

**Increasing the Length of an E2/P4 Timed AI
Protocol with 2 CIDR improves pregnancy per AI
in Lactating Dairy Cows**

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Introduction

Our study group has worked intensively to improve P/AI in E2/P4 protocols in recent years. The studies show that the protocols must have:

- Proestrus period - Alterations regarding protocol length and time of PGF administration during ovulation synchronization protocols are associated with higher P/AI (Peters and Pursley, 2003; Ribeiro et al., 2012; Pereira et al., 2013a) and reduced pregnancy loss between 30 and 60 days of pregnancy (Pereira et al., 2014).
- Circulating P4 concentration at PGF - There are effects of increased circulating progesterone (**P4**) concentrations before AI on P/AI (Pereira et al., 2017b, 2019; Wiltbank et al., 2014).
- Circulating P4 concentration at AI – Reduced P4 concentrations at AI were clearly associated with fertility in TAI cows (Pereira et al., 2013; 2015)
- Estrus expression - Expression of estrus was associated with increased fertility and a reduction in pregnancy loss in protocols that use estradiol and progesterone (P4) to synchronize the time of ovulation (Pereira et al., 2016)

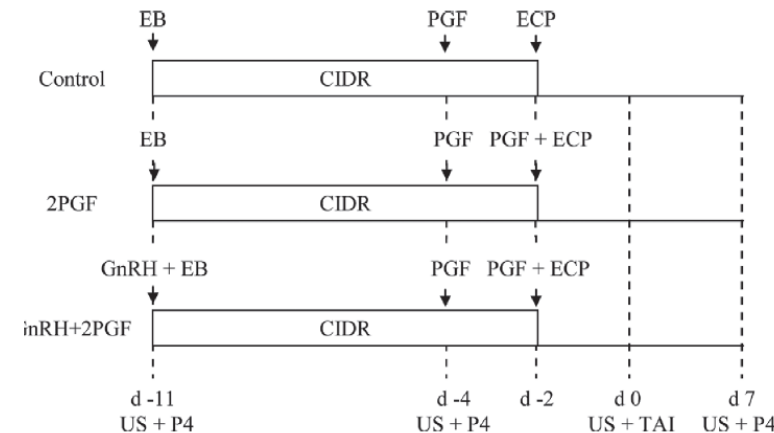
More important combination of these factors: Recent studies suggest that cows ovulating larger follicles in E2/P4-based ovulation synchronization protocols with a CL (Pereira et al., 2013b) or higher circulating P4 concentrations (Pereira et al., 2017a,b; 2019; Melo et al., 2016; Monteiro et al., 2015) at prostaglandin (**PG**) administration had greater P/AI.



Introduction

Effect of adding a gonadotropin-releasing-hormone treatment at the beginning and a second prostaglandin F_{2α} treatment at the end of an estradiol-based protocol for timed artificial insemination in lactating dairy cows during cool or hot seasons of the year

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	E2/P4	E2/P4+2PGF	GnRH+E2+2PGF	P=
Hot season				
P/AI 32d	41.0 (116/283) ^b	44.2 (125/283) ^y	50.9 (148/291) ^{a,x}	0.05
P/AI 60d	32.9 (93/283) ^b	36.4 (103/283) ^{a,b}	41.6 (121/291) ^a	0.09
Cold season				
P/AI 32d	19.0 (61/321)	21.8 (71/326)	23.4 (74/304)	0.40
P/AI 60d	16.2 (52/321)	18.7 (61/326)	19.1 (58/304)	0.59
Combined				
P/AI 32d	30.0 (177/604) ^b	33.2 (196/609) ^{b,y}	37.3 (219/595) ^{a,x}	0.02
P/AI 60d	25.1 (145/604) ^b	28.0 (164/609) ^{a,b}	31.0 (179/595) ^a	0.06



Comparison of 2 protocols to increase circulating progesterone concentration before timed artificial insemination in lactating dairy cows with or without elevated body temperature

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Timed artificial insemination programs during the summer in lactating dairy cows: Comparison of the 5-d Cosynch protocol with an estrogen/progesterone-based protocol

42% (596)

M. H. C. Pereira,* A. D. P. Rodrigues,* T. Martins,* W. V. C. Oliveira,† P. S. A. Silveira,‡ M. C. Wiltbank,§ and J. L. M. Vasconcelos†



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42.7% (492)

Effect of adding a gonadotropin-releasing-hormone treatment at the beginning and a second prostaglandin F_{2α} treatment at the end of an estradiol-based protocol for timed artificial insemination in lactating dairy cows during cool or hot seasons of the year

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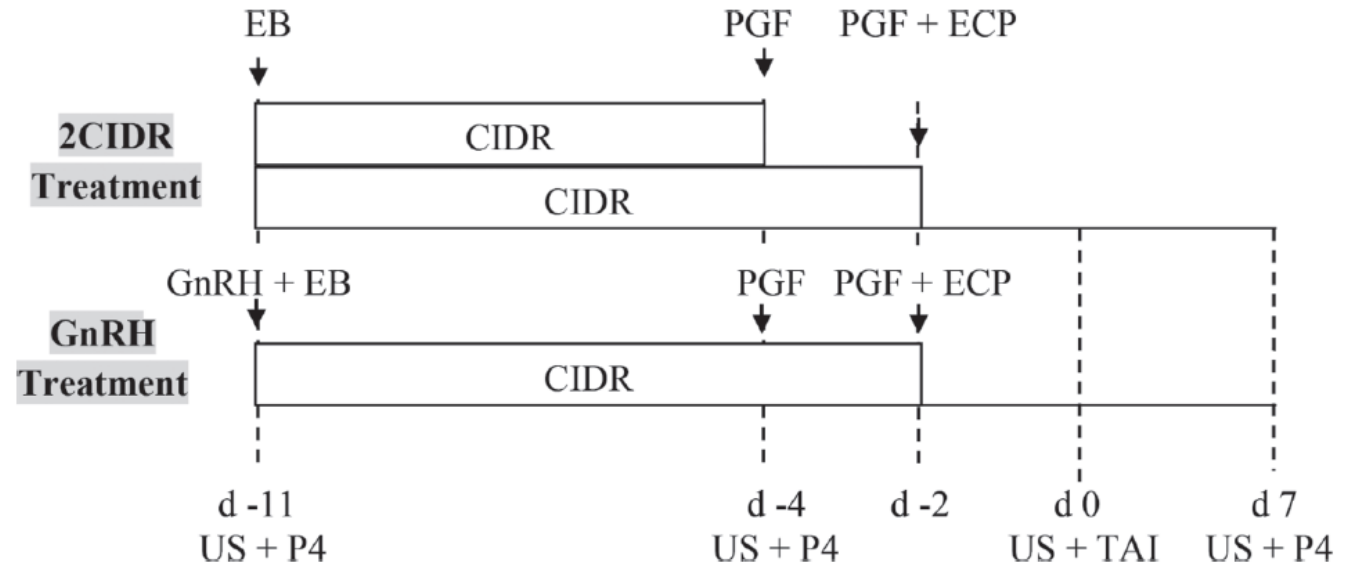
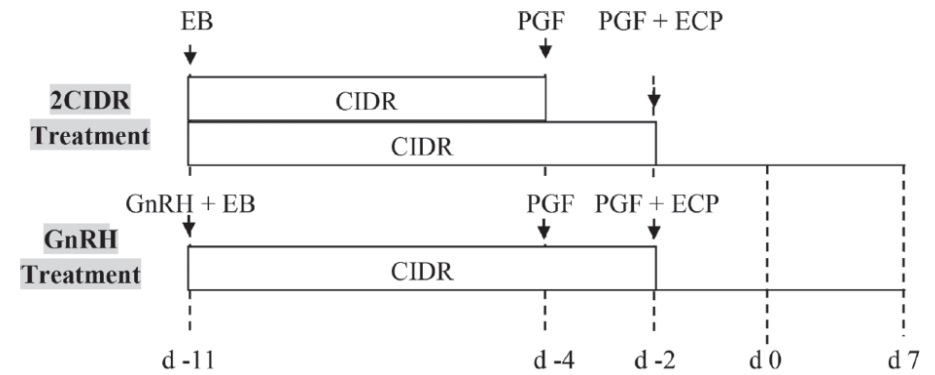


Figure 1. Diagram of activities. Ovaries were evaluated by ultrasound (US) in all cows on d -11, -4, 0 and 7. Cows received the following timed AI (TAI) protocol: an intravaginal progesterone (P4) insert containing 1.9 g of P4 (CIDR, Zoetis, São Paulo, Brazil) and 2.0 mg of estradiol benzoate (EB, i.m.; 2.0 mL of Estrogin, Farmavet, São Paulo, Brazil) on d -11; 25 mg (i.m.) of dinoprost tromethamine (PGF_{2α}; 5.0 mL of Lutalyse, Zoetis) on d -4; CIDR withdrawal, 1.0 mg (i.m.) of estradiol cypionate (ECP; 0.5 mL of ECP, Zoetis) and PGF_{2α} on d -2; and TAI on d 0. Cows were randomly assigned to receive 1 of 2 treatments: (1) GnRH treatment: 100-μg GnRH injection at d -11 (i.m.); 2.0 mL of Cystorelin, Merial, São Paulo, Brazil) or (2) 2CIDR treatment: an additional CIDR at d -11, which was removed at d -4. P4 = blood sampling and analysis of P4 concentration (sample on d -11, -4, and 7); US = ultrasonography of the ovaries (d -11, -4, 0, and 7).

Introduction

GnRH vs 2CIDR

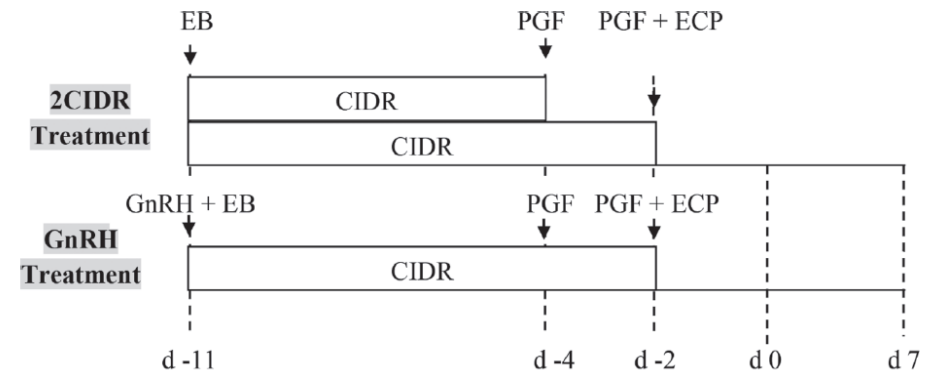


Effect of 2 protocols on synchronization and P/AI

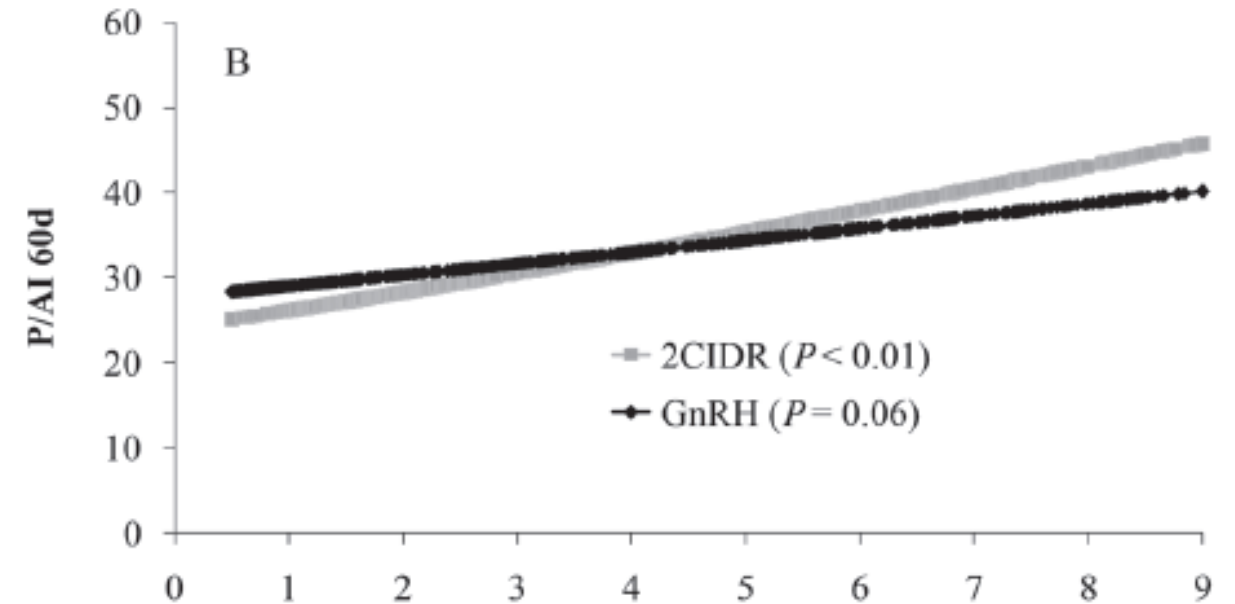
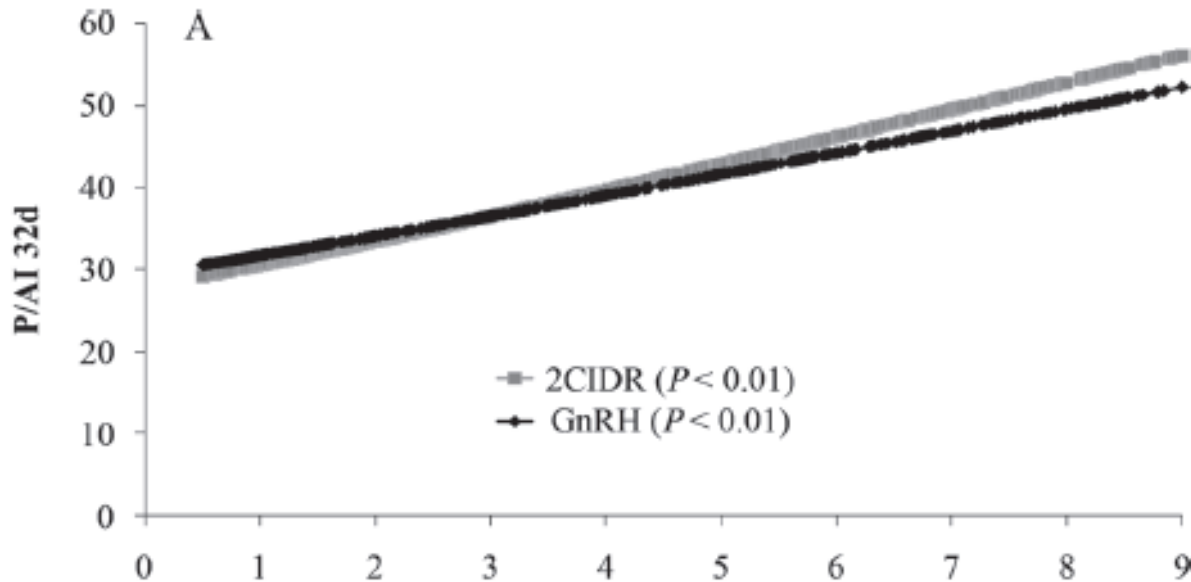
	GnRH	2CIDR	P=
No Heat stress			
Synchronzation	88.1 (355/402)	86.2 (359/415)	0.93
P/AI 32d	39.7 (162/402)	38.9 (164/415)	0.64
P/AI 60d	34.0 (138/402)	33.6 (141/415)	0.91
With Heat Stress			
Synchronzation	83.3 (304/361)	84.8 (298/348)	0.93
P/AI 32d	26.2 (98/361)	29.3 (105/348)	0.64
P/AI 60d	22.0 (81/361)	22.9 (81/348)	0.91

Introduction

GnRH vs 2CIDR



Effect of P4 at PGF on P/AI 32 e 60d



*Item	Tratamento		P =
	GnRH	2CIDR	
CL na PGF ³	73.7 (590/796)	56.5 (455/800)	<0.01
P4 d -4 (ng/mL) ³	3.95 ± 0.10	4.24 ± 0.10	0.03

Introduction



Contents lists available at ScienceDirect

Theriogenology

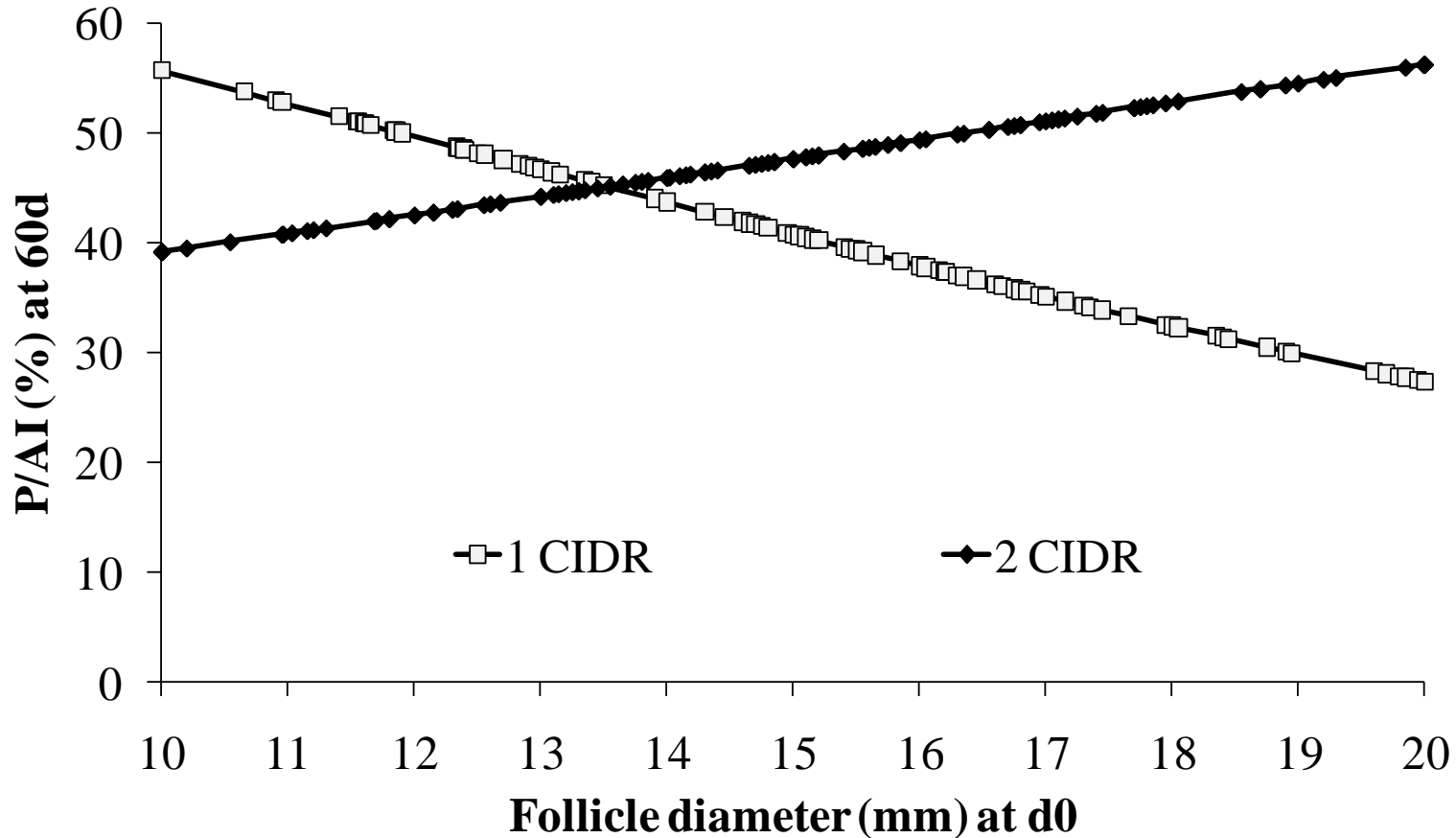
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Comparison of fertility following use of one versus two intravaginal progesterone inserts in dairy cows without a CL during a synchronization protocol before timed AI or timed embryo transfer

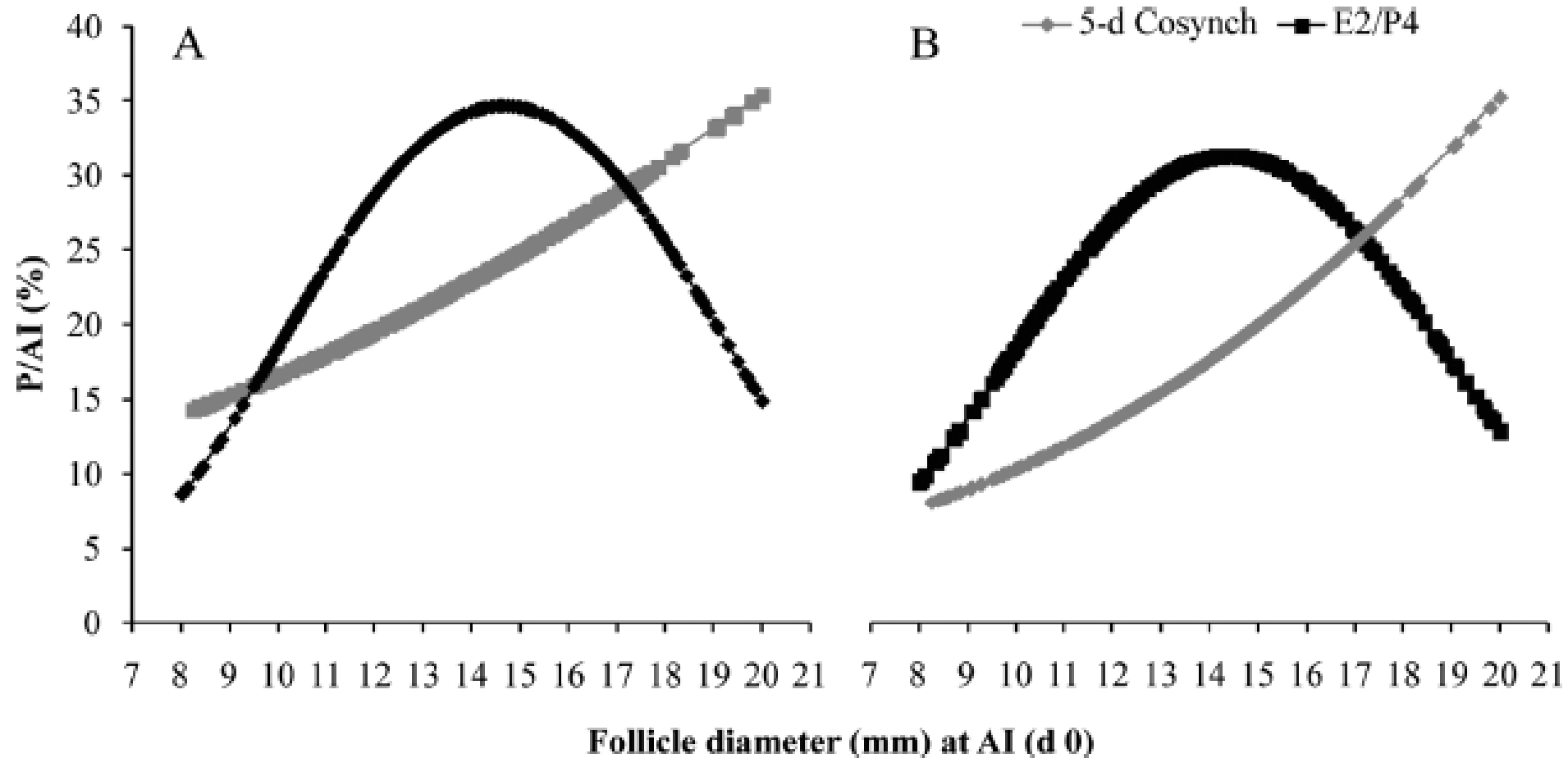


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Timed artificial insemination programs during the summer in lactating dairy cows: Comparison of the 5-d Cosynch protocol with an estrogen/progesterone-based protocol

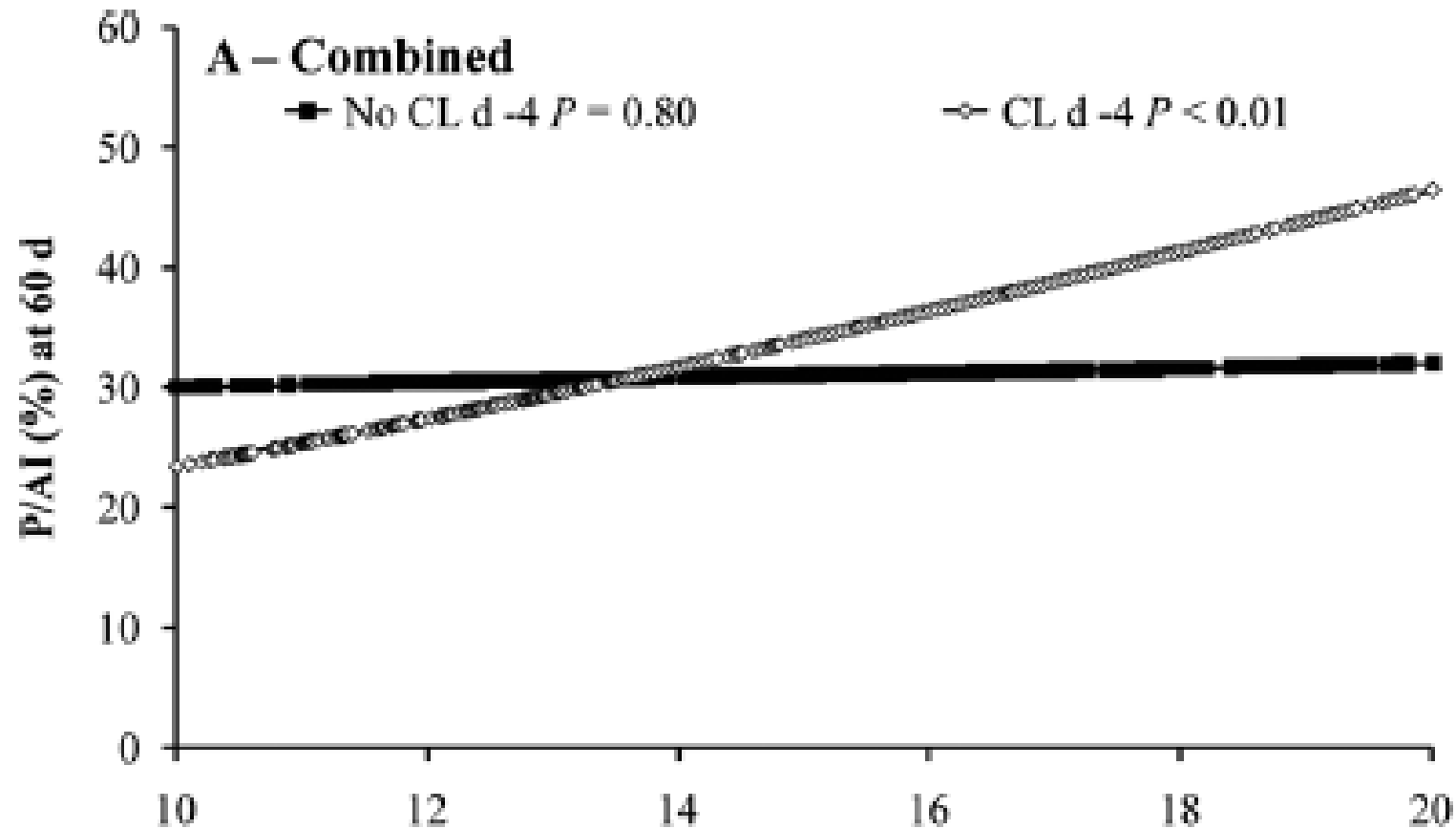
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Effect of adding a gonadotropin-releasing-hormone treatment at the beginning and a second prostaglandin $F_{2\alpha}$ treatment at the end of an estradiol-based protocol for timed artificial insemination in lactating dairy cows during cool or hot seasons of the year

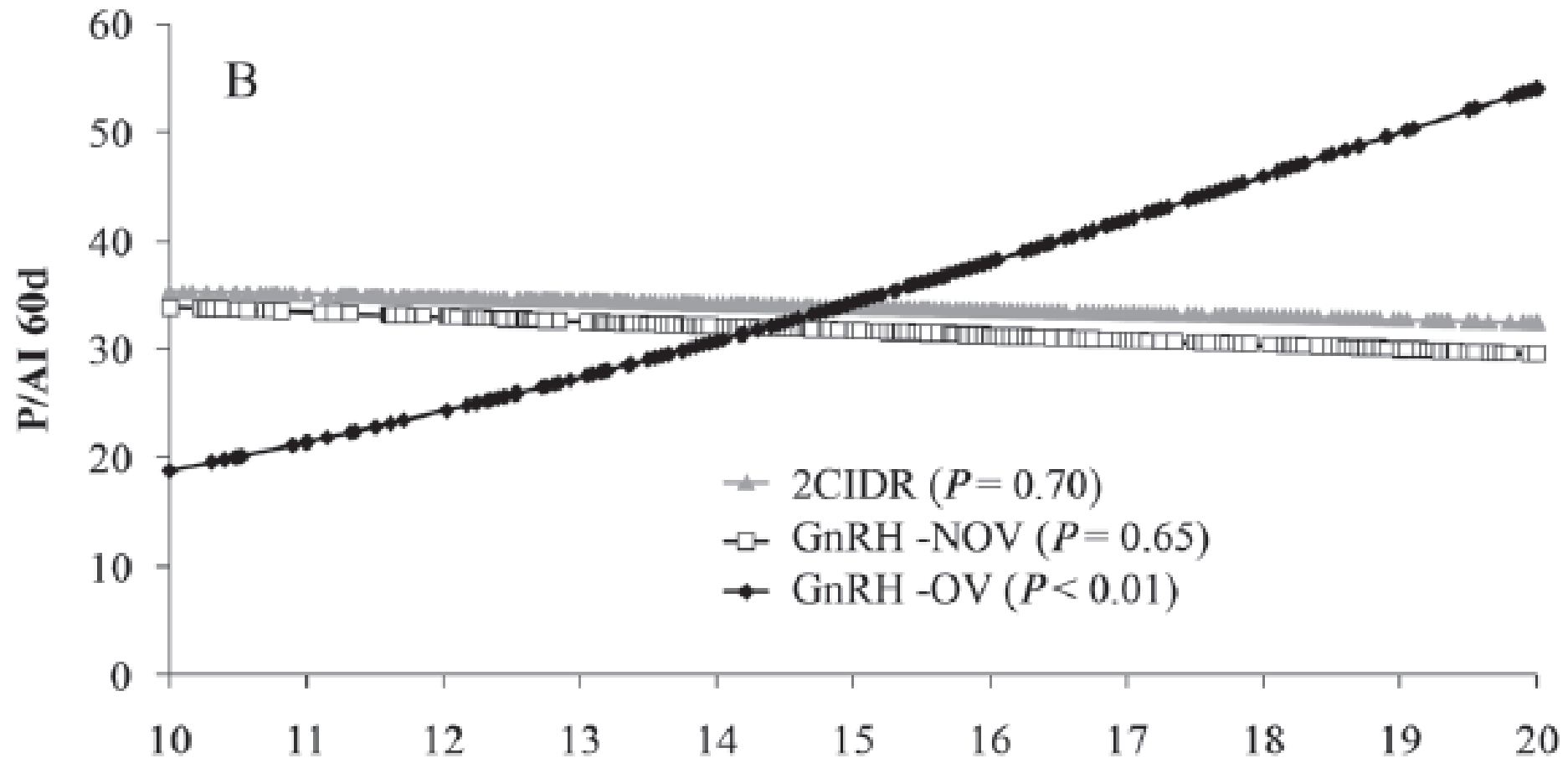
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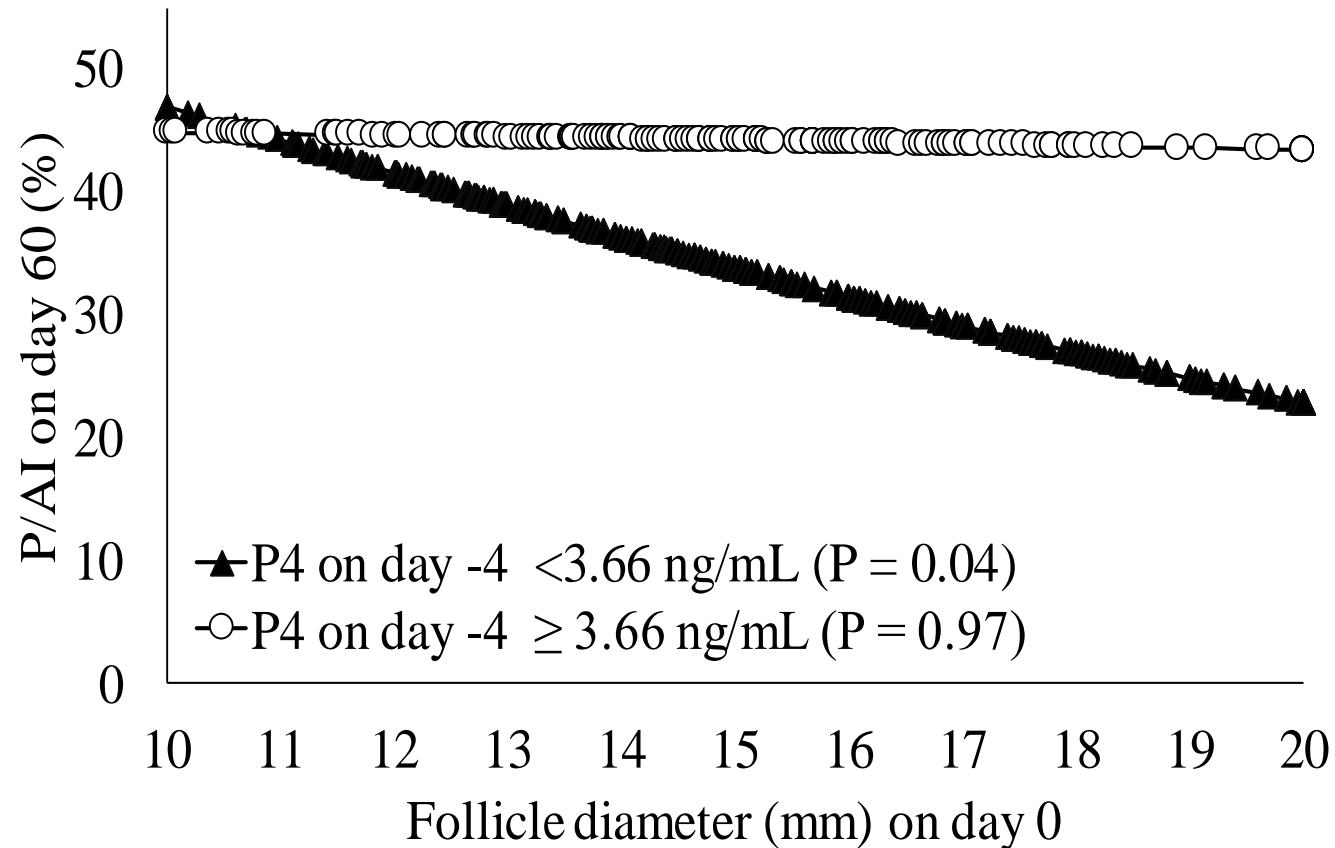
Comparison of 2 protocols to increase circulating progesterone concentration before timed artificial insemination in lactating dairy cows with or without elevated body temperature

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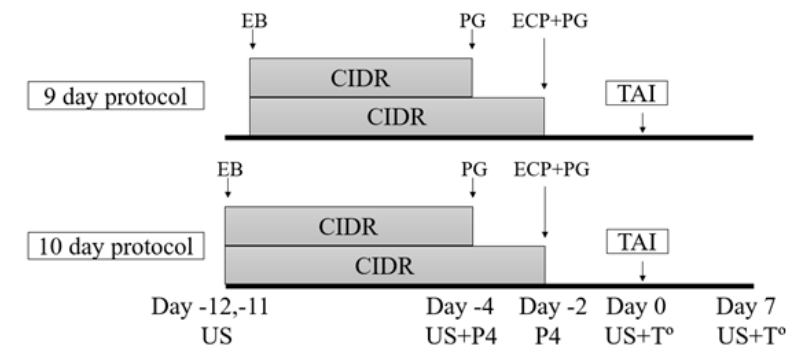


Evaluation of presynchronization and addition of GnRH at the beginning of an estradiol/progesterone protocol on circulating progesterone and fertility of lactating dairy COWS

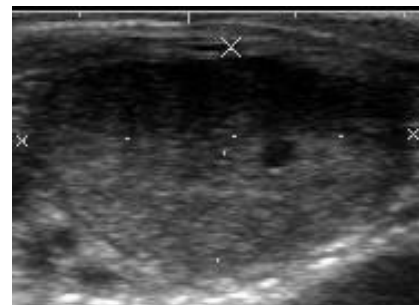
M.H.C. Pereira ^a, M.C. Wiltbank ^b, T.G. Guida ^a, F.R. Lopes Jr. ^a, B.I. Cappelozza ^c, J.L.M. Vasconcelos ^d  



Hipotesis



We hypothesized that increasing the length of an E2/P4-based ovulation synchronization protocol with 2-P4 devices would result in: ovulation of a larger follicle and increased P/AI of lactating dairy cows



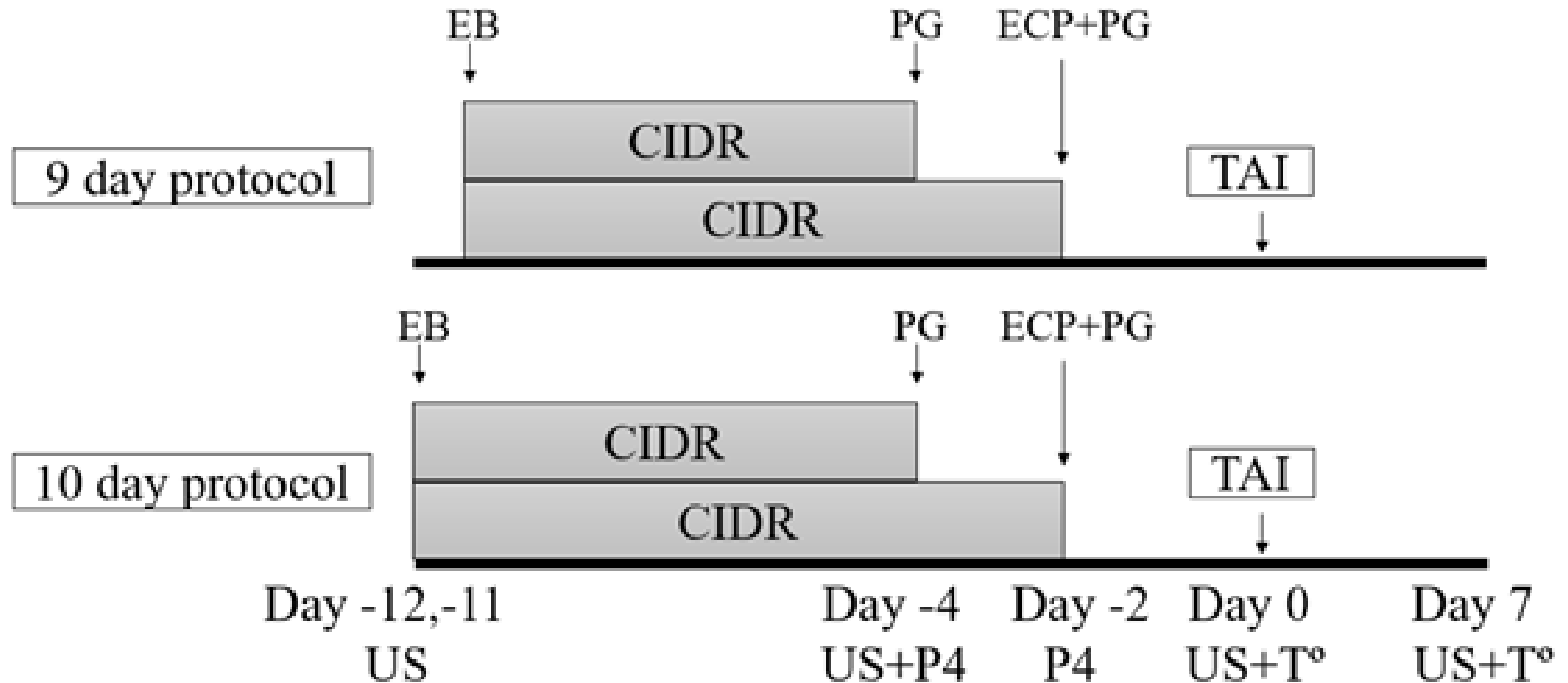
Objectives

The objective of this study are to compare:

- Effects of different lengths of ovulation synchronization protocol using 2-P4 devices on P/AI of lactating dairy cows.
- Evaluate the possible interactions of environment (heat stress) and lactation status (milk production and days in milk) with the protocols used herein on reproductive function of dairy cows.

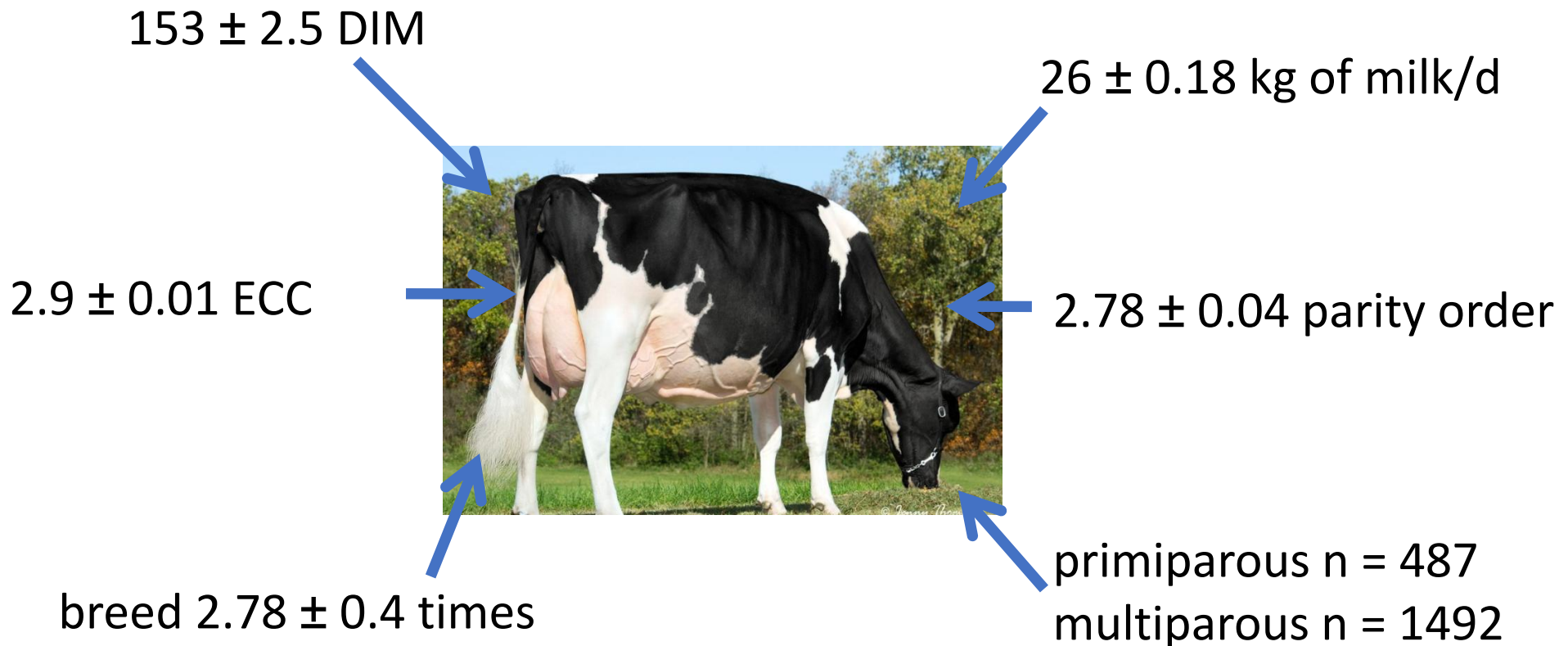
Materials and methods

Within each farm, cows were blocked by parity (primiparous and multiparous), all cows that were past the voluntary waiting period and not pregnant were utilized and randomized into the study, without regard to whether they had been previously utilized in the study



Materials and methods

- 2 farms 1979 lactating dairy cows (minas gerais state)
- At d -12:



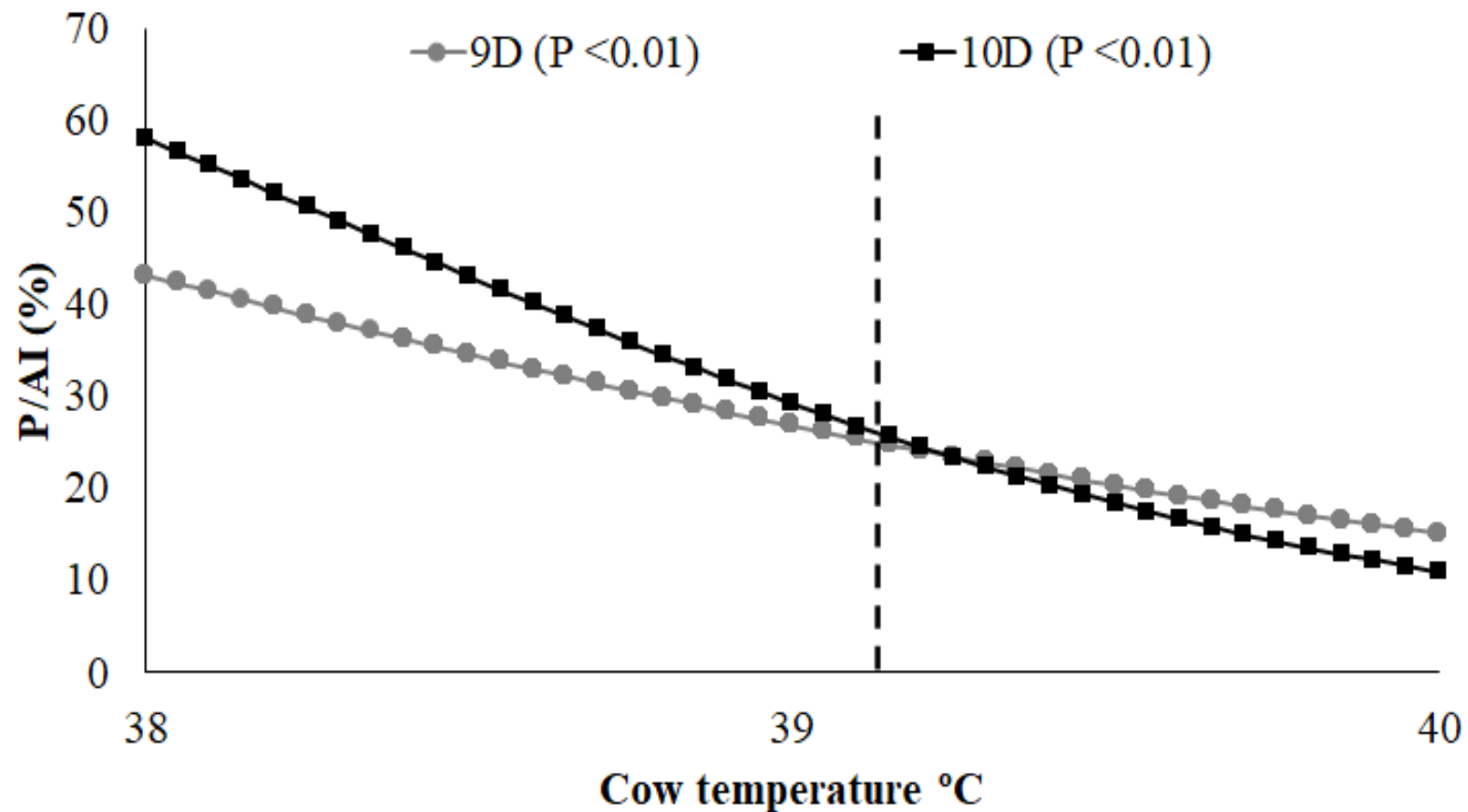
Results

Effect of 9-day (**9D**) or 10-day (**10D**) protocol on estrus detection, ovulation near TAI, and P/AI

Item	Protocol		P- value
	9D	10D	
Estrus detection	79.7 (787/988)	81.2 (805/991)	0.38
Ovulation near TAI	85.6 (846/988)	89.3 (885/991)	0.01
P/AI on day 32	25.4 (251/988)	27.7 (274/991)	0.26
P/AI on day 32 ¹	29.7 (251/846)	31.0 (274/885)	0.56

Results

Logistic regression analysis of the relationship between heat stress and P/AI for cows that were assigned to protocols (**9D** and **10D**).



A protocol × cow temperature ($P = 0.03$) interaction was observed herein.

Results

Effect of 9-day (**9D**) or 10-day (**10D**) protocol on P/AI stratified by incidence of heat stress (> 39.1 °C)

Item*	Number of ≥ 39.1 °C events			P-value
	0	1	2	
P/AI on day 32				
9D	30.2 (95/315) ^a	27.8 (94/338) ^a	18.5 (62/335) ^b	0.01
10D	37.4 (126/337) ^a	28.6 (90/315) ^b	17.1 (58/339) ^c	< 0.01
P-value	0.03	0.82	0.68	

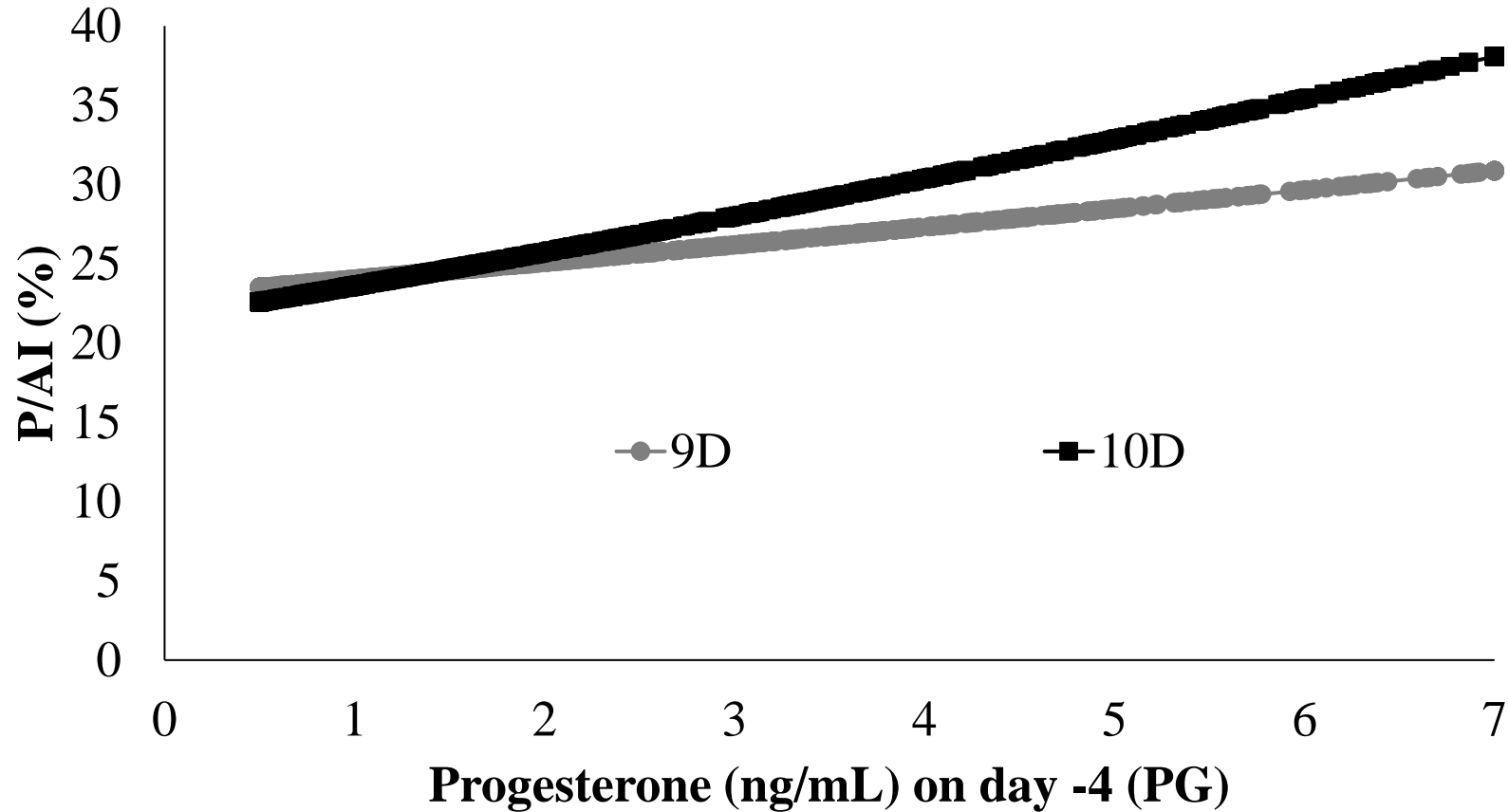
Results

Effect of 9-day (9D) or 10-day (10D) protocol and CL presence at beginning of the protocol on ovulation near TAI, and P/AI

Item	Protocol		P = ²		
	9D	10D	P	V	P × V
Ovulation near TAI			< 0.01	< 0.01	0.09
Without CL	77.8 (283/364)	84.2 (287/341)			
With CL	90.2 (563/624)	92.0 (598/650)			
P/AI			0.66	0.02	0.04
Without CL	25.0 (91/364)	21.7 (74/341)			
With CL	25.6 (160/624)	30.8 (200/650)			
P/AI ¹			0.77	0.40	0.02
Without CL	32.2 (91/283)	25.8 (74/287)			
With CL	28.4 (160/563)	33.4 (200/598)			

Results

Logistic regression analysis of the relationship between circulating P4 concentration on day -4 and P/AI on day 32 for cows that were assigned to protocols (**9D** and **10D**).

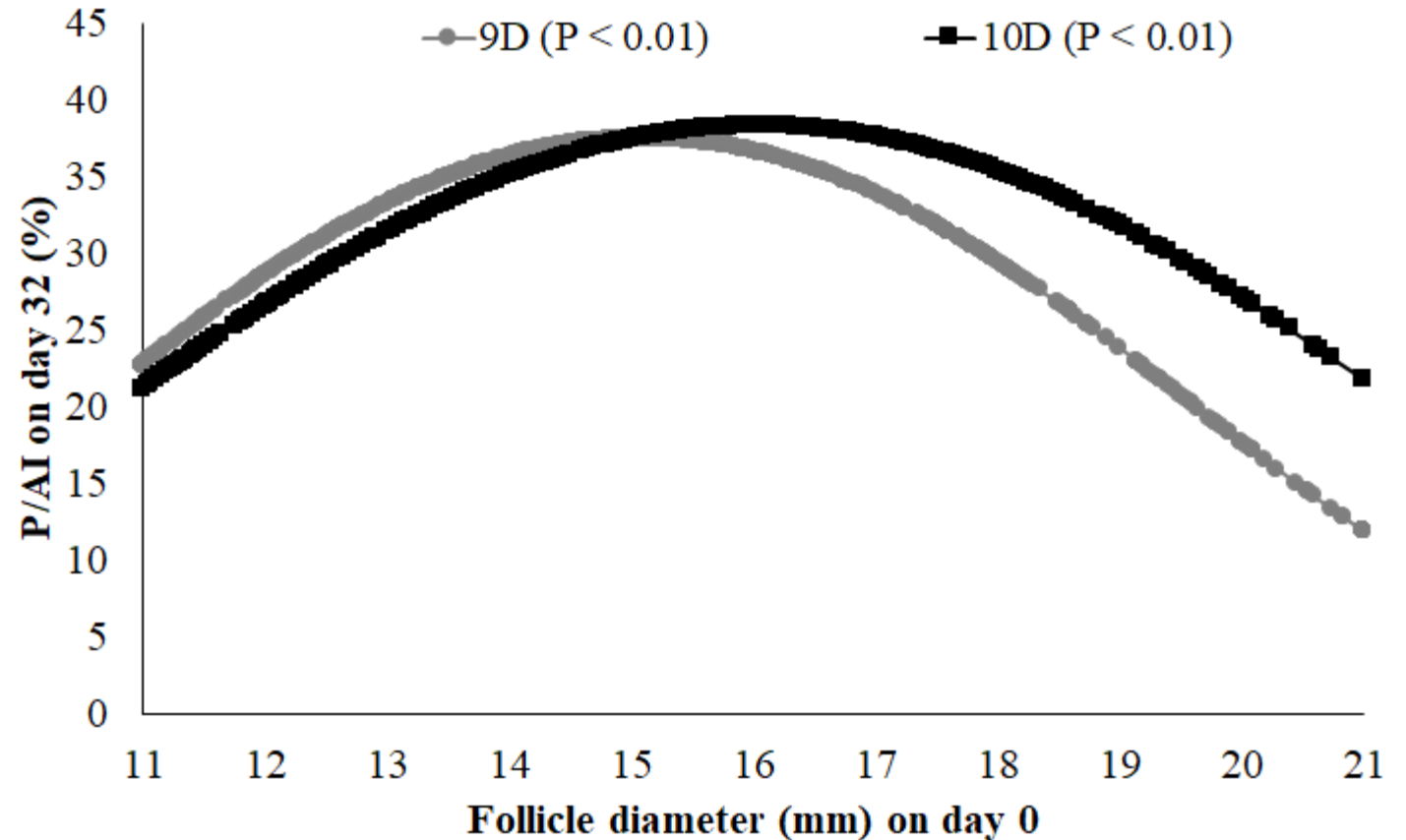
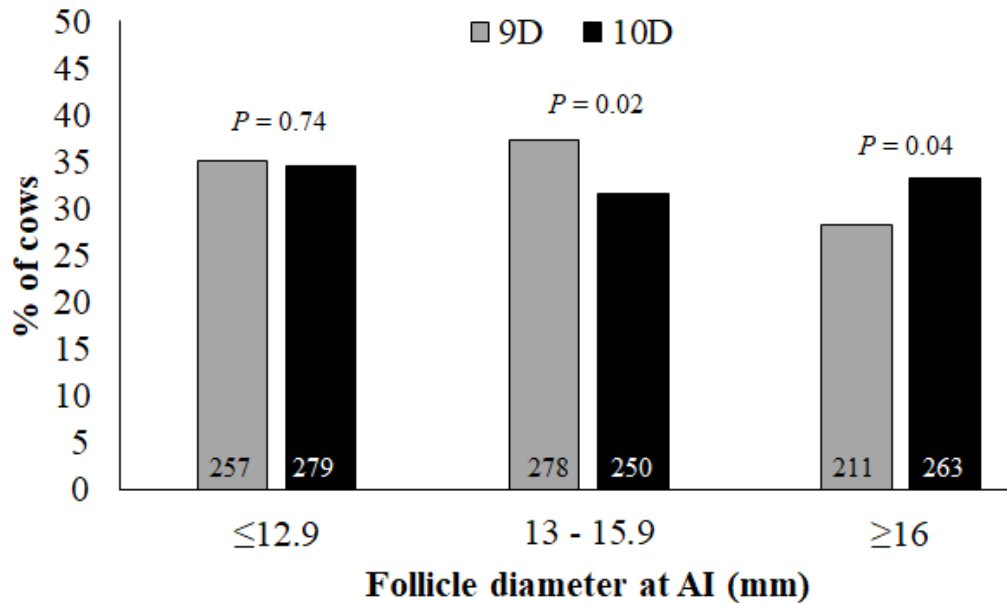


A protocol \times circulating P4 concentration on day -4 interaction was not observed herein ($P = 0.35$), but there was an effect of circulating P4 concentration at PG on P/AI ($P < 0.01$).

Results

logistic regression analysis of the relationship between follicle diameter on day 0 and P/AI on day 32 for cows that were assigned to protocols

Percentage of cows within treatment presenting a small, medium, and large follicle diameter at AI



Conclusion

Strategies to reduce heat stress are necessary to improve P/AI – 50% Cows

Increasing the length of an E2/P4-based ovulation synchronization protocol (10D vs. 9D)

increased:

- the proportion of cows with ovulation near TAI
- the proportion of cows with larger follicles (>16 mm)
- P/AI in cows without heat stress and in cows with a CL at beginning of the protocol.
- Pre synchronization + 10D protocol can be a strategy to improve P/AI