receive E2 in the preovulatory period maintained pregnancy until day 21, however, by day 29 reduced pregnancies were detected when compared to cows that received either ECP or EB to simulate the preovulatory period. In our study, ECP was given to increase circulating E2 in the preovulatory period; however, additional E2 from the follicle may be required to produce estrus and an optimal uterine environment. Alternatively, a greater time for CL regression and reduced circulating P4 near TAI, may increase expression of estrus and potentially improve pregnancy maintenance by enhancing the uterine environment. Development of more optimized protocols, potentially demonstrated by increased expression of estrus, should improve P/AI and reduce pregnancy losses.

In a recent study, single embryos were transferred into recipient beef cows induced to ovulate either small or large follicles using GnRH (Atkins *et al*., 2013; Jinks *et al*., 2013). Concentration of E2 at GnRH treatment in the recipient cows was one of the most important factors that determined pregnancy outcome (Atkins *et al*., 2013). Similarly, circulating E2 at GnRH-induced ovulation in the recipient cows, but not the donor cows, was predictive of pregnancy success at day 27 of gestation (Jinks *et al*., 2013). Further, administration of ECP 24 h before expected time of AI in recipients, increased pregnancy success in cows induced to ovulate a small dominant follicle (<12.2 mm). Thus, the primary benefit of increased preovulatory E2 is mediated through alterations in the maternal environment of the recipient cows. Whether inadequate E2 is responsible for reduced success in lactating dairy cows ovulating small follicles following an ECP-induced ovulation remains to be determined.

Alternatively, ovulation of very large follicle can also be associated with reduced fertility, possibly because of excessive length of dominant follicle persistence (Townson *et al*., 2002; Bleach *et al*., 2004; Cerri *et al*., 2009). An interesting paradox is that although increased circulating P4 after AI can improve fertility (Demetrio *et al*., 2007; Forro *et al*., 2012; Wiltbank *et al*., 2012), cows that ovulate larger follicles (>17 mm) had greater P4 concentrations on day 7 but lower P/AI at 60 day, compared to cows that ovulate follicles between 11 to 17 mm.

Conclusions

Circulating concentrations of progesterone and estradiol prior to and circulating concentrations of progesterone following ovulation can affect fertility in cattle. In addition, small increases in P4 concentrations near the time of AI, due to lack of complete CL regression, result in reductions in fertility. Earlier treatment with PGF2α should allow greater time for CL regression, an increase in estradiol, and subsequent reductions in circulating P4 that could be critical for fertility. Optimization of follicle size in TAI programs is clearly an intricate balance between oocyte quality, adequate circulating E2 near AI, and adequate circulating P4 after AI.

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