

**University of São Paulo
“Luiz de Queiroz” College of Agriculture**

**Strategies for induction of ovulation for fixed-time AI in lactating dairy
cows submitted to a novel presynchronization protocol**

Carlos Eduardo Cardoso Consentini

Dissertation presented to obtain the degree of Master in
Science. Area: Animal Science and Pastures

**Piracicaba
2019**

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Veterinarian

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versão revisada de acordo com a resolução CoPGr 6018 de 2011

Advisor:

Prof. Dr. **ROBERTO SARTORI FILHO**

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DEDICATION

To my grandfather Luiz Morais Cardoso who was, in some way, the responsible to my decision to become a veterinarian.

To my uncle Roberto Rennó Raphaelli that was so proud of my academic career and of my choice to do my Master's at ESALQ/USP. He always thought that I was unbeatable. We really miss you.

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SUMMARY

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1. STRATEGIES FOR INDUCTION OF OVULATION FOR FIXED-TIME AI IN LACTATING DAIRY COWS SUBMITTED TO A NOVEL PRESYNCHRONIZATION PROTOCOL

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Resumo

O estudo avaliou estratégias para induzir a ovulação final em protocolos de IA em tempo fixo (IATF) em vacas leiteiras submetidas a protocolos do tipo Ovsynch iniciados após uma pré-sincronização. Um total de 909 vacas em lactação de seis diferentes fazendas estava com $36,7 \pm 0,28$ d em lactação e escore de condição corporal de $3,16 \pm 0,02$ quando iniciaram o protocolo de pré-sincronização. No D-15 todas as vacas receberam um implante de progesterona (P4) de 1,0 g (novo ou usado) ou de 2,0 g (usado), e 7 d após (D-8), o implante de P4 foi retirado e as vacas receberam 1,0 mg i.m. de cipionato de estradiol (CE) e 0,530 mg de cloprostenol sódico (PGF). No D0, iniciou-se o protocolo de sincronização da ovulação para a IATF e as vacas foram aleatoriamente distribuídas em 1 de 3 grupos experimentais, que diferiam somente na estratégia de indução da ovulação ao final do protocolo. Os protocolos se iniciaram no D0 com 16,8 µg de acetato de busarelina (GnRH) concomitante com a inserção de um dispositivo de P4 de 2,0 g (novo ou usado). No D6, todas as vacas receberam 0,530 mg de PGF seguida de uma segunda PGF no D7, concomitante com a retirada do implante de P4. No **Grupo CE**, as vacas receberam 1,0 mg de CE no D7 (momento da retirada do dispositivo de P4) como indutor de ovulação. No **Grupo CE/G**, as vacas receberam 1,0 mg de CE no D7 e 8,4 µg de GnRH 16 h antes da IATF (56 h após a primeira PGF). Por último, no **Grupo G**, as vacas receberam somente 8,4 µg de GnRH no mesmo momento do grupo CE/G. A IATF foi realizada no D9 (48 h após a retirada do implante de P4) em todos os grupos e o diagnóstico de gestação ocorreu 31 e 60 d após a IATF. A prenhez por IA (P/IA) não diferiu entre vacas com ou sem CL no D-15 (44.7 vs. 38.7%) e no D0 (44.3 vs. 37.3), no entanto, vacas com CL no D6 apresentaram maior P/IA do que vacas sem CL na PGF (45.9 vs. 17.7%). Expressão de cio foi maior em vacas que receberam CE do que vacas que receberam somente GnRH (80.0 vs. 46.1%). Além disso, vacas que expressaram cio tiveram maior P/IA no d31 (50.8 vs. 34.8) e no d60 (48.5 vs. 27.6%), no entanto, expressão de cio não afetou perda gestacional. Não houve diferença entre os tratamentos experimentais na P/IA no d31, e a P/IA média foi 40.4% (367/909). Perda gestacional e P/IA aos 60 d não diferiram entre os grupos. Concluímos que o programa reprodutivo tem potencial para promover alta fertilidade

e que as 3 estratégias para induzir a ovulação final do programa reprodutivo promoveram fertilidade similares. No entanto, é necessário que seja melhor estudado a interação entre expressão de cio e tratamentos nos parâmetros de fertilidade, assim como, estudar mais a fundo os efeitos dos tratamentos (indutores de ovulação) na fertilidade após o d30 e na perda gestacional.

Palavras chave: Vacas leiteira; IATF; Indução da ovulação; Expressão de cio; Fertilidade

Abstract

The study evaluated strategies for induction of ovulation for fixed-time AI (FTAI) in lactating dairy cows submitted to Ovsynch-type protocols initiated after a novel presynchronization strategy. A total of 909 lactating dairy cows from 6 dairy herds were at 36.7 ± 0.28 d in milk, with body condition score of 3.16 ± 0.02 when they underwent presynchronization. On D-15, all cows received an intravaginal progesterone (P4) implant of 1.0 g P4 (new or used) or a used device of 2.0 g P4 and 7 d later (D-8) the P4 implant was removed and cows received 1.0 mg i.m. estradiol cypionate (EC) and 0.530 mg i.m. sodium cloprostenol (PGF). On D0, a synchronization of ovulation protocol for FTAI was initiated and cows were randomly assigned to 1 of 3 experimental groups, that differed only on the strategy to induce ovulation at the end of the protocol. The protocols initiated on D0 with 16.8 µg i.m. of buserelin acetate (GnRH) concomitant with insertion of a 2.0 g (new or used) P4 device. On D6, every cow received 0.530 mg PGF followed by a second PGF on D7, concomitant with P4 device withdrawal. In **Group EC**, cows received 1.0 mg EC on D7 (time of P4 device withdrawal) as inducer of ovulation. In **Group EC/G**, cows received EC on D7 and 8.4 µg GnRH administered 16 h before FTAI (56 h after the first PGF). Finally, in **Group G**, cows only received 8.4 µg GnRH at 56 h after the first PGF. The FTAI was performed on D9 (48 h after P4 device withdrawal) in all experimental treatments and pregnancy diagnosis was performed 31 and 60 d after FTAI. The pregnancy per AI (P/AI) was not different between cows with or without CL on D-15 (44.7 vs. 38.7%) and on D0 (44.3 vs. 37.3), however, cows with CL on D6 had higher P/AI than cows without CL at PGF (45.9 vs. 17.7%). Estrus expression was greater in cows receiving EC compared to cows receiving only GnRH (80.0 vs. 46.1%). Moreover, cows expressing estrus had greater P/AI than cows not showing estrus on d31 (50.8 vs. 34.8) and on d60 (48.5 vs. 27.6%), however, estrus did not affect pregnancy loss. There were no differences between experimental treatments on P/AI on d30 with an overall P/AI of 40.4% (367/909). Pregnancy loss and P/AI on d60 did not differ between treatments. In conclusion, the reproductive program has potential to promote high fertility and the 3 strategies to induce the final synchronized ovulation produced similar fertility. Further research is needed to optimize the presynchronization strategy and to more definitively determine the effect of different methods of ovulation induction on P/AI and in particular on the pregnancy loss after the d30 pregnancy diagnosis.

Keywords: Dairy cow; FTAI; Ovulation induction; Estrus expression; fertility

1.1. Introduction

Reproductive efficiency directly affects profitability of dairy herds and the main objective of reproductive management strategies is to increase the number of cows pregnant early in lactation in order to achieve a profitable calving interval for each dairy herd (Santos et al., 2004; Giordano et al., 2011).

If reproductive management is based on detection of estrus in lactating cows then the shortened period of estrus (Lopez et al., 2004) and the average estrous cycle length of 23 d (Sartori et al., 2004) offer a limited period for detection of estrus and insemination and considerable possibility of missing the estrus and delaying the calving interval. Moreover, cyclicity is delayed in 20-30% cows (Chebel et al., 2010) and many remain anovular even at the end of the voluntary waiting period (**VWP**) (Walsh et al., 2007; Wiltbank et al., 2002; Santos et al., 2016) due to many factors including postpartum diseases (Santos et al., 2010) and BCS loss during the transition period (Barletta et al., 2017). Therefore, there are physiological, health and management aspects that preclude the achievement of high service rates based on detection of estrus and decrease the likelihood of cows becoming pregnant at the optimal stage of lactation.

The use of synchronization of ovulation protocols can allow for fixed-time artificial insemination (**FTAI**) and eliminate the need for detection of estrus, thereby potentially improving reproductive efficiency and profitability of dairy herds (Norman et al., 2009). Use of FTAI programs for first AI can increase the service rate in dairy herds by allowing prompt AI after the voluntary waiting period (Pursley et al., 1997). In addition, recent FTAI programs have been developed that are termed fertility programs because pregnancy/AI (P/AI) can be attained with these programs that exceeds fertility attained after AI to an estrus (Wiltbank and Pursley, 2014, Santos et al., 2017, Carvalho et al., 2018).

South American dairy herds generally utilize protocols based on treatments with estradiol (**E2**) plus progesterone (**P4**) sometimes utilizing gonadotropin releasing hormone (**GnRH**) at the beginning of the protocol and induce final ovulation with estradiol esters (Pereira et al., 2015; Melo et al., 2018). These protocols, however, are not optimized in terms of effectively synchronizing emergence of a new follicular wave or promoting an optimal P4 milieu during development of the preovulatory follicle. For instance, cows that started a FTAI protocol with estradiol benzoate (**EB**) had ~40% luteolysis after D0 of the protocol (Melo et al., 2016). In addition, synchronized emergence of a new follicular wave only occurred in ~60-75% of the cows (Monteiro et al., 2015; Melo et al., 2018), which means that a proportion of cows are ovulating a persistent follicle at the end of the protocol. Melo et al.

(2016) reported 35% of cows submitted to a E2/P4 based protocol ovulated a persistent follicle at the end of the protocol. In addition, lack of ovulation at the end of E2/P4 protocols occurs in about 20% of lactating dairy cows and this substantially impairs fertility (Monteiro et al., 2015; Melo et al., 2018).

On the other hand, North American dairy herds generally use FTAI protocols based on GnRH, such as Ovsynch (Pursley et al., 1995, Wiltbank and Pursley, 2014). In addition, many dairy herds use presynchronization prior to the Ovsynch FTAI program in order to increase fertility (Moreira et al., 2001; Souza et al., 2008; Stevenson, 2016). The FTAI programs that combine presynchronization with optimized Ovsynch programs are the basis for the fertility programs that not only increase service rate but also can increase P/AI (Wiltbank and Pursley, 2014, Carvalho et al., 2018). There are presynchronization programs based on PGF administration, such as Presynch-Ovsynch strategy (Moreira et al., 2001; Navanukraw et al., 2004), or programs that combine the use of GnRH and PGF during presynchronization, such as G-6-G (Bello et al., 2006) or Double-Ovsynch (Souza et al., 2008). Regardless of the presynchronization method used, the objective is to maximize the number of cows in early diestrus (day 6-7 of the estrous cycle), with follicles responsive to GnRH, assuring high synchronization and adequate circulating P4 during development of the preovulatory follicle in most of the cows. Based on the information presented above, a novel presynchronization strategy was utilized in order to optimize fertility of the GnRH/P4 based FTAI protocols to be tested in our study, every cow was submitted to a novel presynchronization strategy.

A synchronized ovulation of the dominant follicle at the end of the FTAI protocol can be induced by estradiol esters, such as estradiol benzoate (**EB**) and estradiol cypionate (**EC**; Melo et al., 2016), or by GnRH, used in Ovsynch-type protocols (Pursley et al., 1995). The use of EC is convenient because it can be administered concomitant to P4 device withdrawal (Melo et al., 2016), however, the timing of ovulation induced by EC is more dispersed than when GnRH is used to induce ovulation (Pancarci et al., 2002; Souza et al., 2009). On the other side, when GnRH is used at the end of FTAI protocols, in addition to the fact that cows need to be handled one more time, estrus expression is reduced, which could compromise fertility, since estrus expression and circulating E2 concentration during proestrus of FTAI protocols have been associated with higher fertility and lower pregnancy loss (Bello et al., 2006; Pereira et al., 2016). Therefore, the aim of the study was to compare 3 strategies to induce/synchronize ovulation of the dominant follicle at the end of the FTAI protocol. One using EC, another using GnRH, and the third combining both, in order to combine the

positive effect of E2 supplementation during proestrus to a more synchronized time of ovulation produced by GnRH treatment prior to a FTAI GnRH to induce final ovulation.

Therefore, 2 main hypotheses were proposed for the study: 1) Expression of estrus at the end of FTAI protocols would positively impact fertility, and 2) The cows in which ovulation was induced by GnRH and also received E2 supplementation would present higher fertility compared to cows with ovulation induced only by EC or GnRH.

1.2. Material and methods

This experiment was conducted in 6 commercial dairy farms located in Southeast and Midwest of Brazil, from August of 2017 to September of 2018. The Animal Care and Use Committee of "Luiz de Queiroz" College of Agriculture of University of São Paulo (ESALQ/USP) approved all procedures involving cows in this study (protocol # 2017.5.11620.11.3).

Animals, housing and diets

The herds were located in Minas Gerais (Farms 1 and 2), Goiás (Farm 3), Paraná (Farm 4) and São Paulo (Farms 5 and 6) states, Brazil, and varied in size between ~400 lactating cows (Farm 6) to over 2000 lactating cows (Farm 5; Table 1). In all herds, cows were housed in free-stall barns and, in farms 5 and 6, they were maintained in cross ventilation facilities. All cows had free access to water and mineral salt and were fed *ad libitum* with a TMR diet based on corn silage and Tifton 85 hay as forages, and concentrate based on corn and soybean meal, minerals and vitamins balanced to meet or exceed the nutritional requirements of lactating dairy cows (National Research Council – NRC, 2001). Throughout the experiment, cows were milked 3 times daily.

Within the study, there were cows enrolled during the warm season of the year (spring and summer) and cool season of the year (autumn and winter), according to farm. In addition, in farm 6, cows were enrolled in the study over an entire year (August of 2017 to September of 2018). Table 1 shows the descriptive data about size of the farms, as well as number and months of the year that cows were enrolled in each farm. It should be noted that more than one third of the cows was from farms 1, 2 and 3, in which cows were enrolled mostly during the summer and had suboptimal management to minimize heat stress.

Table 1. Descriptive data about size of the farms, number of cows and months of the year that cows were enrolled in each farm

Farm	Number of lactating cows	Months of the year that cows were enrolled in the experiment^{1,2}	Number of cows enrolled
1	500-550	October of 2017 to March of 2018	136
2	600-700	September of 2017 to February of 2018	111
3	500-550	October of 2017 to April of 2018	98
4	550-650	May of 2018 to July of 2018	141
5	2000-2100	April of 2018 to August of 2018	233
6	350-450	August of 2017 to September of 2018	190

¹ Warm seasons of the year: Spring (September/22 to December/21) and Summer (December/22 to March/19).

² Cool seasons of the year: Autumn (March/20 to June/20) and Winter (June/21 to September/21).

A total of 909 lactating Holstein cows (primiparous and multiparous) were used for their first postpartum AI in the study. At the beginning of the experiment (D-15, Figure 1), cows were at 36.7 ± 0.28 d in milk (**DIM**), yielding 38.9 ± 0.64 kg of milk/d, with BCS of 3.16 ± 0.02 (scale from 1 to 5 according to Ferguson et al., 1994). On D0 (Figure 1), cows yielded 39.8 ± 0.67 kg of milk/d and the BCS was 3.22 ± 0.02 .

Treatments and experimental design

Weekly, a cohort of cows was submitted to a presynchronization protocol that was the same for all cows. This presynchronization initiated (D-15) with 1.0 g P4 device (Reproneo, GlobalGen, Jaboticabal, Brazil) previously used once or a 2.0 g P4 device (Reprosync, GlobalGen) previously used once, twice or thrice disinfected according to Melo et al. (2018), and 7 d later (D-8), the P4 implant was removed and cows received 1.0 mg i.m. EC (Cipion, GlobalGen) and 0.530 mg i.m. sodium cloprostenol (PGF; Induscio, GlobalGen). Eight d after the end of the presynchronization protocol (D0), it was initiated a synchronization of ovulation protocol for FTAI and cows were randomly assigned to 1 of 3 experimental groups, that differed only in the strategy to induce ovulation at the end of the protocol. The protocols initiated on D0, when all cows received 16.8 µg i.m. of buserelin acetate (GnRH; Maxirelin, GlobalGen) concomitant with insertion of a 2.0 g (new or previously used-once) P4 device. On D6, every cow received 0.530 mg PGF followed by a second PGF on D7, concomitant

with P4 device withdrawal. On **Group EC**, cows received 1.0 mg EC at D7 (time of P4 device withdrawal) as ovulation inductor. On the **Group EC/G**, cows received EC on D7 and 8.4 µg GnRH administered 16 h before FTAI (56 h after the first PGF). Lastly, on **Group G**, cows only received 8.4 µg GnRH at the same time as for the EC/G group. The FTAI was performed on D9 (48 h after P4 device withdrawal) in all experimental treatments (Figure 1).

BCS, ovarian structures, estrus expression and pregnancy diagnosis

The BCS of cows was evaluated on D-15 (765 cows) and D0 (751 cows) of the experimental design. In order to check for presence and number of CL, and to evaluate the response to hormonal treatments throughout the experiment, ultrasound evaluations were performed on D-15 (631 cows), D0 (535 cows) and D6 (376 cows). The ultrasound was performed with a transrectal ultrasonography of reproductive tract with an 8-5 MHz multi-frequency linear-array transducer (Ibex Lite, E.I. Medical Imaging, Loveland, CO, USA).

A subset of cows (n = 368) received a tail-head device for estrus detection (BOViFLAG - Bovitime Animal Products LTD, Stellenbosch, South Africa) on D7, and were considered expressing estrus when the paint of the device had been removed by D9 (Time of AI).

Pregnancy diagnosis was performed at 31.0 ± 3.0 d after AI by transrectal ultrasonography of reproductive tract by confirming an embryo heartbeat. Pregnant cows were reconfirmed at 60.0 ± 3.0 d after AI (farms 3, 5 and 6). Pregnancy per AI was calculated at d31 and d60, as well as pregnancy loss between these two evaluations.

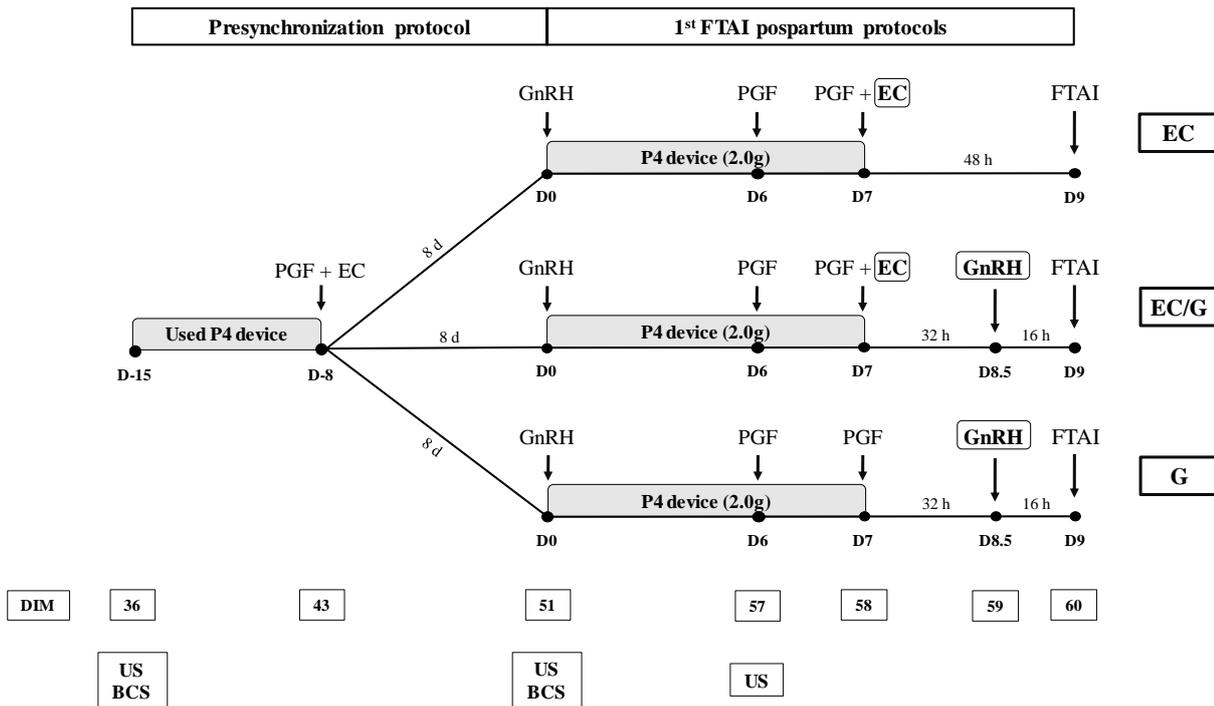


Figure 1. Experimental design with hormonal treatments and procedures performed during the presynchronization protocol and FTAI protocols.

Presynchronization protocol: on D-15, all cows received a 1.0 g (used once) or a 2.0 g (used once, twice or thrice) P4 intravaginal device, and 7 d later (D-8), P4 implant was removed concomitant with the administration of 1.0 mg estradiol cypionate (EC) and 0.530 mg sodium cloprostenol (PGF).

For the FTAI protocols, cows from all groups received 16.8 µg buserelin acetate (GnRH) and a 2.0 g P4 device (new or once used). On D6, the first PGF injection was administered, followed by a second dose on D7, concomitant with P4 device withdrawal.

Group EC: cows received 1.0 mg EC on D7.

Group EC/G: cows received 1.0 mg EC on D7 and 8.4 µg GnRH on D8.5.

Group G: cows only received 8.4 µg GnRH on D8.5.

Ultrasound (US) evaluations were performed at D-15, D0 and D6. The body condition score (BCS) was measured on D-15 and D0.

Statistical analysis

Statistical analyses were performed using the Statistical Analysis System (SAS, Version 9.4 for Windows SAS Institute Inc., Cary, NC).

Statistical analysis for continuous variables (DIM on D-15, BCS on D-15, BCS on D0, milk production on D-15 and D0) were performed using the GLIMMIX procedure fitting a *Gaussian* distribution. All data were tested for normality of residuals using UNIVARIATE procedure according to Shapiro-Wilk test. Non-normally distributed data were transformed before analysis if improved residual distribution, in addition, outliers were removed when necessary. Effect was determined by one-way ANOVA using Type III sums of squares. The final model for these continuous variables included effect of treatment, farm, parity and BCS on D-15 or BCS on D0, depending which day the variable had been studied.

Analysis of binomial variables (presence of CL on D-15, D0 and D6, ovulation after D0, estrus expression, P/AI on d31 and d60, and pregnancy loss) were performed using the GLIMMIX procedure fitting a *Binomial* distribution with *Logit Link* function. Additionally, the option *ddfm = kenwardroger* was included to the model statement to adjust the degrees of freedom for variances. The selection of the model that best fitted each variable of interest was performed by finding the model with the lowest value for the *Akaike Information Criterion Corrected (AICC)* using the *forward* selection procedure that removed variables with $P > 0.20$ from the model.

Tukey honest significant difference post hoc test was performed to determine differences. Values are presented as means \pm SEM (continuous variables) or as percentage (%; binomial variables). Significant differences were declared when $P \leq 0.05$, whereas tendencies were considered when $0.10 \geq P > 0.05$.

The final model for presence of CL on D-15, D0 and D6 included effects of treatment, farm and BCS on D-15 (for presence of CL on D-15) or BCS on D0 (for presence of CL on D0 and D6). Parity was studied in all models, as well as its interaction with BCS. For presence of CL on D6, ovulation after D0 was evaluated, as well as its interaction with parity and BCS.

Final model for ovulation after D0 included treatment, farm, and BCS on D0. Presence of CL on D0 and parity were evaluated, as well as their interaction with each other and with BCS on D0.

Regarding estrus expression, the final model included effects of treatment, parity and BCS on D0. Presence of CL on D0 and D6, and ovulation after D0 was evaluated. The interaction between treatment and parity, BCS, CL on D0, CL on D6 and ovulation after D0 was studied.

The final model for P/AI on d31 included effects of treatment, estrus expression, the interaction between treatment and estrus expression, farm, parity, BCS on D0, ovulation to D0 and CL on D6. It was evaluated the interaction of treatment with farm, parity BCS on D0, ovulation to D0 and CL on D6. Interaction of BCS on D0 with estrus expression and ovulation to D0 was studied.

The final model for P/AI on d60 included effects of treatment, estrus expression, farm, parity, BCS on D0, ovulation to D0 and CL on D6. It was evaluated the interaction of treatment with farm, parity BCS on D0, ovulation to D0 and CL on D6.

For pregnancy loss, the final model included effects of treatment, farm, BCS on D0, estrus expression and ovulation to D0. The interactions of treatment with BCS on D0 and ovulation to D0 was studied.

The LOGISTIC procedure was used for logistic regression to model the probability of presence of CL on D-15, D0 and D6 as a function of BCS on D-15 (for presence of CL on D-15) or BCS on D0 (for presence of CL on D0 and D6). In addition, it was performed a logistic regression to evaluate the probability of estrus expression, P/AI (31d), P/AI (60d) and pregnancy loss according to BCS on D0. Logistic regression curves were created using the coefficients provided by the interactive data analysis from SAS and the formula $Y = \exp(\alpha \times X + \beta) / [1 + \exp(\alpha \times X + \beta)]$, where Y = probability of occurrence; exp = exponential; α = slope of the logistic equation; β = intercept of the logistic equation; and X = variable analyzed.

1.3. Results and discussion

Fertility according to estrus expression and experimental treatments

Other studies that compared EC vs. GnRH to induce/synchronize ovulation and/or used another source of E2 during proestrus to increase circulating E2 before AI, administered GnRH at the time of FTAI as a Cosynch strategy (Hillegass et al., 2008; Ferreira et al., 2015), or used EC concomitant with GnRH given 48 h after PGF and 24 h before FTAI (Sellars et al., 2006). Other studies used EC 24 h after PGF or after P4 device withdrawal as a Heatsynch strategy, but administered GnRH 48 h after PGF (Pancarci et al., 2002; Stevenson et al., 2004), or administered E2 8 h before (Souza et al., 2007) or at the time of GnRH administered 16 h prior to FTAI (Brusveen et al., 2009). In our study, in order to establish a fair comparison to evaluate fertility regarding different ovulation inducers and the effect of E2 supplementation, we designed experimental treatments in which the ovulation inducers were administered at their traditional and ideal times in FTAI protocols.

We confirmed our hypothesis that cows expressing estrus would achieve higher fertility, because expression of estrus increased P/AI on d31 in 69% and on d60 in 57% (Table 2). It is well-known that estrus expression at the end of FTAI protocols increases fertility parameters as shown by Bisinotto et al. (2015b), who reported that expression of estrus increased ~10 percentage points P/AI on d32 (50.2 vs. 40.4%) and on d60 (46.0 vs. 35.8) in cows submitted to the Ovsynch protocol. Likewise, in a retrospective study with more the 5,430 FTAIs analyzed, Pereira et al. (2016) reported that in protocols using EC as ovulation

inducer, estrus expression increased P/AI on d30 and d60 in 13 percentage points. In addition, cows that expressed estrus had lower (6 percentage points) pregnancy loss (Pereira et al., 2016). However, in our study, we could not detect difference, maybe due to a low number ($n = 35$) of experimental units not detected in estrus (Table 2).

Table 2. Pregnancy per AI 31 and 60 d after FTAI and pregnancy loss according to estrus expression at the end of breeding protocols.

Item	Estrus expression		P-value
	No estrus	Estrus	
P/AI (31d), %	34.8 (39/112)	50.8 (130/256)	0.02
P/AI (60d), %	27.6 (19/105)	48.5 (94/194)	0.02
Pregnancy loss, %	17.1 (6/35)	11.3 (12/106)	0.82

One of the reasons why estrus is associated with improved fertility in FTAI protocols is ovulation rate. For example, Galvão et al. (2004) reported that cows expressing estrus had greater ovulation rate at the end of the FTAI protocol compared to cows not showing estrus (94.2 vs. 63.1%, respectively). However, it should be noted that our study used EC to induce final ovulation, differently than the aforementioned study. Another reason why fertility is greater in cows displaying estrus is likely due to a higher circulating E2 concentration during proestrus. In fact, Bello et al. (2006) demonstrated that the probability of cows being pregnant on d35 increased as circulating E2 increased at the time of the second GnRH treatment of the Ovsynch protocol.

In our study, there was a tendency ($P = 0.07$) for interaction between estrus expression and treatment on P/AI (31d), in which, apparently, the effect of estrus expression was relevant when cows received EC at the time of P4 device withdrawal, since in G group, the P/AI on d31 was similar between cows with or without estrus (Figure 2).

P/IA (31d) according to experimental group and estrus expression

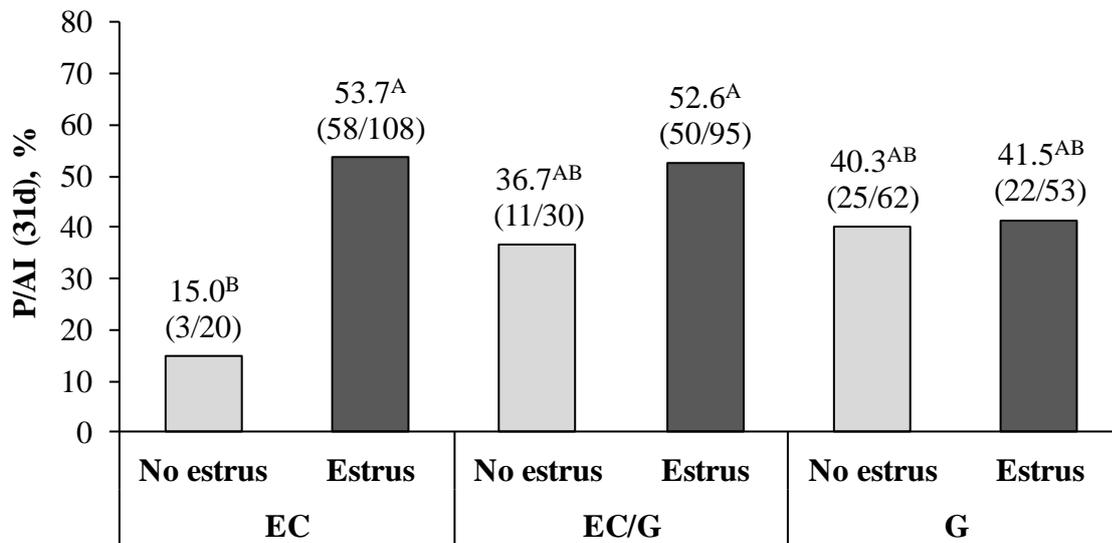


Figure 2. Pregnancy per AI 31 d after FTAI according to experimental group and estrus expression. There was a tendency ($P = 0.07$) for interaction between estrus expression and treatment.

Although studies reported improved P/AI in cows that expressed estrus and in which GnRH was used as ovulation inducer (Bisinotto et al., 2015b), several other authors reported no influence of estrus on fertility of Ovsynch type protocols. For instance, Bisinotto et al. (2010), in cows submitted to a Presynch-Ovsynch program, and the breeding Ovsynch being a traditional Ovsynch-56 or Cosynch-72, reported similar P/AI in cows with or without estrus at AI, either on d32 (45.6 vs. 46.2%, respectively) or d60 (39.6 vs. 39.9%, respectively). Similarly, Souza et al. (2007), using a Presynch-Ovsynch-56 program, also reported no effect of estrus expression on P/AI 38 and 61 d after FTAI. So, it seems that estrus expression impacts the fertility in different ways depending on type of protocols, as well as, ovulation inducers.

Regarding fertility according to the experimental treatments, our hypothesis that EC/G group would achieve the highest fertility/AI, due to a well synchronized ovulation in response to GnRH plus higher estrus expression because of the E2 supplementation, was not supported. Instead, considering all herds used in the study, the experimental groups had similar ($P = 0.45$) P/AI 31 d after FTAI (Figure 3). Other studies that compared EC to GnRH as ovulation inducers also reported similar fertility (Pancarci et al., 2002; Souza et al., 2009).

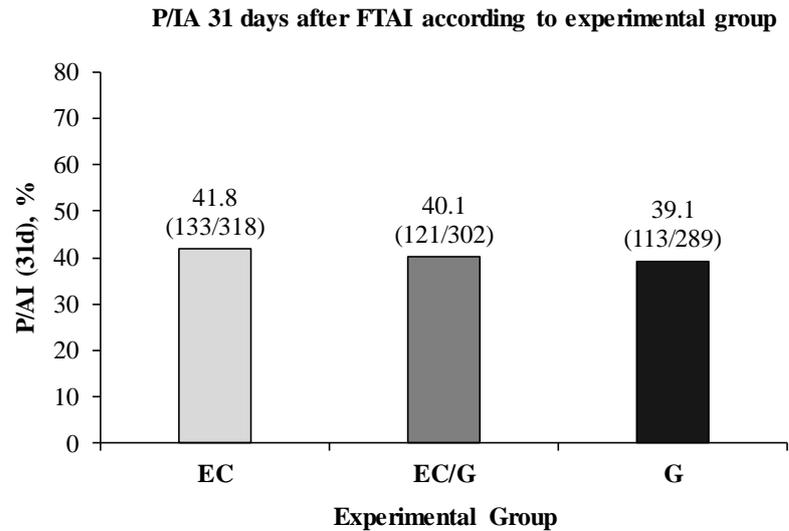


Figure 3. Pregnancy per AI 31 d after FTAI according to experimental group ($P = 0.45$). For this analysis, all herds were included.

To analyze data on P/AI on d60 and pregnancy loss, only farms (farms 3, 5 and 6) that provided information of the second pregnancy diagnosis were included. Considering data from only these farms, there was still no difference ($P = 0.32$) among experimental treatments on P/AI 31 d after FTAI. We also did not detect difference on P/AI on d60 ($P = 0.54$) or pregnancy loss ($P = 0.22$), although we cannot ignore the numerical differences in cows in P/AI on d60 and pregnancy loss between cows from the G group and the other 2 groups (Figure 4).

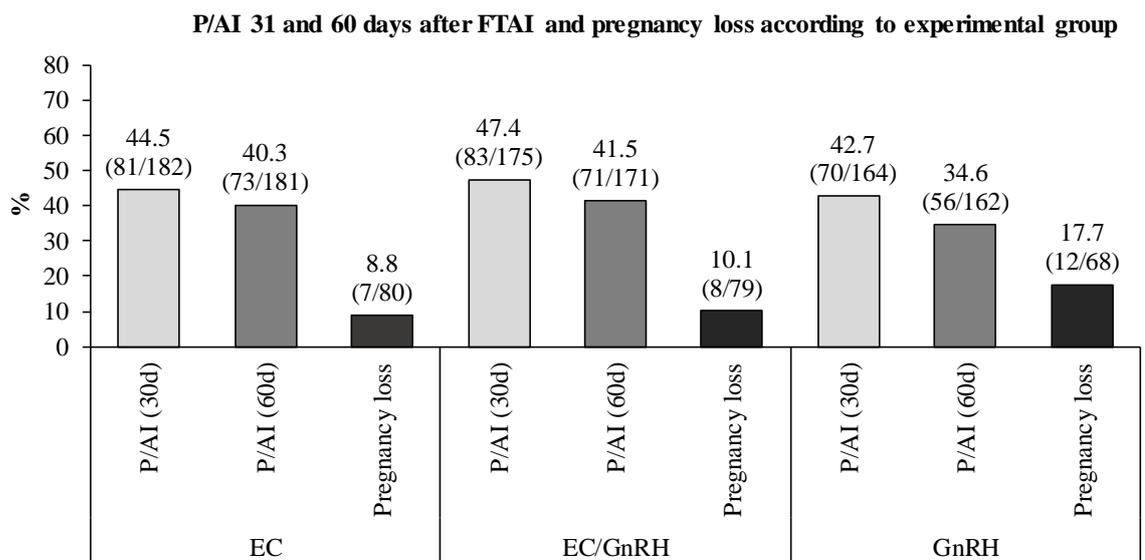


Figure 4. Pregnancy per AI 31 and 60 d after FTAI and pregnancy loss according to experimental group. There was no effect of treatment on P/AI on d31 ($P = 0.32$) or d60 ($P = 0.54$) and on pregnancy loss ($P = 0.22$). In this analysis were used only data from farms 3, 5 and 6 that provided information of second pregnancy diagnosis.

Regarding effects of E2 supplementation in cows also receiving GnRH to synchronize ovulation, there was no detectable effect on fertility parameters, which was similar to several studies. Brusveen et al. (2009) reported higher estrus expression but no improvement on fertility when cows received 0.5 mg estradiol-17 β concomitant with the second GnRH of the Ovsynch. Similarly, in cows presynchronized with 2 PGF and starting the breeding protocols 13 d after the second PGF, Hillegass et al. (2008) administered 1.0 mg EC 24 h after the PGF of Consynch-48 or Cosynch-72 and reported increased estrus but no effect on P/AI on d30 and d60 or on pregnancy loss. Souza et al. (2007), using the Presynch-Ovsynch-56 program for first FTAI postpartum, reported no effect of 1.0 mg of estradiol-17 β given 8 h before the second GnRH on P/AI 30d or 60d and pregnancy loss, despite the higher estrus expression in cows supplemented with E2.

Therefore, even developing an experimental design different from those studies mentioned above, which we considered adequate to evaluate the EC or GnRH as ovulation inducer and the effect of E2 supplementation, we could not detect any difference between ovulation inducers or effect of E2 supplementation. However, it is worthy to mention that some results suggested a potential need for higher E2 concentration at the end of the FTAI protocols in high producing dairy cows, in order to maximize fertility.

Ovarian dynamics and estrus expression

The proportion of cows with CL on D-15, which was the onset of the presynchronization protocol (~37 DIM), was not different ($P = 0.63$) between experimental groups as shown in Table 3. Overall percentage of cows without CL on D-15 was 32.3% (204/631; Table 3), and it was similar to other studies that reported anovulatory conditions of about 30% in early lactation and at the end of VWP (Chebel et al., 2010).

Table 3. Percentage of cows with CL on D-15, D0, D6, and ovulation rate to D0 of the breeding protocols according to experimental group.

Item	Experimental group			Overall % (n/n)	P-value
	EC	EC/G	G		
Cows with CL on D-15, %	65.2 (144/221)	69.7 (147/211)	68.3 (136/199)	67.7 (427/631)	0.63
Cows with CL on D0, %	80.0 (152/190)	81.5 (145/178)	81.4 (136/167)	80.9 (433/535)	
Cows with CL on D6, %	88.7 (118/133)	91.3 (115/126)	93.2 (109/117)	91.0 (342/376)	0.34
¹ Ovulation after D0, %	58.1 (75/129)	65.0 (78/120)	67.3 (76/113)	63.3 (229/362)	0.34

¹Ovulation to D0 was considered based on appearance of a new CL on D6 that was not present on D0 and if the cow had a cavity CL on D6 in the same ovary that had a compact CL on D0.

Ciclicity was not affected by parity, with primiparous and multiparous cows presenting similar proportions of CL on D-15 [66.5 (137/206) vs. 67.1% (161/240), respectively; $P = 0.71$]. On the other side, BCS impacted ciclicity status on D-15, in which cows with higher BCS presented greater likelihood of being cyclic on D-15 (Figure 5). Likewise, BCS affected probability of cows having a CL present on D0 ($P = 0.05$), or on D6 ($P = 0.02$) as shown in Figure 5. Other studies reported greater ciclicity in early lactation cows with higher BCS (Santos et al., 2009). The variation in BCS on D-15 (~37 DIM) can be a reflection of the transition period. Cows with better energy balance and healthy status during the transition period, have higher BCS and, consequently, resume their ciclicity earlier. In fact, Barletta et al. (2017) reported that cows that gained BCS during the transition period presented fewer clinical diseases and resumed ciclicity earlier postpartum compared to cows that maintained or lost BCS during the transition period.

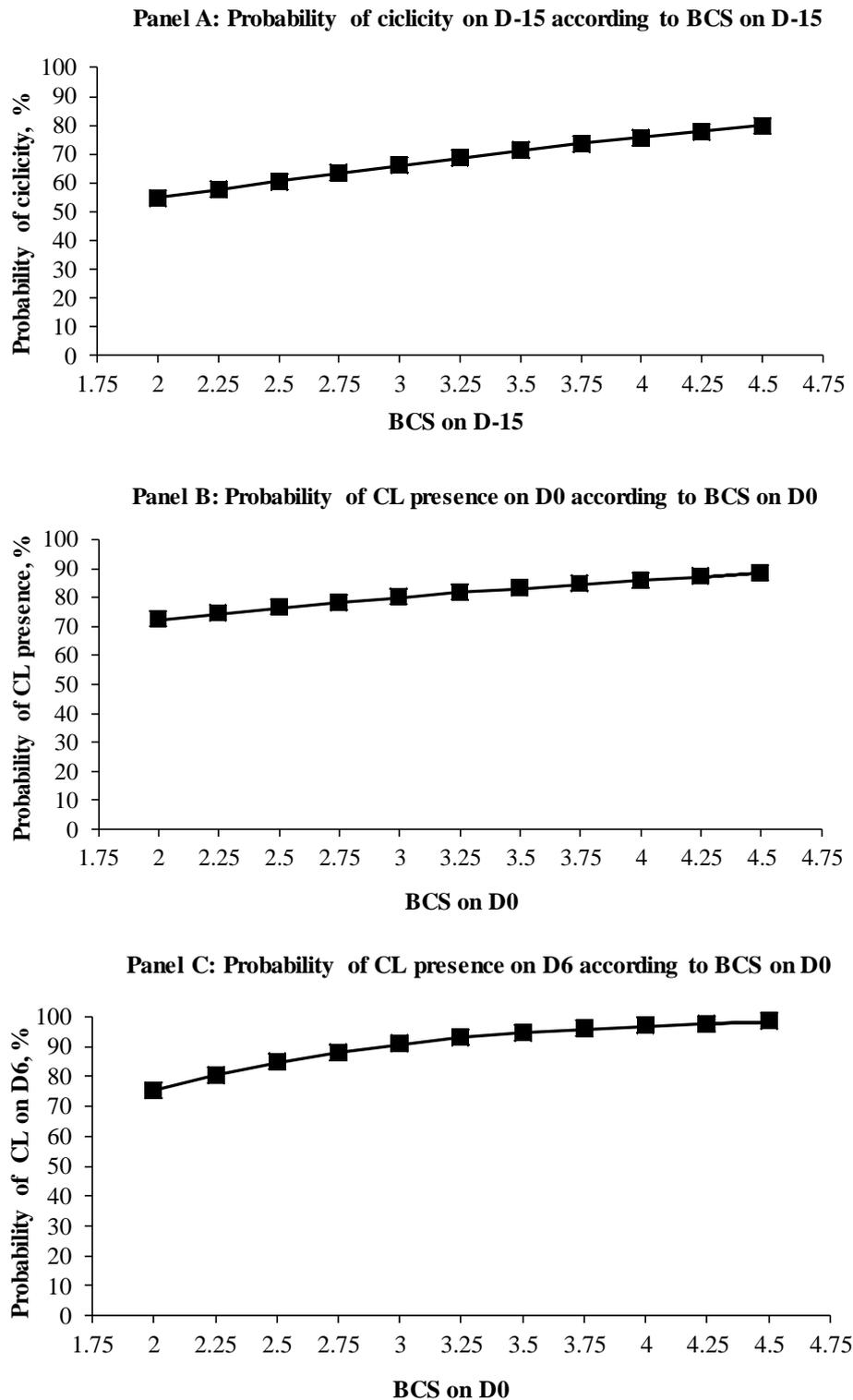


Figure 5. Probability of cows being cyclic on D-15 [panel A (n = 625)], and have a CL on D0 [panel B (n = 525)], or D6 [panel C (n = 356)] according to BCS on D-15, and D0. There was a linear relationship between BCS on D-15 and cyclicity on D-15 ($P = 0.008$), BCS on D0 and probability of having a CL on D0 ($P = 0.05$), and BCS on D0 and presence of CL on D6 ($P = 0.02$).

On D0, the cows had already been submitted to the presynchronization protocol and had chance to ovulate. The fact that BCS also affected the probability of cows to have a CL

on D0 suggests that BCS and/or the energy balance during early lactation could have influenced the response of the cows to the presynchronization strategy used in the study. Nevertheless, the proportion of cows that initiated FTAI protocols with CL was 80.9% (Table 3), similar to what has been reported in other reproductive programs for first FTAI that also used a presynchronization protocol. For example, Ayres et al. (2013) and Dirandeh et al. (2015) reported 68% and 77% of cows with CL at the onset of the Ovsynch protocol in the Presynch-Ovsynch program, respectively. Moreover, studies using Double-Ovsynch (Ayres et al., 2013; Carvalho et al., 2015) reported over 90% of cows starting the breeding Ovsynch with CL.

Ovulation at the onset of Ovsynch protocols has been associated with higher P/AI (Giordano et al., 2013; Carvalho et al., 2015). It is known that early diestrus, between day 5 and 9 of the estrous cycle, is the optimal stage of the estrous cycle to initiate Ovsynch protocols, in order to maximize the ovulatory response to GnRH (Vasconcelos et al., 1999).

In our study, based on the proposed methodology, overall ovulation rate to the GnRH administered on D0 of the protocol (GnRH1) was 63.3% (229/362), and did not differ ($P = 0.34$) between experimental groups (Table 3). Since a presynchronization strategy was used in the study, the ovulation in response to GnRH of the Ovsynch protocol could be higher, as reported by other authors that obtained over 80% of ovulation rate to the first GnRH of the FTAI protocol in Presynch-Ovsynch or Double-Ovsynch-synchronized cows (Gumen et al., 2012; Ayres et al., 2013). However, the ovulation rate of the present study was similar to other studies that reported rates around 60-70% (Souza et al., 2008; Giordano et al., 2013; Giordano et al., 2016). It should be mentioned that the 16.8 μg dose of buserelin acetate used on D0 of our study represents 68% more in relation to the recommended dose of 10.0 μg (Monteiro et al., 2015). This dose was increased based on preliminary data from our laboratory and data from others that have shown a decreased ovulation rate and lower GnRH-induced LH surge when cows are treated with a recommended dose of gonadorelin (100 μg) in the presence of a CL (Giordano et al., 2012; Melo et al., 2016). Besides, ovulation failure on D0 could also have occurred due to an inadequate response of cows to the presynchronization protocol. In fact, in contrast to Double-Ovsynch, the ovulation inducer used in our presynchronization protocol was EC, that synchronizes ovulation at ~72 h after EC treatment and P4 device withdrawal (Souza et al., 2009). Despite that, the dispersion of ovulation using EC is higher than when GnRH is used to induce ovulation (Pancarci et al., 2002; Souza et al., 2009). Therefore, considering the 8 d interval between P4 device withdrawal of the presynchronization protocol and treatment with GnRH on D0 of the FTAI

protocol, some cows may not have had ovulatory-size follicles at the time of GnRH (Sartori et al., 2001).

There is another aspect that is worthy to mention regarding ovulation rate to the first GnRH of the protocol. Traditionally, the Ovsynch protocol after a presynchronization does not use P4 devices, differently than the breeding protocols of the present study that used a P4 device concomitant with the GnRH treatment, promoting a relatively low, but sharp increase in circulating P4 concentration. It has been shown, for example, in cows submitted to a Presynch-Ovsynch protocol, that cows in the experimental groups with inclusion of a 1.38 g P4 device concomitant with GnRH of the Ovsynch had lower ovulation rate to GnRH compared to cows submitted to the traditional Ovsynch (67.7 vs. 87.1%; Galvão et al., 2004).

The proportion of cows with CL on D6, which was the moment of first PGF of the FTAI protocols, was high (~90%) and did not differ ($P = 0.34$) between experimental groups (Table 3). Studies using Presynch-Ovsynch or Double-Ovsynch also reported high (87 to 100%) proportion of cows with CL on that day (Ayres et al., 2013; Carvalho et al., 2015; Dirandeh et al., 2015; Giordano et al., 2016). Therefore, in our study, more than 90% of the cows had at least one CL during development of the preovulatory follicle, providing an adequate circulating P4 milieu for optimized fertility.

There was no effect of presence of CL on D0 ($P = 0.88$) or ovulation after D0 ($P = 0.21$) on estrus expression at the end of the FTAI protocol. As expected, cows from EC or EC/G groups expressed more estrus than cows from G group (Figure 6). The 80% (203/253) of cows in estrus from EC and EC/G groups was similar to reported by studies that also used EC at the time of P4 device withdrawal. For example, Pereira et al. (2015) reported 83.2% of estrus expression in 1780 cows submitted to a FTAI protocol that used EC as ovulation inductor. In relation to EC/G group, several studies reported higher estrus expression in cows that had their ovulation induced by GnRH but also received E2 supplementation (Souza et al., 2007; Brusveen et al., 2009). Lastly, the estrus expression in cows from the G group was higher compared to other studies that used GnRH to induce ovulation and reported 24 to 35% of estrus (Ribeiro et al., 2012; Bisinotto et al., 2013, 2015a, 2015b), but was similar to the 44% obtained by Souza et al. (2007). The possible increased estrus expression in group G could be due to the fact that in our study we used two treatments with PGF, and the first one was administered on D6 of the protocol and not on D7 as in the traditional Ovsynch protocols. This earlier PGF initiated an earlier decrease in circulating P4, leading to an earlier increase in LH pulsatility, allowing for the preovulatory follicle to produce more E2, and therefore, inducing more cows to manifest estrus behavior.

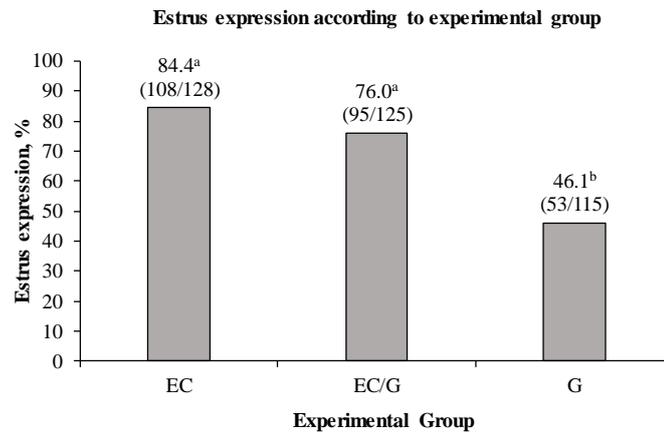


Figure 6. Estrus expression at the end of the FTAI protocols according to experimental treatments ($P < 0.001$). Cows in EC and EC/G group received 1.0 mg of estradiol cypionate on D7 (time of P4 device withdrawal) and G cows receive only GnRH on D8.5. FTAI was performed on D9 in all cows.

Parity influenced ($P = 0.002$) expression of estrus, with first and second lactation cows expressing more estrus [76.3% (184/241)] than cows in third or more lactations [58.1% (61/105)]. One reasonable explanation for this variation is the fact that older cows produce more milk than cows in the first two lactations. Therefore, they have lower circulating E2 because of higher dry matter intake and increased steroid catabolism by the liver (Sangristavong et al., 2002; Sartori et al., 2004).

There was no interaction between BCS and treatment in estrus expression, however BCS on D0 of the protocol influenced ($P = 0.02$) estrus expression, in which there was a positively linear effect of BCS on D0 on the probability of cows expressing estrus at the end of the FTAI protocol (Figure 7). The lower estrus expression in cows with low BCS has been reported by others (Souza et al., 2007; Hillegass et al., 2008).

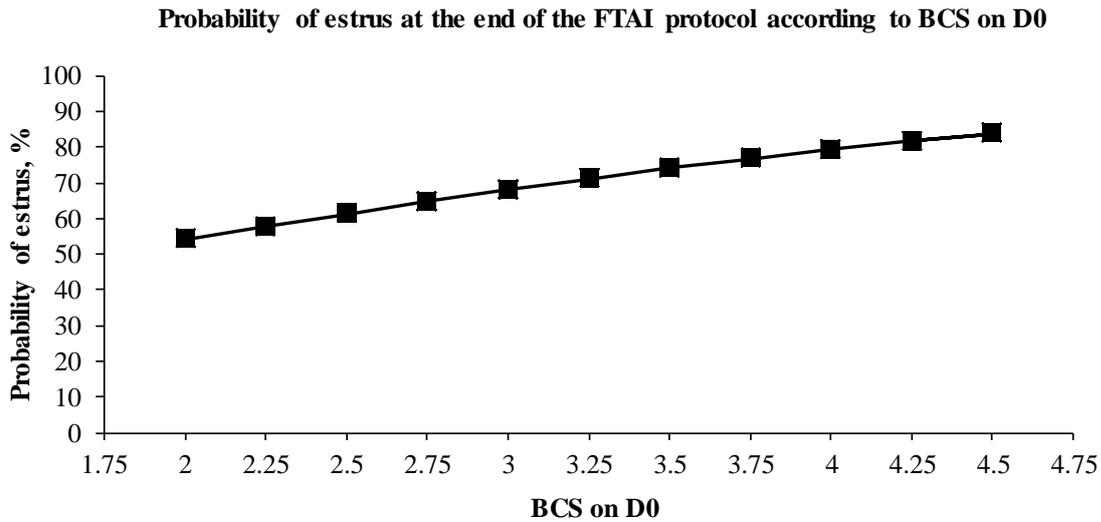


Figure 7. Probability of cows ($n = 346$) expressing estrus at the end of the FTAI protocols according to BCS on D0. There was a positive linear relationship between BCS on D0 and estrus expression ($P = 0.02$). There was no interaction ($P = 0.36$) between treatment and BCS on estrus expression.

Fertility according to farm, parity, BCS, CL status and ovulation to D0

Regarding P/AI on d31 and d60, as well as, pregnancy loss, there was no interaction between farm and any of variables as treatment, parity, BCS, CL on D0 or D6 and ovulation after D0 ($P > 0.10$). The P/AI 31 days after FTAI was higher ($P = 0.002$) for farm 6 compared to the other herds (Figure 8). The 60 d pregnancy diagnosis was performed only in farms 3, 5 and 6, and likewise, farm 6 had the highest ($P = 0.02$) P/AI on d 60 compared to the other two herds [49.5 (91/184) vs. 34.5 (80/232) vs. 29.6% (29/98) for farms 6, 5 and 3, respectively].

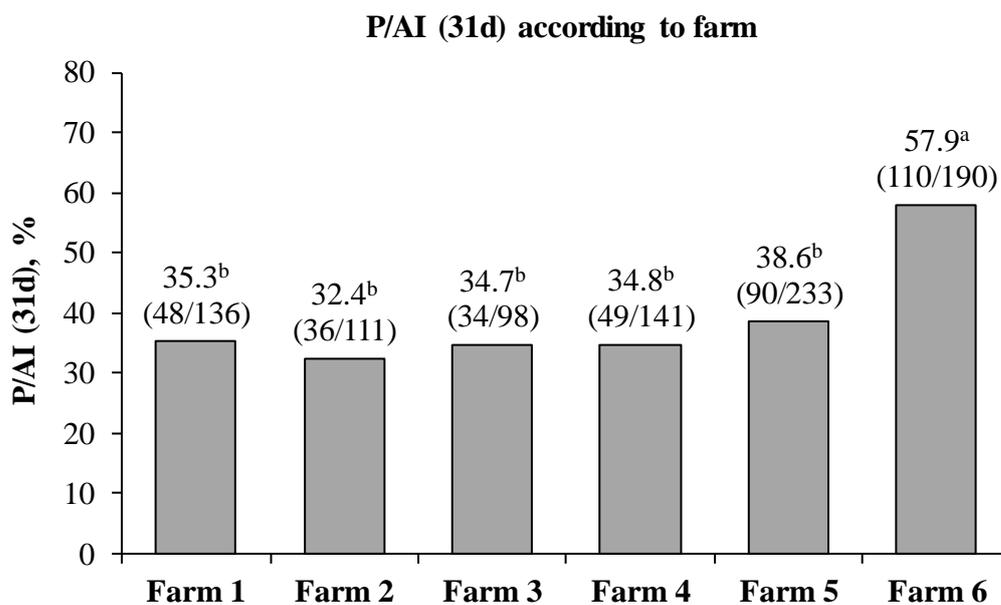


Figure 8. Pregnancy per AI 31 d after FTAI according to farm. ($P < 0.001$).

There was no interaction ($P = 0.46$) between parity and treatment on P/AI 31 d after FTAI, however primiparous had higher ($P < 0.001$) P/AI than multiparous cows in the 3 experimental treatments (Figure 9). In several studies that used primiparous and multiparous cows, the fertility was higher for primiparous (Pereira et al., 2016), even more in first postpartum FTAI (Carvalho et al., 2014; Giordano et al., 2016), since primiparous are producing less milk because they are in first lactation and may have fewer health problems postpartum such as subclinical hypocalcemia (Reinhardt et al., 2011) and metabolic or clinical disorders (Cheong et al., 2011; Pascottini et al., 2017). Another aspect that was recently discussed in the literature about fertility in primiparous and multiparous cows is uterine size. Baez et al. (2016), using Double-Ovsynch, reported higher P/AI on d32 and d67 for primiparous compared to multiparous cows. In addition, the authors reported a larger uterus size for multiparous cows and also established a negative impact on fertility as uterine size of the animals increased, particularly for multiparous cows. In our study, the E2 supplementation did not improve fertility of any category submitted to a Ovsynch type protocol, on the other hand, Souza et al. (2007), giving 0.5 mg of 17β estradiol 8 hours before final GnRH of Ovsynch, reported a positive effect of E2 supplementation on P/AI of primiparous cows.

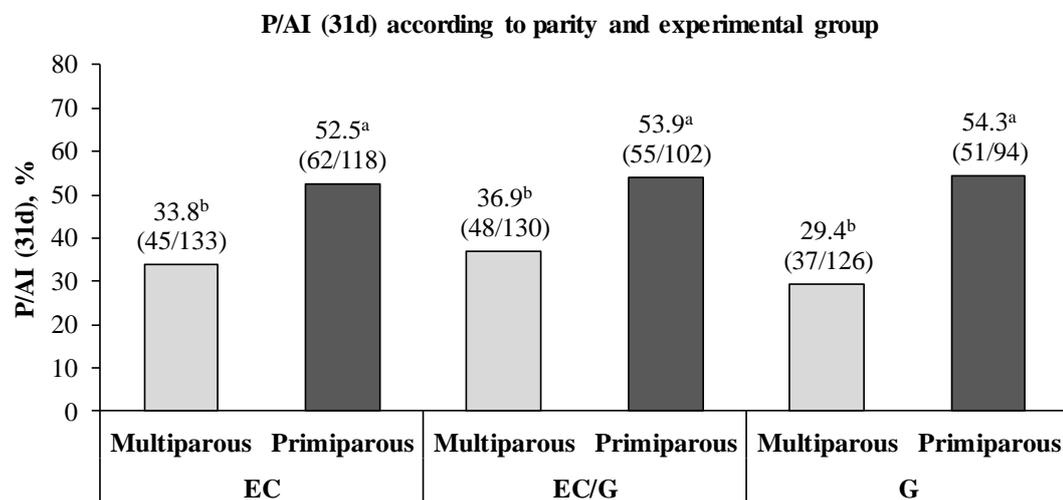


Figure 9. Pregnancy per AI 31 days after FTAI according to parity and experimental group. There was no interaction ($P = 0.46$) between parity and experimental group, however primiparous had greater P/AI than multiparous ($P < 0.001$)

When logistic regression was performed to evaluate the effect of BCS at the beginning of the breeding protocol on fertility parameters, we detected a positive linear effect of BCS on D0 on pregnancy risk at d31 ($P = 0.004$) and d60 ($P = 0.002$) after FTAI (Figure 10) and a tendency ($P = 0.08$) for a negative linear effect of BCS on D0 on pregnancy loss (Figure 10).

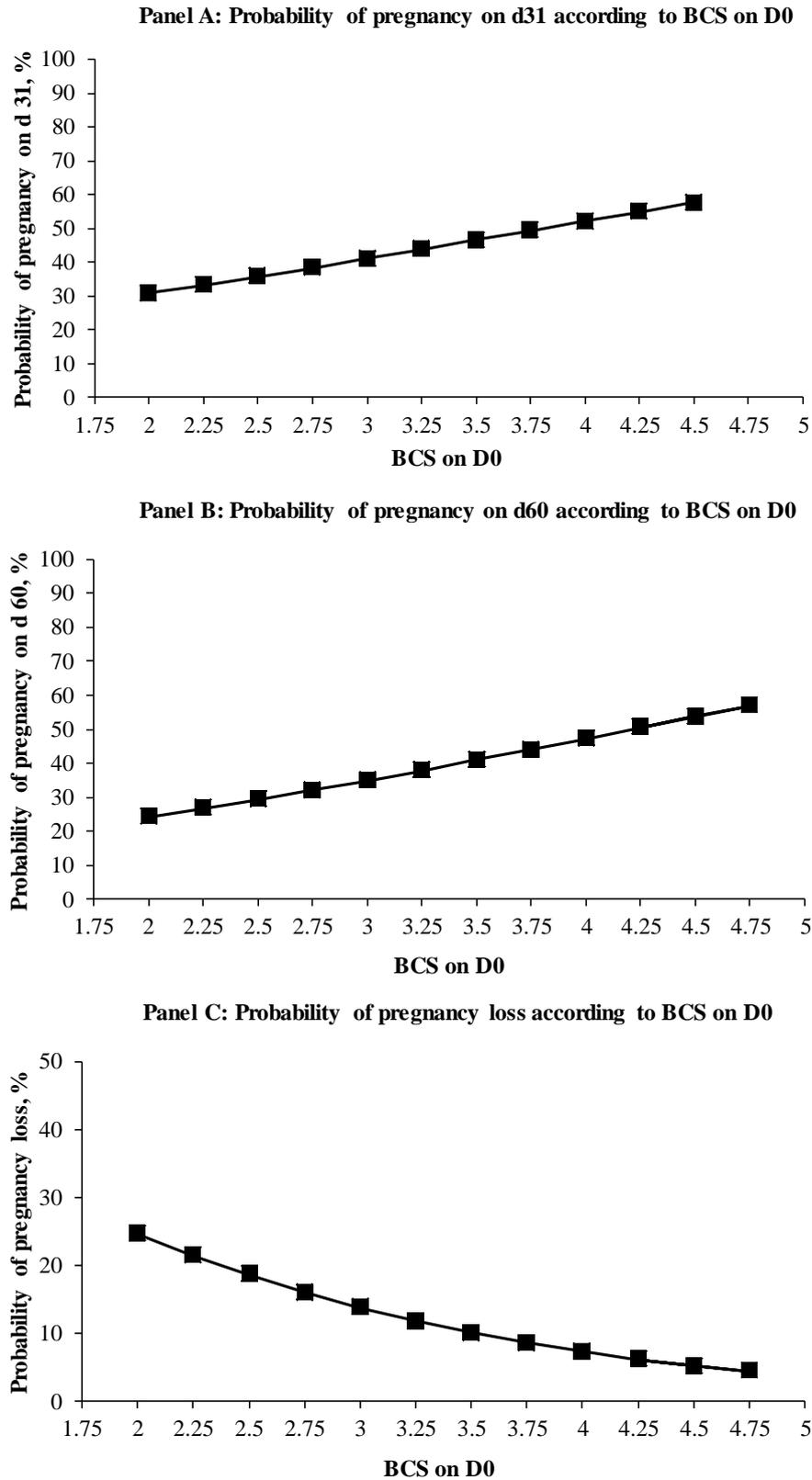


Figure 10. Probability curves for pregnancy on d31 [panel A (n = 500)] and d60 [panel B (n = 494)] after FTAI, and for pregnancy loss [panel C (n = 213)] according to BCS on D0. There was a linear effect of BCS on pregnancy on d30 ($P = 0.004$) and on d60 ($P = 0.002$), and there was a tendency ($P = 0.08$) for a linear effect of BCS on pregnancy loss.

It is well documented that BCS near the time of FTAI influences P/AI in cows submitted to first (Carvalho et al., 2014a) or later postpartum AIs (Pereira et al., 2016). Using more than 1.100 cows submitted to Double-Ovsynch for first FTAI postpartum, Carvalho et al. (2014a) reported that cows with BCS < 2.75 near the time of AI had the lowest P/AI on d70 compared to cows with BCS 2.75, 3.0, or ≥ 3.5 . Moreover, cows with BCS < 2.75 became pregnant later in lactation compared to cows with BCS ≥ 2.75 (median days open of 146 vs. 113, respectively). In our study, cows with BCS < 2.75 at the onset of the breeding protocol had lower ($P < 0.001$) P/AI compared to cows initiating the protocol with BCS ≥ 2.75 (16.7 vs. 43.4%, respectively). Despite studies have reported positive effects of E2 supplementation in cows with BCS < 2.75 (Souza et al., 2007), in our study there was no interaction ($P = 0.21$) between BCS and experimental treatment (Figure 11).

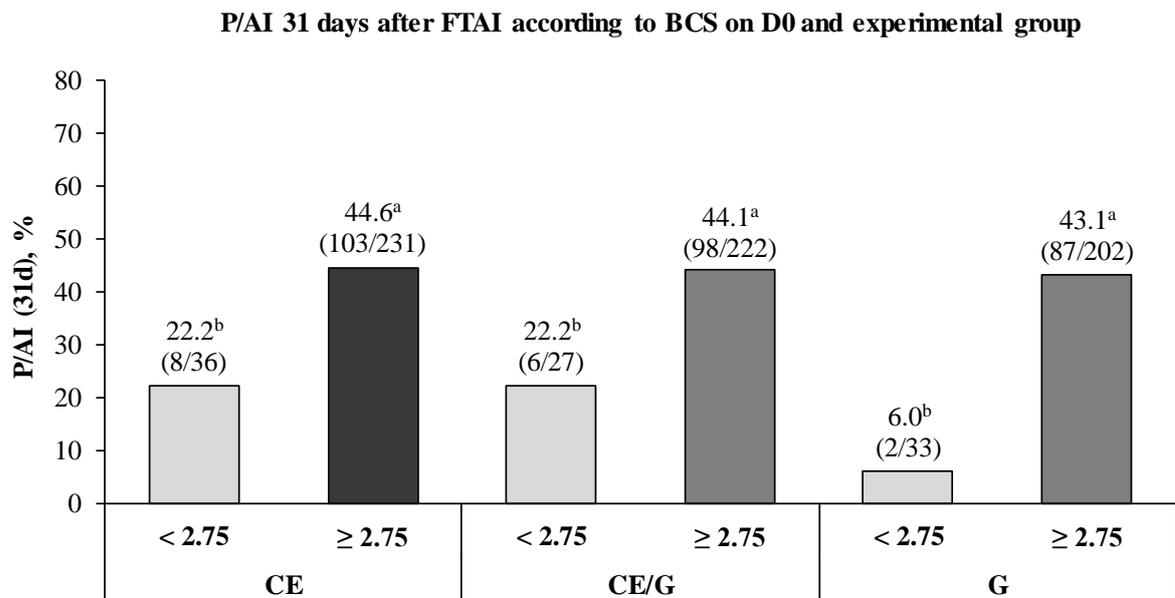


Figure 11. Pregnancy per AI 31 after FTAI according to BCS on D0 and experimental group. There was effect of BCS on D0 ($P < 0.0001$), but there was no interaction ($P = 0.21$) between BCS on D0 and experimental treatment.

It should be noted that in our study, BCS were positively related to the presence of CL on D0 and at the PGF of the breeding protocol. So, the better fertility of cows with higher BCS can be, at least, partially explained by the greater probability of higher BCS cows having a CL at the onset and at the time of PGF of the protocol, which is also associated with better fertility (Herlihy et al., 2012; Melo et al., 2016).

Regarding effects of presence of CL on D-15, D0 and D6, as well as, number of CL on D6, there were no interactions ($P > 0.10$) of these variables with treatment, BCS, parity,

ovulation after D0, and estrus. Figure 12 shows the P/AI 30 d after dos números FTAI according to presence of CL on D-15, D0, D6 and number of CL on D6, which was the time of PGF treatment of the breeding protocols.

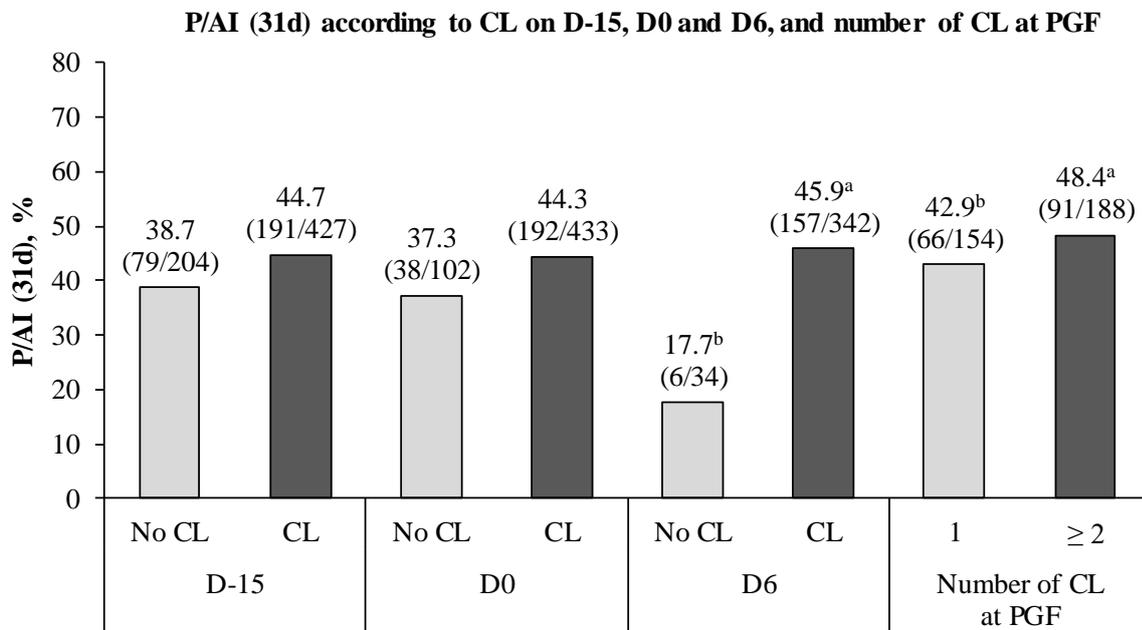


Figure 12. Pregnancy per AI 31 d after FTAI according to the presence of CL on D-15, D0 and D6, and according to number of CL on D6 (time of PGF treatment of breeding protocols). There was no effect of CL on D-15 ($P = 0.26$) or D0 ($P = 0.49$) on P/AI (31d). Cows with CL on D6 had higher ($P = 0.003$) P/AI (31d) than cows with no CL, and cows with ≥ 2 CL at PGF had greater P/AI (31d) compared to cows with 1 CL.

Despite the difference of 6-7 percentage points in P/AI (31d) between cows that had CL on D-15 or D0 compared to cows with no CL at these moments, there was no detectable effect of presence of CL on D-15 ($P = 0.26$) or D0 ($P = 0.48$) on P/AI 31 d after FTAI. The statistical difference could not be detected maybe due to the limited number of animals, since it is reported that cows that resume their cyclicity earlier in the postpartum period have higher fertility (Ribeiro et al., 2016) and cows that initiate the FTAI protocol without CL or with low P4 concentrations have lower fertility (Chebel et al., 2010; Herlihy et al., 2012; Ribeiro et al., 2012; Bisinotto et al., 2015a). It should be mentioned that the negative effect of absence of CL on D0 on P/AI may have been diminished in our study due to the fact that all cows received a 2.0 g P4 device (new or used once) on D0 of the protocol. In fact, Bisinotto et al. (2015a) reported that supplementation with two 1.38 g P4 devices in cows without a CL at the beginning of the Ovsynch protocol reestablished the fertility of these cows, promoting P/AI similar to cows that initiated the Ovsynch with CL.

As shown, 90% of the cows had CL on D6 of our study, and P/AI on d31 was higher ($P = 0.003$) for cows with CL on D6 compared to those cows with no CL at the time of PGF (Figure 12), which makes sense, since the presence of CL on D6 indicates that the follicle developed under higher circulating P4 than cows with no CL on D6. Supporting this finding, other studies reported higher fertility in cows with CL compared to cows with no CL or low P4 concentrations at PGF (Chebel et al., 2006; Giordano et al., 2013; Carvalho et al., 2014b; Melo et al., 2016).

Another analysis that we performed in order to highlight the importance of P4 during development of the preovulatory follicle was number of CL at PGF affecting P/AI 31 d after FTAI. For this purpose, we categorized the cows in two groups: cows with one CL at PGF or cows with ≥ 2 CL (Figure 12). It should be noted that this analysis was based only on presence of one or more CLs and did not consider whether the CL was from the presynchronization program or from ovulation after D0, which will be discussed ahead.

As mentioned earlier, cows with CL on D6 had good fertility. However, considering only cows with CL at PGF, those with 2 or more CL had greater ($P = 0.006$) fertility than cows with only one CL at PGF (Figure 12). The presence of two CL at PGF can be result of one CL generated by presynchronization and another from ovulation of the dominant follicle present at the first GnRH of the breeding protocol, which would be the cows perfectly synchronized by the reproductive program used in the study. Indeed, several studies reported that the best fertility is achieved by those synchronized cows that have high P4 (produced by two CL) at PGF (Giordano et al., 2013; Carvalho et al., 2014b, 2015). Giordano et al. (2013) and Carvalho et al. (2015) reported higher P4 concentrations at the PGF and greater synchronization rate in cows ovulating to the GnRH, thus, having two CL at PGF, as well as, P/AI was greater for these cows that ovulated in these studies.

In order to better describe the synchronization efficiency of the reproductive program used in our study, and to refine the understanding of circulating P4 effects during the protocol, we performed another analysis, now based on type of CL present on D6. Cows were categorized in four classes as shown in Figure 13. Those without CL on D6, cows with only a CL from the presynchronization protocol, cows with only a CL originated by the ovulation to GnRH on D0, and cows with two CL (one from presynch and another from ovulation to GnRH). Reinforcing, the fertility was higher in cows with CL on PGF compared to cows without CL, but, considering only cows with CL at PGF, the synchronized cows, which had 2 CL at PGF, one from presynch and another from ovulation in response to GnRH, had the greatest fertility (Figure 13). As discussed earlier, synchronized cows or cows that started the

protocol with CL and had a CL present at PGF had the highest fertility in other studies (Giordano et al, 2013; Melo et al., 2016).

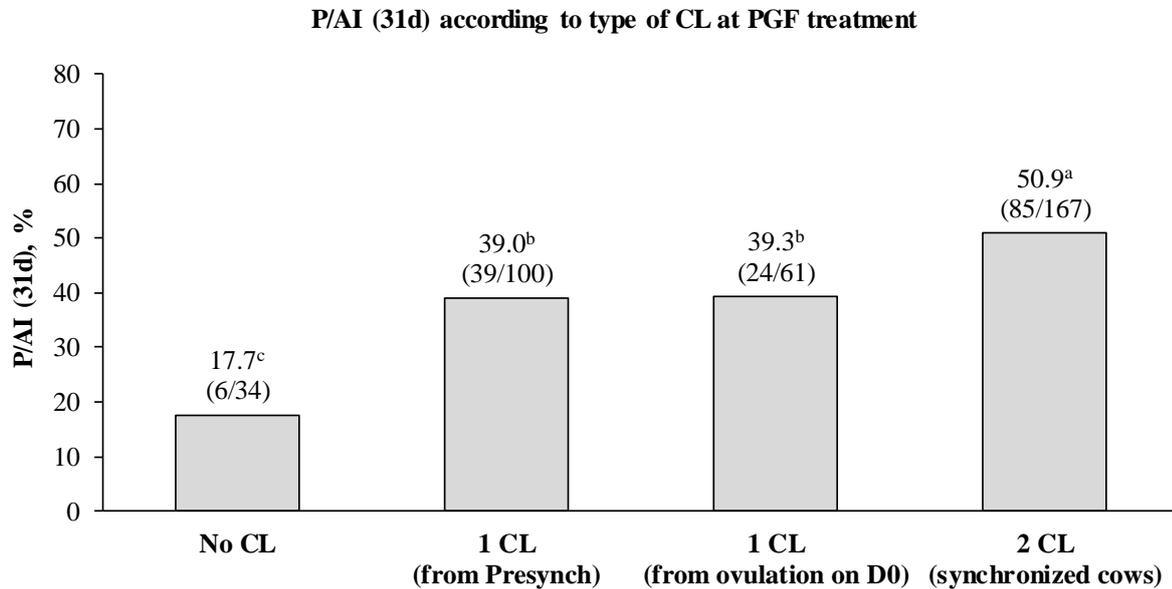


Figure 13. Pregnancy per AI according to type of CL at the time of PGF treatment of the breeding protocols ($P = 0.03$). The highest P/AI (31d) was achieved by cows considered perfectly synchronized, which was cows with 2 CL at PGF, one from the presynch program and another generated by ovulation on D0.

Although studies reported no effect of ovulation in response to first GnRH of the Ovsynch protocol on fertility, especially in cows with high P4 at the beginning of the protocol (Giordano et al., 2016), generally, cows that ovulated at the onset of the protocol had higher P/AI 30 d (Giordano et al., 2013; Bisinotto et al., 2015b; Carvalho et al., 2015) and 60 d (Chebel et al., 2006; Bisinotto et al., 2015b) after FTAI. In our study, there were no interactions between ovulation on D0 and presence of CL ($P = 0.40$) or treatment ($P = 0.44$) on fertility responses. Ovulation in response to GnRH administered at the beginning of breeding protocols affected ($P = 0.03$) P/AI 30 d after FTAI, in which cows ovulating achieved higher P/AI [47.8 (109/228) vs. 33.6% (45/134)], however, did not affect P/AI on d60 ($P = 0.24$) and only tended ($P = 0.0935$) to decrease pregnancy loss [3.3 (1/30) vs. 14.6% (12/82), for cows with and without ovulation on D0, respectively]. However, farm 5 that was one of the farms with lower pregnancy loss, did not provide information regarding ovulation after D0, thus, we have to be careful to make inferences about the effect of ovulation to D0 on P/AI (60d) and pregnancy loss.

Ovulation at the initiation of FTAI protocols can increase fertility due to the generation of a new CL during the protocol, which increases circulating P4 during follicle

development, in fact, studies reported higher P4 in cows that ovulated to first GnRH of the Ovsynch (Giordano et al., 2013). Another potential benefit of the ovulation on D0 is the emergence of a new follicular wave, which leads to ovulation of follicles with adequate age at the end of the protocol, assuring reasonable fertility, since cows that ovulate persistent follicles had decreased fertility compared to cows that had emergence of follicular wave at the beginning of the protocol (Monteiro et al., 2015).

1.4 Conclusions

In conclusion, this study has shown that it is possible to implement a reproductive program for first FTAI postpartum in commercial dairy herds that included a novel presynchronization strategy prior to the breeding protocols and achieve noteworthy fertility, especially in farms well managed and with good environmental conditions, such as fertility of farm 6. Regarding experimental treatments, the 3 strategies used to induce final ovulation of the reproductive program promoted similar fertility, thus, it may be used a strategy to induce ovulation that decreased estrus expression, since this is a desirable situation in numerous dairy herds. However, more studies must be performed, with a larger number of cows, in order to better evaluate aspects that we could not be conclusive in our study, such as the interaction between estrus expression and treatment on fertility parameters, as well as, deeply study the effect of treatments (ovulation inductors) on pregnancy per AI after d30 and on pregnancy loss.

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