Aus der
Nutztierklinik – Abteilung Wiederkäuer und Kameliden,
Arbeitsgruppe Reproduktionsmedizin und Eutergesundheit
des Fachbereichs Veterinärmedizin
der Freien Universität Berlin

Optimization of Ovsynch protocols in lactating dairy cows using modified prostaglandin treatments and progesterone intravaginal devices

Inaugural-Dissertation
zur Erlangung des Grades eines
Doktors der Veterinärmedizin
an der
Freien Universität Berlin

vorgelegt von
Marvin Hölper
Tierarzt aus Hachenburg

Berlin 2024
Journal-Nr.: 4454

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PUBLICATION II

Effect of a progesterone-releasing intravaginal device (PRID) for 8 days during a modified Ovsynch protocol on pregnancy outcomes in lactating Holstein cows.

ABBREVIATIONS

2PGF	Ovsynch with a second $PGF_{2\alpha}$ dose on d 8
AAM	Automated activity monitoring
AI	Artificial insemination
AUC	Area under the curve
BCS	Body condition score
°C	Celsius
CI	Confidence interval
CIDR	Controlled internal drug release
CL	Corpus luteum
Control	Control group
d	Day(s)
DIM	Days in milk
DMI	Dry matter intake
DoubleDose	Ovsynch with a double $PGF_{2\alpha}$ dose on d 7
ECM	Energy corrected milk
e.g.	Exempli gratia
et al.	Et alii/aliae/alia
e.V.	Eingetragener Verein
FN	False negative
FP	False positive
g	G force
G1	First GnRH treatment
G2	Second GnRH treatment
GnRH	Gonadotropin-releasing Hormone
h	Hour(s)
i.e.	Id est
IFNτ	Interferone tau
kg	Kilogram(s)

LH	Luteinizing hormone
mg	Milligram(s)
MHz	Megahertz
mL	Milliliter
mm	Millimeter
n	Number of samples
ng	Nanogram(s)
NRC	Nutrient requirements of dairy cattle
P	Probability
P4	Progesterone
P/AI	Pregnancy per artificial insemination
PG	$PGF_{2\alpha}$ treatment on d 7 of the protocol
$PGF_{2\alpha}$	Prostaglandin $F_{2\alpha}$
PRID	Progesterone-releasing intravaginal device
PRIDsynch	Ovsynch with addition of a PRID
ROC	Receiver-operating-characteristics
SD	Standard deviation
SEM	Standard error of the mean
TAI	Timed artificial insemination
TMR	Total mixed ration
TN	True negative
TP	True positive
TRM	Targeted reproductive management
Trt	Treatment
US	Ultrasonography
vs.	Versus
VS0	Vaginal discharge score 0
VS1	Vaginal discharge score 1
VS2	Vaginal discharge score 2
µg	Microgram(s)

1 INTRODUCTION

Reproductive management has changed significantly in the last decades, with breeding strategies for dairy cows shifting over the years from predominantly natural service to a greater adoption of artificial insemination (**AI**), pioneered by E. I. Ivanoff (Perry, 1960) and later complemented by more refined breeding strategies like ovulation-synchronization (**Ovsynch**) protocols (Stevenson and Britt, 2017). As reproductive performance is contributing to milk production in a major way, AI service rate and fertility to AI are key factors in determining the profitability of dairy farms. While cows can be classified as spontaneous ovulators, with ovulation occurring after behavioral estrus caused by a cascade of endocrine events, the success of AI in lactating dairy cows was limited by efficient estrus detection for many years. Due to physiologic variability and management factors, estrus detection remained a challenging endeavor for dairy farmers (Senger, 1994; Lopez et al., 2004). To circumvent this limitation, Pursley et al. (1995) developed a hormonal protocol, utilizing coordinated applications of gonadotropin releasing hormone (**GnRH**) and prostaglandin $F_{2\alpha}$ (**$PGF_{2\alpha}$**). This protocol aimed at turning cows into a type of induced ovulator, from a management perspective (Wiltbank and Pursley, 2014; Fricke and Wiltbank, 2022). The Ovsynch protocol has been widely adopted in the dairy industry (Caraviello et al., 2006). It consists of a GnRH treatment at protocol initiation (**G1**), followed by a $PGF_{2\alpha}$ injection on d 7 and another GnRH treatment on d 9 (**G2**), allowing for timed artificial insemination (**TAI**) approximately 16 hours after G2 without the need for estrus detection (Pursley et al., 1997). The protocol assumes that GnRH at a random stage of the estrous cycle induces ovulation of a follicle, therefore creating a corpus luteum (**CL**) and emergence of a new follicular wave. However, the efficiency of GnRH induced ovulation after G1 in cows submitted to a standard 7-d Ovsynch protocol at a random stage of the estrus cycle varies on average between 54 to 64 % (Vasconcelos et al., 1999; Bello et al., 2006). Injection of $PGF_{2\alpha}$ 7 d later is supposed to induce luteal regression of the CL, which allows for further development and maturation of a dominant follicle. After subsequent administration of GnRH at G2, the follicle is estimated to ovulate 24 h later, thereby allowing fertilization after AI. The optimal timing for TAI seemed to be 16 h after G2 in order to achieve optimum results for reproductive performance (Pursley et al., 1998). This precise sequence of hormonal treatments was developed to coordinate reproductive hormones with follicle development and corpus luteum, creating a somewhat

optimized hormonal environment and synchronized ovulation, aiming to result in pregnancy per AI (**P/AI**) comparable to P/AI in cows inseminated after estrus detection. The Ovsynch protocol initially increased the AI service rate, however, the risk of pregnancy per AI was not affected.

Five physiological principles have been identified that influence fertility in timed AI protocols (Cardoso Consentini et al., 2021): 1) synchronous emergence of a new follicular wave by ovulation of a dominant follicle after G1; 2) maintenance of elevated circulating P4 concentrations during development of the new preovulatory follicular wave; 3) efficient regression of the CL using $PGF_{2\alpha}$, therefore minimizing P4 and enhancing circulating estradiol near TAI; 4) synchronous ovulation of a follicle with adequate size and age after G2 to correspond with proper scheduling of TAI; and 5) provision of elevated and consistent circulating P4 from a properly functioning CL generated after the final ovulation.

One possibility to increase ovulation response after G1 is presynchronization, by decreasing cycle variation at the start of the TAI protocol. Several presynchronization strategies, such as Presynch-Ovsynch (Moreira et al., 2001), G6G (Bello et al., 2006) and Double-Ovsynch (Souza et al., 2008), have been developed in order to enhance the protocols efficacy by beneficially influencing hormonal environment at protocol initiation. For presynchronization of the cow's cycle, Presynch-Ovsynch utilizes two $PGF_{2\alpha}$ treatments 14 days apart and an 11 (Galvão et al., 2007), 12 (Moreira et al., 2001) or extended 14 day interval (Navanukraw et al. 2004) between the second $PGF_{2\alpha}$ injection and the start of the breeding Ovsynch. Theoretically, cows presynchronized this way should be on d 5 to 9 of the estrous cycle at G1, seemingly favorable for P/AI and synchronization rate (Vasconcelos et al., 1999) and increased probability of ovulation after G1. Other presynchronization strategies like G6G and Double-Ovsynch utilize GnRH additionally to the $PGF_{2\alpha}$ treatments, potentially making them more viable in anovular cows compared with Presynch-Ovsynch only utilizing $PGF_{2\alpha}$. These methods result in greater rates of ovulation in response to G1, because breeding Ovsynch should be synchronized to start at day 6 or 7 (Bello et al., 2006) of the cow's cycle, when responsive follicles of approximately 10 mm and above should be present on the ovaries and a GnRH induced LH surge should be sufficient in inducing ovulation.

Although ovulation after G1 is related to greater reproductive performance, luteolysis after $PGF_{2\alpha}$ treatment and a low progesterone concentration near TAI seem to have an even greater impact on P/AI (Stevenson, 2016). Incomplete luteolysis, determined by circulating P4 blood concentrations ranging from above 0.3 - 0.5 ng/mL at G2, occurs in 12 – 21 % of cows submitted to TAI protocols with a single $PGF_{2\alpha}$ treatment (Borchardt et al., 2018). These elevations in P4 result in a decreased magnitude of GnRH induced LH surge and delayed ovulation, ultimately compromising fertility (Stevenson and Pulley, 2016; Carvalho et al., 2018). Especially cows that do not bear a functional CL at protocol initiation, which are more likely to ovulate after G1, suffer from delayed or incomplete luteolysis after a single $PGF_{2\alpha}$ treatment because the newly formed CL may be reluctant to regression until d 7 after G1 (Momont and Seguin, 1984). Even though prostaglandin receptors are expressed earlier on the newly formed CL (Wiltbank et al., 1995), resistance to luteal regression seems inherent unless an older, functional CL is present simultaneously. If the older CL is present at the same time, luteolysis occurs in the newly formed CL after a single $PGF_{2\alpha}$ treatment as well (Stevenson, 2016). The exact mechanism for this phenomenon has not been determined. With approximately 30 % of first service cows (Stevenson et al., 2008) and 22 to 46 % of resynchronized cows (Fricke et al., 2003) not bearing a CL at initiation of a TAI protocol, these animals resemble a low fertility cohort more prone to incomplete luteolysis and subsequently decreased P/AI.

Two strategies have been developed to overcome incomplete luteal regression in cows submitted to TAI protocols, one of them consisting of an increased dose of $PGF_{2\alpha}$ at d 7, (Giordano et al., 2013), the other one of an additional $PGF_{2\alpha}$ treatment at d 8 of the protocol (Brusveen et al., 2009). Several studies have shown that adding a second $PGF_{2\alpha}$ treatment 24 h after the first application can reduce incomplete luteal regression and increase reproductive performance (Brusveen et al., 2009; Wiltbank et al., 2015). Additionally, Giordano et al. (2013) described comparable results regarding luteolysis and P/AI in multiparous cows when using an increased $PGF_{2\alpha}$ dose. However, the direct comparison of these alterations with a control group and inclusion of cyclic status by ovarian ultrasonography and hormonal blood sampling is only occasionally described (Barletta et al., 2018; Tippenhauer et al., 2021b).

Although marginally increased P4 concentrations at G2, caused by incomplete luteal regression, can have tremendous negative effects on fertility (Carvalho et al., 2018), high blood P4 concentrations at initiation of the protocol and during the phase of follicular

development are desirable. The negative feedback mechanism of progesterone depresses the LH pulse frequency in cattle (Kinder et al., 1996), most likely mediated by the kisspeptin/GPR54 system depressing GnRH release at the hypothalamus (Clarke and Pompolo, 2005; Dungan et al., 2006). This ensures an appropriate growth of the preovulatory follicle and avoids premature oocyte maturation, which otherwise would lead to impaired embryo quality (Rivera et al., 2011) and a decrease in fertility (Wiltbank et al., 2011). Once again, especially cows that do not bear a functional CL at protocol initiation suffer from insufficient P4 concentrations. However, it is known that high producing dairy cows with an elevated dry matter intake (**DMI**) have an increased liver blood flow and therefore greater steroid hormone catabolism, causing decreased circulating P4 concentrations (Sangsritavong et al., 2002). This leads to the assumptions that cows with a functional CL at protocol initiation may suffer from suboptimal P4 concentrations during follicular development as well. For optimization of the hormonal milieu und synchronization of ovulation, progesterone-impregnated inserts such as the progesterone-releasing intravaginal device (PRID) can be applied, releasing P4 via direct surface area contact with the vaginal mucosa. As a reaction to the P4 devices, a local inflammation can occur, leading to secretion of various amounts of vaginal discharge in a subset of cows (Santos et al., 2016), with a potential influence on reproductive performance.

A recent meta-analysis has summarized the effect of P4 supplementation in TAI protocols on fertility (Bisinotto et al., 2015b), with data from 25 randomized controlled studies including 8,285 supplemented cows and 8,398 untreated controls. Cows treated with a P4 device had a tendency for reduced pregnancy loss and increased P/AI, especially in cows without a CL at the start of the protocol. However, the analysis only included studies with a shortened or regular 7-day P4 application, mostly provided by a controlled internal drug release (**CIDR**) device with 1.38g of P4. One can only speculate about the outcomes when utilizing another P4 regimen, like an extended duration or a more frequent usage of other devices like the PRID, containing 1.55g of P4.

As luteal status seems to have such an enormous influence on fertility in cows submitted to TAI protocols, the ability to diagnose the presence or absence of a functional CL, characterized by a cutoff value of 1.0 ng/mL serum progesterone (Silva et al., 2007), is of particular importance. While transrectal palpation is still a commonly used method for detection of functional structures on the ovaries, ultrasonography examinations have shown to be a more reliable diagnostic tool to detect a functional CL (Bicalho et al., 2008). However,

specificity of CL detection, or rather the ability to detect the absence of a functional CL, seems to be challenging as corpora lutea can be observed in ultrasound examinations, although P4 does not exceed the 1 ng/mL threshold. This underlines the necessity for a more precise specification of a functional CL, based on the correlation between the extent of a CL and the serum P4 concentration.

It is obvious that circulating P4, emergence of luteal tissue as well as its timely regression and a precise determination of cyclic status is of major concern when submitting cows to TAI protocols, as the decision for potential protocol modifications, in order to increase fertility, may be based on these factors.

Therefore, the overall objectives of this thesis were 1) to compare the effect of an additional $PGF_{2\alpha}$ treatment and an increased dosage of $PGF_{2\alpha}$ on d 7 during an Ovsynch protocol with an untreated control group, regarding luteolysis and reproductive performance; 2) to determine the effect of P4 supplementation by means of a PRID insert for 8 days during an Ovsynch protocol on P/AI and pregnancy loss; 3) to evaluate vaginal discharge at device removal on fertility, and 4) to evaluate the diagnostic performance of transrectal ultrasound to detect a functional CL based on circulating P4 levels.

Publication I was published in the Journal of Dairy Science (Impact Factor 3.5 – 2022) and Publication II was published in JDS Communications.

2 PUBLICATION I

Effect of dose and frequency of prostaglandin F2α treatments during a 7-d Ovsynch protocol with an intravaginal progesterone releasing device on luteal regression and pregnancy outcomes in lactating Holstein cows.

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2.1 Abstract

Our objective was to evaluate the effect of 3 different Ovsynch protocols on progesterone (P4) and pregnancies per artificial insemination (P/AI), where all cows received a P4 releasing intravaginal device (PRID) from d 0 until d 8. We hypothesized that (1) both modified $PGF_{2\alpha}$ treatments lead to decreased P4 at the second GnRH treatment (G2), resulting in greater P/AI, (2) the treatment effect is influenced by the presence of a corpus luteum (CL) at the beginning of the protocol, and (3) potential vaginal discharge caused by the PRID does not have a negative influence on fertility. Lactating Holstein cows (n = 1,056) were randomly assigned to 1 of 3 treatment groups on a weekly basis (n = 356; control: d 0, 100 μg of GnRH + PRID; d 7, 25 mg of dinoprost; d 8, PRID removal; d 9, 100 μg of GnRH). Cows in the second group (n = 353) received an Ovsynch protocol with a double dose of $PGF_{2\alpha}$ (DoubleDose: d 0, 100 μg of GnRH + PRID; d 7, 50 mg of dinoprost; d 8, PRID removal; d 9, 100 μg of GnRH). Cows in the third group (n = 347) received an Ovsynch protocol with a second $PGF_{2\alpha}$ treatment 24 h after the first one (2PGF: d 0, 100 μg of GnRH + PRID; d 7, 25 of mg dinoprost; d 8, 25 mg of dinoprost and PRID removal; d 9, 100 μg of GnRH). All cows had their ovaries scanned to determine the presence of a CL at the beginning of the Ovsynch protocol. Vaginal discharge score (VS) was evaluated at PRID removal. All cows received timed artificial insemination approximately 16 h after G2. Pregnancy diagnosis was performed via transrectal ultrasonography (d 38 ± 3 after timed artificial insemination) and rechecked on d 80 ± 7 after timed artificial insemination. Blood samples were collected on d 0, 7, and 9 of the protocol to determine P4 concentrations. Treatment affected P4 at G2. Progesterone was lower for 2PGF and DoubleDose cows compared with cows in the control group (control 0.35 ± 0.02 ng/mL; DoubleDose 0.29 ± 0.02 ng/mL; 2PGF 0.30 ± 0.02 ng/mL). Overall, P/AI did not differ among treatments. We found, however, an interaction between treatment and CL at the first GnRH treatment. Cows lacking a CL at the first GnRH treatment in the 2PGF group had greater P/AI (47.9%) compared with the same type of cows in the DoubleDose group (32.7%). We observed an effect of VS on P4 concentration at d 7. We found an increase in P4 with greater VS. Vaginal discharge score at PRID removal tended to have a positive effect on P/AI at d 38 (VS0: 36.5%; VS1: 41.3%; VS2: 49.7%). In conclusion, the addition of a second $PGF_{2\alpha}$ treatment on d 7 and 8 of a 7-d Ovsynch protocol increased luteal regression and decreased mean P4 at G2. Cows treated with $PGF_{2\alpha}$ 2 times 24 h apart showed greater P/AI, compared with cows treated with an increased dose of $PGF_{2\alpha}$.

2.2 Key words

prostaglandin; luteal regression; timed artificial insemination; Ovsynch; dairy cow

2.3 Introduction

Protocols for timed AI (TAI) have been widely adopted in the dairy industry since their development in the late 1990s (Pursley et al., 1995; Caraviello et al., 2006). The Ovsynch protocol consists of a consecutive treatment with GnRH and $PGF_{2\alpha}$ in a certain time interval, allowing AI that is not dependent on estrus detection (Pursley et al., 1997) due to synchronization of ovulation. Initiating an Ovsynch protocol at a random stage of the estrus cycle may lead to suboptimum fertility because a proportion of cows will not ovulate after the first GnRH treatment (G1), and hence have a low concentration of circulating progesterone (P4) during the follicle growth phase. This results in reduced oocyte quality, a higher risk for pregnancy loss, and double ovulation (Revah and Butler, 1996; Martins et al., 2018; Carvalho et al., 2019). Intravaginal P4 devices can be used to counteract this effect, providing high P4 levels by mucosal absorption and therefore improving reproductive performance (Bisinotto et al., 2014, 2015b). As a reaction to the devices, a proportion of cows show vaginal discharge (Walsh et al., 2008; Santos et al., 2016b). Also, strategies for presynchronization, such as Presynch-Ovsynch (Moreira et al., 2001; Galvão et al., 2007), Double-Ovsynch (Souza et al., 2008; Herlihy et al., 2012), and G6G (Peters and Pursley, 2002; Bello et al., 2006), have been developed to ensure that cows are in an optimal stage of the estrus cycle to initiate the breeding Ovsynch protocol. This leads to more cows ovulating after G1 and results in a newly formed corpus luteum (CL) at d 7 of the Ovsynch protocol. Despite the beneficial effect of providing higher P4 levels during the follicle growth phase (Bisinotto et al., 2010; Wiltbank et al., 2014), the young CL does not necessarily regress completely after a single treatment with $PGF_{2\alpha}$, supposing that a mature CL is not simultaneously present at that time (Nascimento et al., 2014; Carvalho et al., 2018). Although the exact mechanism remains unclear, a co-existing mature CL seems to induce luteal regression after a single $PGF_{2\alpha}$ treatment in the young CL as well (Stevenson, 2016). Incomplete luteal regression, ranging from 0.3 to 0.5 ng/mL (Brusveen et al., 2009; Fricke et al., 2016; Carvalho et al., 2018), at the time of the second GnRH treatment (G2) can cause a dramatic decrease in pregnancies per AI (P/AI; Giordano et al., 2012b; Wiltbank et al., 2014; Borchardt et al., 2018; Carvalho et al., 2018). Modifications of

dose and timing of $PGF_{2\alpha}$ treatments in an Ovsynch protocol have been tested to increase reproductive performance by decreasing the percentage of cows without complete luteal regression at the end of the protocol. One of the strategies was implementing an additional $PGF_{2\alpha}$ treatment 24 h after the first treatment at d 7 of the Ovsynch protocol, increasing the percentage of cows with complete luteal regression and subsequently P/AI (Brusveen et al., 2009; Carvalho et al., 2015a; Wiltbank et al., 2015; Santos et al., 2016b; Rheinberger et al., 2020).

Alternatively, a greater dose of $PGF_{2\alpha}$ at the scheduled time (Giordano et al., 2013) was applied, which increased luteal regression in multiparous cows in a 7-d Ovsynch and tended to increase P/AI, but failed to reproduce similar results in a 5-d Ovsynch protocol, compared with cows receiving 2 $PGF_{2\alpha}$ treatments 24 h apart (Ribeiro et al., 2012; Stevenson et al., 2018). Despite several studies examining 1 of the 2 methods, there are only a few studies comparing these different modifications directly and with a control group, regarding reproductive performance and P4 concentrations (Barletta et al., 2018; Minela et al., 2021; Tippenhauer et al., 2021).

The objective of this study was to compare the effect of 2 $PGF_{2\alpha}$ treatments 24 h apart (25 mg of dinoprost) and the treatment of a double $PGF_{2\alpha}$ dose (50 mg of dinoprost) with a control group (25 mg of dinoprost at d 7) during a 7-d Ovsynch protocol with inclusion of a P4 releasing intravaginal device (PRID) on luteal regression and P/AI in lactating Holstein cows. We set out to test 3 hypotheses: (1) the risk of complete luteal regression and P/AI would increase in the modified Ovsynch protocols compared with the traditional 7-d Ovsynch protocol, (2) the treatment effect is influenced by the presence of a CL at the beginning of the protocol, and (3) eventual vaginal discharge due to intravaginal insertions does not have a negative effect on reproductive performances.

2.4 Material and Methods

All experimental procedures were approved by the Institutional Animal Care and Use Committee of the Freie Universität Berlin (Reference number 2347-A-3–2-2020).

2.4.1 Cows, Housing, and Feeding

The experiment was conducted from October 2020 until July 2021. Lactating Holstein cows (n = 1,056; 404 primiparous and 652 multiparous cows) from 1 commercial dairy farm in Northeast Germany were used in this study. The farm consisted of 1,600 Holstein Friesian cows with an average 305 ECM yield of 9,809 kg. Lactating cows were housed in a freestall barn with slatted floors and beds equipped with rubber mats. Group composition was dynamic, with cows entering and leaving the study barn depending on their calving dates. Cows were fed a TMR twice a day in a 12-h interval with free access to feed and water. The TMR consisted mainly of corn, alfalfa, and rye silage with brewer's grain, extruded flax, canola meal, and soda gain with an additional mineral mix. The rations were formulated to meet or exceed the requirements according to the NRC (2001). Lactating cows were milked 2 times daily, starting at 07:30 and 20:00 h.

2.4.2 Reproductive Management and Experimental Treatments

First postpartum AI was executed using a modified Presynch-Ovsynch protocol ($PGF_{2\alpha}$, 14 d later $PGF_{2\alpha}$, 12 d later start of an Ovsynch protocol) with cows receiving AI either at detected estrus after the first $PGF_{2\alpha}$ treatment (50 ± 3 DIM) or after second $PGF_{2\alpha}$ treatment (64 ± 3 DIM). Cows not detected in estrus during this presynchronization (41.5%; n = 465) received TAI according to the following Ovsynch protocol (86 ± 3 DIM). For the subsequent AI, nonpregnant cows not reinseminated based on estrus detection (46.0%; n = 591) received an Ovsynch protocol starting at the day of nonpregnancy diagnosis, at d 38 ± 3 post AI. All cows receiving TAI were randomly assigned within parity to 1 of 3 Ovsynch protocols on a weekly basis (Figure 1). Cows in the control group (n = 356) received an Ovsynch protocol with a single $PGF_{2\alpha}$ treatment (control: d 0, 100 µg of GnRH + PRID; d 7, 25 mg of dinoprost; d 8 PRID removal, d 9, 100 µg of GnRH). Cows in the second group (n = 353) received an Ovsynch protocol with a double dose of $PGF_{2\alpha}$ (DoubleDose: d 0, 100 µg of GnRH + PRID; d 7, 50 mg of dinoprost; d 8 PRID removal, d 9, 100 µg of GnRH). Cows in the third group (n = 347) received an Ovsynch protocol with a second $PGF_{2\alpha}$ treatment 24 h after the first one (2PGF: d 0, 100 µg of GnRH + PRID; d 7, 25 mg of dinoprost; d 8, 25 mg of dinoprost and PRID removal; d 9, 100 µg of GnRH). Cows that lost the PRID before scheduled removal (n = 56; control: 20;

DoubleDose: 23; 2PGF: 13), cows that left the herd before pregnancy diagnosis (n = 47; control: 16; DoubleDose: 16; 2PGF: 15), and cows that were not treated or inseminated according to the protocol (n = 23; control: 14; DoubleDose: 6; 2PGF: 3) were excluded. Gonadotropin-Releasing-Hormon (gonadorelin diacetate tetrahydrate; Ovarelin 50 µg/mL), $PGF_{2\alpha}$ (dinoprost trometamol; Enzaprost T 5 mg/mL), and P4 devices (P4; PRID Delta 1.55g) were from Ceva Santé Animale. Approximately 16 h after the G2, all cows received TAI by a trained AI technician. Conventional semen of multiple Holstein sires chosen by the farmer was used for AI.

2.4.3 Body Condition Scoring, Ovarian Ultrasonography, Evaluation of Vaginal Discharge

On study d 0, transrectal ultrasound (Easi-Scan:GO, IMV Imaging) assessment of the ovaries was performed to determine the absence or presence and size of a CL. Luteal structures were identified based on their echogenicity (i.e., isoechogenic). Dimensions were estimated by visualization of the luteal tissue on a grid of 5-mm squares displayed in the background of the ultrasound device screen. Number and size of every CL on each ovary was recorded. Cavities within the CL filled with anechogenic texture were also recorded and subsequently subtracted from the CL total size. Body condition score was evaluated at enrollment using a 5-point scale with 0.25 increments whereas 1 = thin to 5 = fat (Ferguson et al., 1994). Vaginal discharge at the day of the PRID removal was evaluated and scored from 0 to 2, as described by Walsh et al. (2007) and modified by Santos et al. (2016b), consisting of 0 = no debris, 1 = small flecks of purulent debris, and 2 = abundant amount of purulent debris on the intravaginal device and vulva. Determination of BCS, vaginal discharge score (VS), and CL measurement were performed by the same, trained individual (M.H.) throughout the study.

2.4.4. Blood collection and P4 Analysis

Blood samples were collected by venipuncture of the coccygeal vessels, using a 20-gauge, 1.5-inch hypodermic needle (Vacuette, Greiner Bio-One GmbH) at d 0, 7, and 9, to analyze the course of P4 concentration during the different protocols. Blood was extracted

into a sterile, plastic, evacuated serum collection tube with no anticoagulant (8 mL, Vacuette, Greiner Bio-One GmbH), and centrifuged after collection on the dairy farm at 2,000 × g for 10 min at approximately 20°C, then stored on ice for transport to the laboratory of Clinic of Animal Reproduction, where serum was transferred into sterile vials (2 mL, Cryovial, Simport) and stored at −18°C until shipment to a commercial laboratory (Segalab, accreditation number L0295). Serum P4 concentrations were determined, using an Enzyme labeled chemiluminescent competitive immunoassay (Immulite Progesterone Enzym, Siemens Healthcare). Intra- and interassay coefficients of variation for 20 assays for repeated samples averaged 7.35 ± 2.44% and 8.08 ± 2.42%, respectively. The lower limit of detection was 0.2 ng/mL.

2.4.5. Pregnancy Diagnosis

Pregnancy diagnosis was performed using transrectal ultrasound d 38 ± 3 after AI and reconfirmed d 80 ± 7 after AI by a veterinarian using a portable ultrasound device (Easi-Scan:GO, IMV Imaging), equipped with a 7.5-MHz linear-array transducer. A positive pregnancy diagnosis was based on visualization of an embryo with a heartbeat. Nonpregnancy was based on absence of pregnancy at the day of examination or a rebreeding to an estrus before pregnancy diagnosis. Pregnancy loss included cows that experienced pregnancy loss from d 38 to d 80.

2.4.6. Statistical Analysis

The results of pregnancy diagnosis and additional breeding information such as parity and number of AI were obtained from the herd management software (HerdeW, dsp agrosoft GmbH) and transferred to Excel (Microsoft Office 2016, Microsoft Deutschland Ltd.). Based on a statistical power calculation using MedCalc (version 15.6.1, MedCalc Software), enrollment of 350 cows per treatment allowed for detection of a 10 percentage-point treatment difference in P/AI (95% confidence; 80% power) based on a 1-tailed test. All data were analyzed using SPSS for Windows (version 25.0, SPSS Inc., IBM). Logistic regression models for P/AI and pregnancy loss analysis were built using the GENLINMIXED procedure of SPSS. Cow

was the experimental unit. Model building was conducted as recommended by (Dohoo et al., 2009), where each parameter was first analyzed separately in an univariable model. Only parameters resulting in univariable models with $P \leq 0.10$ were included in the final mixed model. Selection of the model that best fit the data was performed by using a backward stepwise elimination procedure that removed all variables with $P > 0.10$ from the model. The initial model included the following explanatory variables as fixed effects: treatment (control vs. DoubleDose vs. 2PGF), parity (primiparous vs. multiparous), BCS (≤2.75 vs. 2.75 to 3.5 vs. ≥ 3.5), AI number (first service vs. subsequent AI), and CL at G1 (yes vs. no), as well as interactions between these variables. Treatment and CL at G1 were forced to stay in the final model, regardless of their significance level. Insignificant effects and interactions were left out of the model. Therefore, the final model consisted of the following fixed effects: treatment, CL at G1, parity, and the interaction between treatment and CL at G1.

To evaluate the dynamics of P4 during the protocol (d 0, 7, 9), repeated measures ANOVA with first-order autoregressive covariance structure were performed using the GENLINMIXED procedure of SPSS. The outcome variable was serum P4 (ng/mL). The initial model contained the following explanatory variables as fixed effects: sampling time (d 0, 7, 9), treatment (control vs. DoubleDose vs. 2PGF), parity (primiparous vs. multiparous), AI number (first service vs. subsequent AI), CL at G1 (yes vs. no), and interactions between these variables. Insignificant effects and interactions were left out of the model. Therefore, the final model consisted of the following variables as fixed effects: treatment, CL at G1, parity, AI number, their interactions with sampling time, sampling time, and the interaction of CL at G1 and treatment. Due to a significant interaction of CL at G1 and treatment, we calculated 2 separate models for cows with and without a CL at G1.

A Bonferroni adjustment was used to account for multiple comparisons. Variables were declared to be significant when $P < 0.05$. A statistical tendency was declared when $P \geq 0.05$ and $P \leq 0.10$.

Cows were stratified into 6 categories (Figure 2) using P4 concentrations (≤0.2 to ≥0.6 ng/mL in 0.1 ng/mL increments) at G2. Pregnancy per AI was calculated for cows in each P4 section to determine a threshold with the greatest decrease in P/AI, using a chi-squared test. The threshold was used to categorize a cutoff value for incomplete luteal regression at G2.

The effect of VS on P/AI was evaluated using the GENLINMIXED procedure as described above. The initial model contained the following fixed effects: parity (primiparous vs. multiparous), BCS (≤2.75 vs. 2.75 to 3.5 vs. ≥ 3.5), AI number (first service vs. subsequent AI), CL at G1 (yes vs. no), and VS (0 vs. 1 vs. 2). Insignificant effects and interactions were left out of the final model; therefore, the final model consisted of VS and parity.

The effect of vaginal discharge on P4 dynamics was evaluated by repeated measures ANOVA using the GENLINMIXED procedure as described above. The initial model contained the following explanatory variables as fixed effects: sampling time (d 0, 7, 9), parity (primiparous vs. multiparous), AI number (first service vs. subsequent AI), CL at G1 (yes vs. no), VS (0 vs. 1 vs. 2), and interactions between these variables. Insignificant effects and interactions were left out of the model. Therefore, the final model consisted of the following variables as fixed effects: sampling time, VS, parity, AI number, CL at G1, and the interaction between VS and sampling time.

To determine the accuracy to identify a functional CL at the beginning of the Ovsynch protocol using transrectal ultrasound, we used a similar procedure as described by Sauls-Hiesterman et al. (2020). Accuracy, sensitivity, specificity, positive predictive value, and negative predictive value were calculated for the visual ability of the veterinarian to detect a CL by transrectal ultrasonography. The threshold for comparison of a functional CL at G1 was defined as P4 above 1 ng/mL. Positive detection of a CL and serum P4 above 1 ng/mL was defined as a true positive (TP). Absence of a CL and serum P4 below 1 ng/mL was considered to be a true negative (TN). A false positive (FP) was defined as detection of a CL when P4 was below 1 ng/mL. A false negative (FN) was defined as absence of a CL when serum P4 was above 1 ng/mL. Accuracy was calculated by dividing the TP by all positives [TP/(TP + FP)]. Sensitivity was calculated by dividing the TP by the sum of the TP and FN [TP/(TP + FN)], whereas specificity was determined by dividing the TN by the sum of the FP and TN [TN/(FP + TN)]. The positive predictive value was determined by dividing the TP by all test positives [TP/(TP + FP)]. The negative predictive value was determined by dividing the TN by all test negatives [TN/(TN + FN)]. To define reference criteria for identifying cows with a functional CL based on the CL diameter, we used a receiver operating characteristic (ROC) analysis. The continuous variable was CL diameter, and the classification variable was P4 concentration above 1.0 ng/mL. The ROC curves compare sensitivity with 100 − specificity.

The point on the ROC curve with the highest combined sensitivity and specificity was considered the critical threshold. Interpretation of this critical threshold was based on the area under the curve (AUC), according to Swets (1988), as noninformative (AUC = 0.5), accurate (0.5 < AUC ≤ 0.7), very accurate (0.7 < AUC ≤ 0.9), highly accurate (0.9 < AUC < 1), and perfect (AUC = 1).

2.5. Results

2.5.1. Milk Yield, BCS, and AI in Estrus

Descriptive data are summarized in Table 1. Average BCS did not differ among treatments (*P* = 0.840). Primiparous cows had greater (*P* = 0.001) BCS compared with multiparous cows (3.09 ± 0.3 vs. 2.77 ± 0.4). The proportion of cows with low BCS (≤2.50) was higher in multiparous cows (37.5%) than in primiparous cows (6.6%). However, the distribution of cows with low BCS did not differ among treatments (*P* = 0.633). Average milk production did not differ among treatments (*P* = 0.788, control: 34.9 ± 0.4; DoubleDose: 34.6 ± 0.4; 2PGF: 34.5 ± 0.4). The proportion of cows receiving first postpartum AI, thus having presynchronization before the Ovsynch protocol, did not differ (*P* = 0.603) among treatments. Days in milk at enrollment did not differ among treatments (*P* = 0.466).

2.5.2. P4 Concentrations

In total, 1,011 cows had a complete set of P4 concentrations from all 3 time points (Table 2). Mean P4 serum concentrations at enrollment did not differ (*P* = 0.630) among treatments (control 2.31 ± 0.11 ng/mL; DoubleDose 2.38 ± 0.11 ng/mL; 2PGF 2.24 ± 0.11 ng/mL) or parity (*P* = 0.222; primiparous cows 2.37 ± 0.09 ng/mL; multiparous cows 2.25 ± 0.07 ng/mL). Cows receiving first AI had lower (*P* = 0.001) P4 concentrations at G1 (1.94 ± 0.08 ng/mL) compared with cows receiving subsequent AI (2.68 ± 0.08 ng/mL). Cows with a CL at G1 had greater (*P* = 0.001) P4 concentrations at initiation of the protocol (4.2 ± 0.06 ng/mL) compared with cows lacking a CL at G1 (0.41 ± 0.11 ng/mL). On study d 7, mean P4 concentrations did not differ (*P* = 0.282) among treatments (control 3.65 ± 0.14 ng/mL; DoubleDose 3.54 ± 0.14 ng/mL; 2PGF 3.33 ± 0.15 ng/mL), but differed among parity (*P* =

0.001), with primiparous cows having greater P4 concentrations (3.82 ± 0.13 ng/mL) than multiparous cows (3.12 ± 0.1 ng/mL). Cows receiving first AI had greater (*P* = 0.001) P4 concentrations on study d 7 (4.24 ± 0.11 ng/mL) compared with cows receiving subsequent AI (2.8 ± 0.11). Cows with a CL at initiation of the protocol had greater (*P* = 0.001) P4 concentrations on study d 7 (4.34 ± 0.09 ng/mL) compared with cows lacking a CL at G1 (2.68 ± 0.14 ng/mL). Distribution of cows having P4 concentrations above 1.0 ng/mL, as considered a cutoff level for presence of a CL, at first $PGF_{2\alpha}$ application did not differ (*P* = 0.904) among treatments (control 85.7%; DoubleDose 84.7%; 2PGF 85.4%), but was greater (*P* = 0.001) for primiparous cows (359/389; 93.3%) compared with multiparous cows (504/622; 81.0%), and also (*P* = 0.001) for cows receiving first AI (402/443; 90.7%) compared with cows receiving subsequent AI (461/568; 81.2%). Proportion of cows with luteolysis differed among treatments (*P* = 0.025; control 84.8%; DoubleDose 91%; 2PGF 92.6%) considering only cows with P4 concentrations above 1.0 ng/mL (indicative of presence of a CL) at first $PGF_{2\alpha}$ (863/1011) and 0.5 ng/mL at G2 (indicative of complete luteolysis). Cows in the 2PGF group (*P* = 0.010) and in the DoubleDose group (*P* = 0.033) had a greater proportion of cows with complete luteolysis compared with control cows. The proportion of cows with complete luteolysis did not differ between DoubleDose and 2PGF cows (*P* = 0.525). The proportion of cows with complete luteal regression did not differ among parity (*P* = 0.229) or luteal status at protocol initiation (*P* = 0.267). We found, however, an interaction between treatment and CL at G1 (*P* = 0.045), with a greater proportion of cows with complete luteolysis in the 2PGF group (*P* = 0.06) and DoubleDose group (*P* = 0.017) compared with control cows, when no CL was present at G1. The proportion of cows with complete luteolysis differed (*P* = 0.032) among AI number (first AI 87.7%; subsequent AI 91.7%). Mean P4 concentrations at G2 differed (*P* = 0.037) among treatments (control 0.35 ± 0.02 ng/mL; DoubleDose 0.29 ± 0.02 ng/mL; 2PGF 0.30 ± 0.02 ng/mL) and were lower for 2PGF cows (*P* = 0.043) and DoubleDose cows (*P* = 0.023) compared with cows in the control group. Progesterone at G2 did not differ for DoubleDose and 2PGF cows (*P* = 0.753). Progesterone concentrations at G2 did not differ among parity (*P* = 0.425; multiparous 0.27 ± 0.01 ng/mL; primiparous 0.29 ± 0.02 ng/mL). Cows receiving first AI tended (*P* = 0.087) to have greater P4 concentrations at G2 (0.36 ± 0.01 mg/mL), compared with cows receiving subsequent AI (0.26 ± 0.03 ng/mL). Luteal status at G1 had no influence on P4 concentrations (*P* = 0.700). We found, however, an interaction between treatment and CL at G1 (*P* = 0.087). Cows without a CL at initiation of the protocol had decreased (*P* = 0.014)

P4 concentrations when treated with a double dose of $PGF_{2\alpha}$ and tended (P = 0.062) to have a decreased P4 concentration at G2 when receiving 2 consecutive treatments 24 h apart.

2.5.3. P/AI and Pregnancy Loss

Overall P/AI at d 38 ± 3 was 42.3%. When all 3 treatments were considered in the model, an effect of treatment on P/AI at d 38 ± 3 (Table 3) was not observed (P = 0.119). In pairwise comparison, however, cows that received an additional treatment 24 h apart had greater P/AI (46.5%) compared with DoubleDose cows (37.7%; P = 0.045), but did not differ compared with cows in the control group (40.3%; P = 0.144). Treatment effect for cows receiving a double dose compared with control cows was not significant (P = 0.548). Primiparous cows (44.6%; 185/404) tended (P = 0.051) to have greater P/AI at d 38 ± 3 post AI than multiparous cows (38.4%; 262/652). Service number had no effect (P = 0.867) on P/AI at d 38 ± 3 post AI.

Pregnancy per AI at d 80 ± 7 after AI was significantly greater for primiparous cows (42.9%) compared with multiparous cows (35.4%; P = 0.015). Treatment did not affect (P = 0.134) P/AI at d 80 ± 7 post AI. However, pairwise comparison indicated that cows in the 2PGF group tended to have greater (44.1%) P/AI than cows in the DoubleDose group (35.6%; P = 0.055) but did not differ compared with control cows (37.7%; P = 0.139). Pregnancy per AI did not differ between cows in the control group and DoubleDose cows (P = 0.620). Service number had no effect (P = 0.993) on P/AI at d 80 ± 7 post AI.

Treatment had no effect on pregnancy loss [P = 0.471; control 6.9% (10/144) vs. DoubleDose 3.6% (5/137) vs. 2PGF 5.8% (9/156)]. Parity tended to affect pregnancy loss (P = 0.09). Primiparous cows tended to have reduced pregnancy loss compared with multiparous cows [3.3% (6/181) vs. 7.0% (18/256)]. Service number had no effect (P = 0.514) on pregnancy loss.

2.5.4. Effect of Presence of a CL at G1

Of all the cows included in this study, 74% (781/1056) had a functional CL, based on serum P4 levels of 1.0 ng/mL or above at G1. Distribution of CL among treatments (P = 0.305)

did not differ (Table 1). Distribution of CL did not differ (P = 0.133) between primiparous and multiparous cows [76% (307/404) vs. 72.7 (474/652)] and also (P = 0.315) between cows receiving first service and subsequent AI [73.1% (340/465) vs. 74.6% (441/591)]. Overall P/AI at d 38 ± 3 after AI did not differ (P = 0.121) between cows with and without a CL at G1. For cows with a CL at G1, P/AI did not differ among treatments (P = 0.868). For cows without a CL at G1, an effect of treatment on P/AI was not observed (P = 0.110). Pairwise comparison, however, indicated that cows within the 2PGF group had greater (47.9%; P = 0.043) P/AI when compared with DoubleDose cows (32.7%), but did not differ compared with control cows (36.1%; P = 0.109). Pregnancy per AI did not differ between DoubleDose and control cows (P = 0.623). Presence of a CL had no significant effect on P/AI at d 80 ± 7 after AI (P = 0.280) as well as pregnancy loss (P = 0.203). Pregnancy per AI at d 80 ± 7 did not differ among treatments when considering the presence (P = 0.854) or absence (P = 0.130) of a CL. Cows in the 2PGF group, however, had greater P/AI (P = 0.047) if no CL was present at G1 compared with cows in the DoubleDose group, but did not differ for cows in the control group (P = 0.162). Pregnancy per AI did not differ when comparing cows in the DoubleDose group with control cows (P = 0.504).

2.5.5 Accuracy to Detect a Functional CL

The overall accuracy was 87.2% to identify a functional CL using transrectal ultrasound at the beginning of the protocol. The AUC was 0.901 and can be considered highly accurate. The optimum cutoff was a 20-mm diameter of the CL. Sensitivity and specificity was 89.6 and 80.0%, respectively. The positive predictive value was 92.8%. The negative predictive value was 73.1%.

2.5.6. Effect of Vaginal Discharge at PRID Removal on P/AI and P4

Overall, 6.5% (69/1056) of cows had a VS of 0, 73.9% (780/1056) had a VS of 1, and 19.5% (207/1056) had a VS of 2 (Figure 3). The distribution of VS at PRID removal did not differ among treatments (P = 0.265) or luteal status at G1 (P = 0.579). The distribution of VS was affected by parity (P = 0.001; primiparous cows: 3.7% VS 0, 72.3% VS 1, 24.0% VS 2 vs. multiparous cows: 8.3% VS 0, 74.8% VS 1, 16.9% VS 2) and service number (P = 0.046; first AI: 6.5% VS 0, 70.5% VS 1, 23% VS 2 vs. subsequent AI: 6.6% VS 0, 76.5% VS 1, 16.9% VS 2).

Vaginal discharge score had a significant association (P = 0.001) with P4 concentrations at the first $PGF_{2\alpha}$ treatment (Figure 4). Cows assigned to a VS of 2 had greater (4.3 ± 0.2 ng/mL) serum P4 levels than cows assigned to score 0 (3.0 ± 0.3 ng/mL; P = 0.001) and 1 (3.7 ± 0.1 ng/mL; P = 0.002). Progesterone concentration at d 7 was greater (P = 0.027) for cows assigned to VS 1 compared with cows assigned to VS 0. Vaginal discharge score at PRID removal tended to have an association with P/AI at d 38 after AI (Figure 3; P = 0.072). Pregnancy per AI did not differ (P = 0.459) among cows scored with a VS of 0 and 1 [36.5% (25/69); 41.3% (320/780)], but was significantly greater [49.7% (102/207)] for cows having a VS of 2, compared with VS 1 (P = 0.039), and tended to be greater compared with VS 0 (P = 0.066). Parity had a significant effect (P = 0.032), with primiparous cows having greater (45.9%) P/AI at d 38 ± 3 post AI compared with multiparous cows (39%).

Vaginal discharge had no relationship with P/AI at d 80 ± 7 after AI (P = 0.116). Pregnancy per AI did not differ (P = 0.848) among cows scored with a VS of 0 and 1 [37% (22/65); 38.2% (287/754)], but was greater [46.5% (91/196)] for cows having a VS of 2 compared with VS 1 (P = 0.045). Pregnancy per AI did not differ for cows with a VS of 0 and 2 (P = 0.190) and a VS of 1 and 2 (P = 0.123). Parity had a significant effect (P = 0.008) on P/AI at d 80 ± 7 post AI, with primiparous cows having greater (44.9%) P/AI compared with multiparous cows (36.2%).

Pregnancy loss was not affected by VS (P = 0.461).

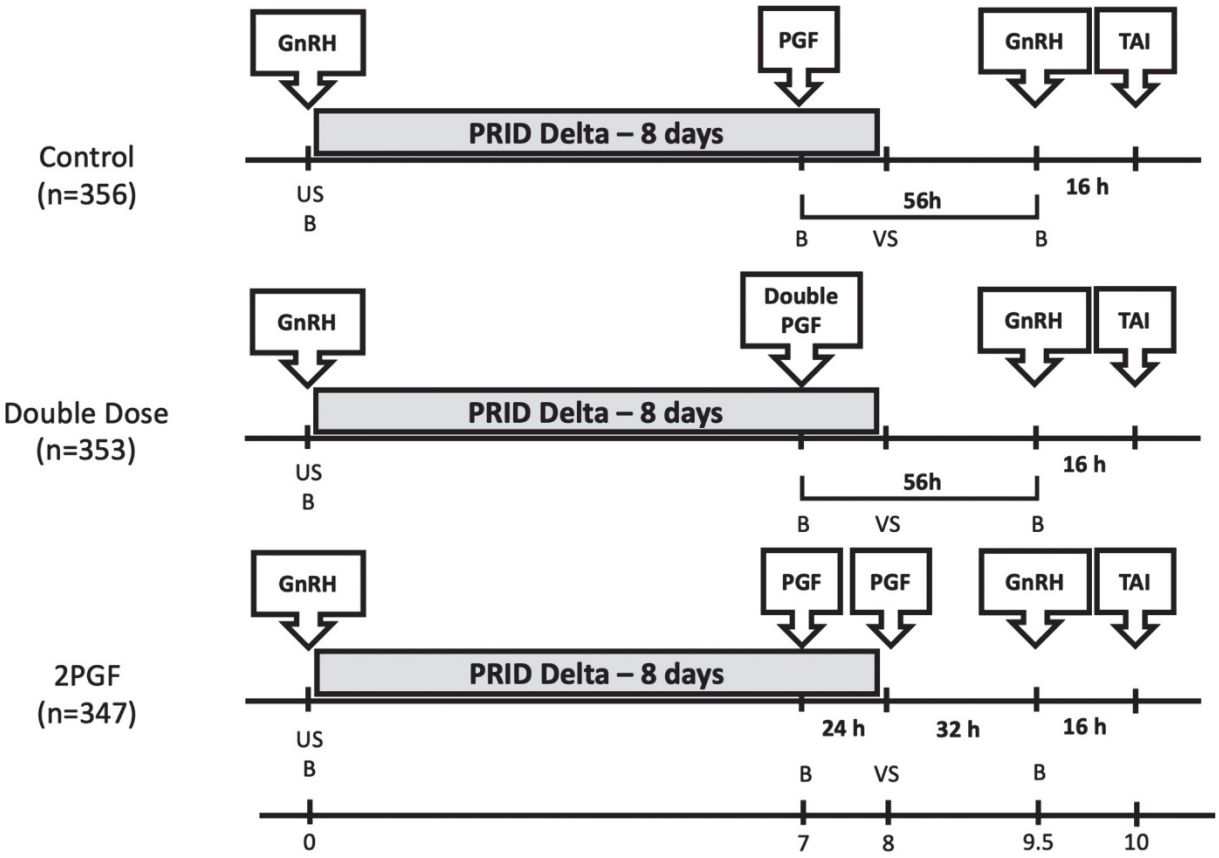

Figure 1. Schematic representation of the study design. Cows in the control group (n = 356) received an Ovsynch protocol with a single prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) treatment (control). Cows in the second group (n = 353) received an Ovsynch protocol with a double dose of $PGF_{2\alpha}$ (DoubleDose). Cows in the third group (n = 347) received an Ovsynch protocol with a second $PGF_{2\alpha}$ treatment 24 h after the first one (2PGF). All cows received a progesterone-releasing intravaginal device (PRID) at the start of the Ovsynch protocol, which was removed at d 8. Approximately 16 h after the second GnRH treatment, all cows received timed AI (TAI). Blood samples were collected on d 0, 7, and 9, and ultrasound examination was performed on d 0 of each protocol. B = blood sample; US = ultrasonography; VS = vaginal discharge score.

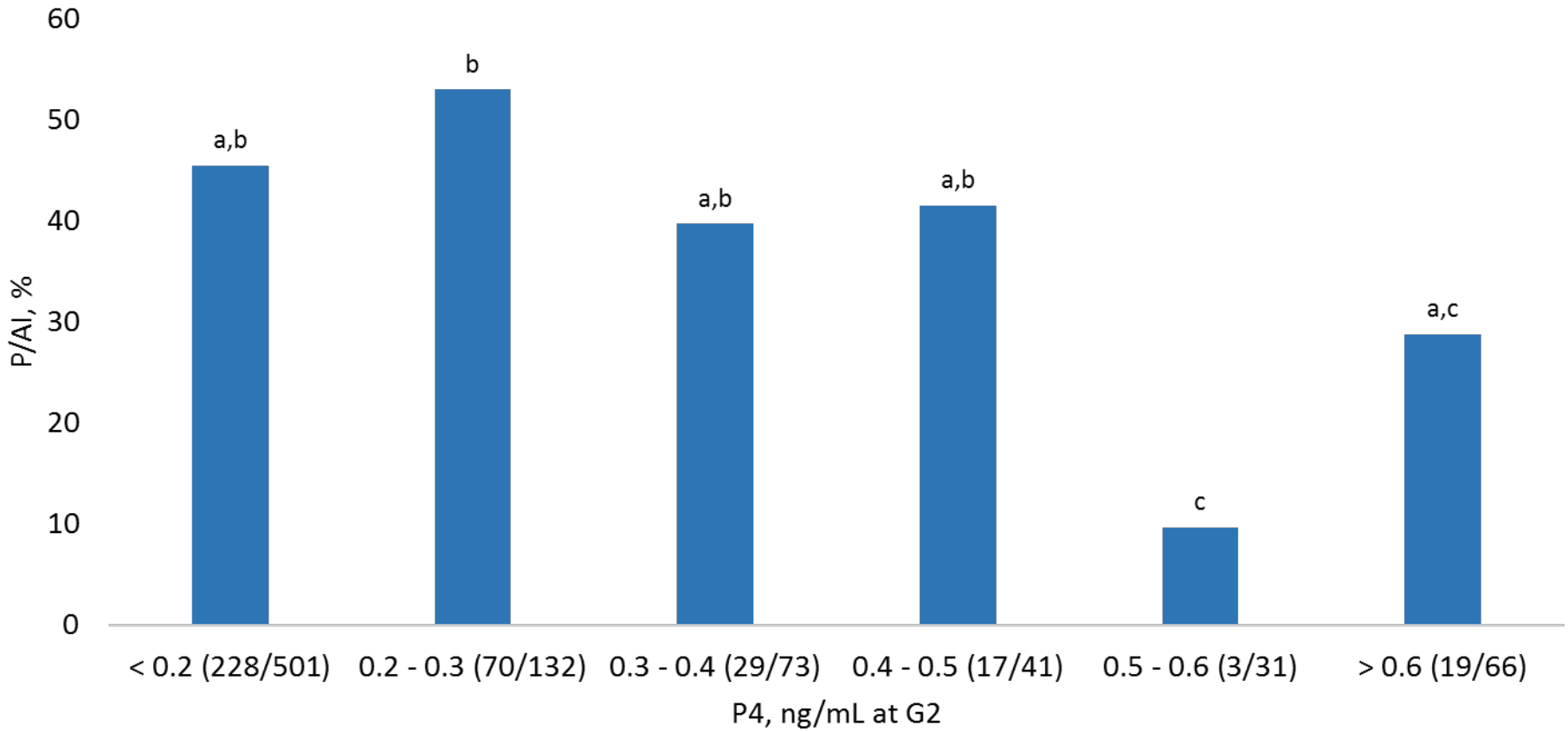

Figure 2. Comparison of pregnancy per AI (P/AI) among different progesterone (P4) concentrations at the second GnRH treatment (G2). Cows were stratified into 6 categories using P4 concentrations (≤0.2 to ≥0.6 ng/mL in 0.1 ng/mL increments) at G2. Pregnancy per AI was calculated for cows in each P4 section to determine a threshold with the greatest decrease in P/AI, using a chi-squared test. Bars with different letters (a–c) differed significantly ($P \leq 0.05$).

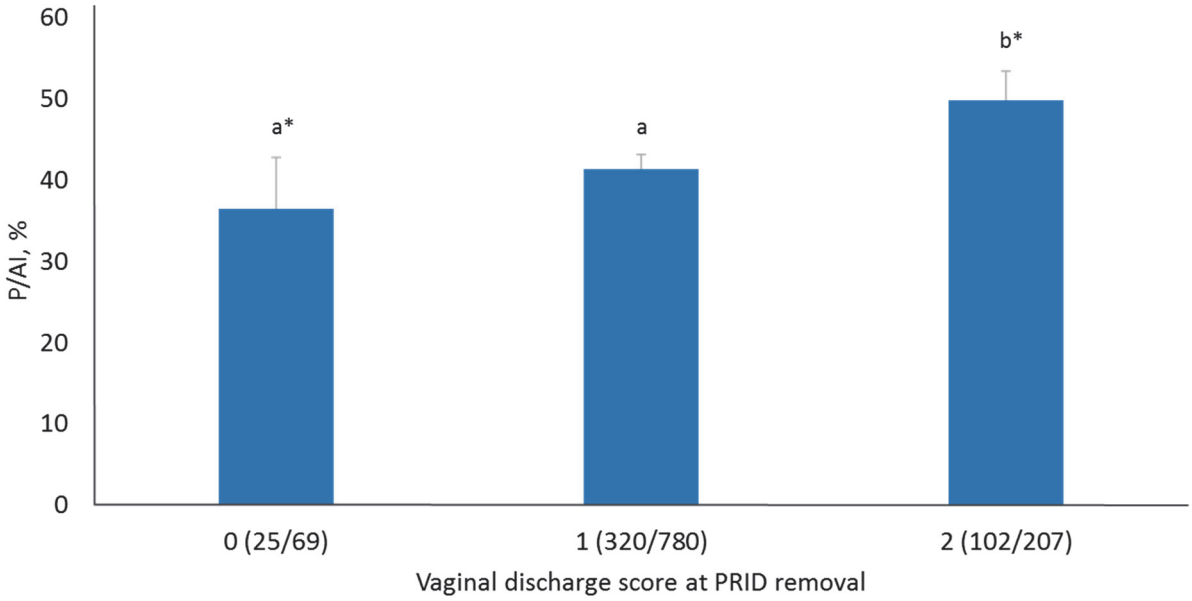

Figure 3. Comparison of pregnancy per AI (P/AI; ± SEM) at d 38 ± 3 post AI, depending on vaginal discharge score at removal of the progesterone-releasing intravaginal device (PRID). Vaginal discharge was evaluated in all cows at PRID removal using a scale of 0 to 2. A score of 0 indicated clear or no debris on the PRID (6.6% of cows), a score of 1 indicated small flecks of purulent debris on the PRID (73.8% of cows), and a score of 2 indicated abundant purulent mucus on the PRID (19.6% of cows). Treatment had no effect on vaginal discharge score (P = 0.265). Percentages for P/AI were derived from the GENLINMIXED model including vaginal discharge score and parity. Bars with different letters (a, b) differed significantly (P ≤ 0.05). Bars with * tended to differ ($P \leq 0.1$).

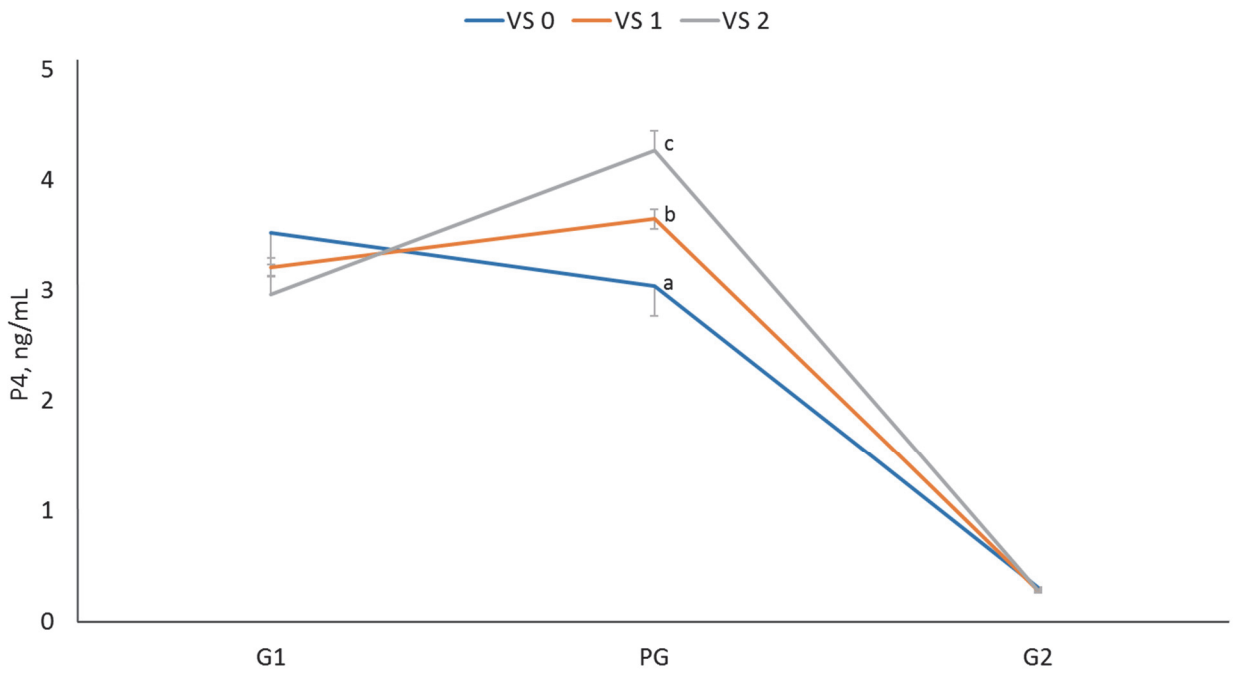

Figure 4. Comparison of progesterone concentration (P4, ng/mL ± SEM) at 3 different time points during a 7-d Ovsynch protocol depending on vaginal discharge score (VS) at removal of the P4-releasing intravaginal device (PRID). Vaginal discharge was evaluated in all cows at PRID removal using a scale of 0 to 2. A score of 0 (n = 69) indicated clear or no debris on the PRID (6.6% of cows), a score of 1 (n = 780) indicated small flecks of purulent debris on the PRID (73.8% of cows), and a score of 2 (n = 207) indicated abundant purulent mucus on the PRID (19.6% of cows). Treatment had no effect on VS (P = 0.265). Progesterone concentrations were derived from the GENLINMIXED model including sampling time, VS, parity, AI number, corpus luteum at G1, and the interaction between VS and sampling time. G1 = d 0 of the protocol; PG = d 7 of the protocol; G2 = d 9 of the protocol. Lines with different letters (a–c) differ significantly ($P \leq 0.05$) within each sampling time (i.e., G1, PG, G2).

Table 1. Comparison of descriptive data (±SEM) for cows enrolled in this experiment[1]

Item	**Treatment[2]**			***P*-value**
	Control	**DoubleDose**	**2PGF**	
DIM at enrollment	141.6 ± 4.2	136.1 ± 4.0	143.4 ± 5.0	0.466
BCS at enrollment	2.89 ± 0.02	2.90 ± 0.02	2.91 ± 0.02	0.840
Primiparous cows, %	37.9	38	38.9	0.955
Mean lactation number	2.24 ± 0.07	2.21 ± 0.07	2.27 ± 0.08	0.513
First service, %	43.5	46.2	42.7	0.603
Mean service number	2.24 ± 0.08	2.20 ± 0.1	2.31 ± 0.11	0.704
Active CL at enrollment[3], %	71.1	75.1	75.8	0.305

[1] Lactating Holstein cows were randomly assigned to receive (1) control: d 0, 100 µg of GnRH + progesterone-releasing intravaginal device (PRID); d 7, 25 mg of dinoprost; d 8, PRID removal; d 9, 100 µg of GnRH; (2) DoubleDose: d 0, 100 µg of GnRH + PRID; d 7, 50 mg of dinoprost; d 8, PRID removal; d 9, 100 µg of GnRH; (3) 2PGF: d 0, 100 µg of GnRH + PRID; d 7, 25 mg of dinoprost; d 8, 25 mg of dinoprost and PRID removal; d 9, 100 µg of GnRH. All cows received timed AI approximately 16 h after the second GnRH treatment (G2).

[2] DoubleDose = an Ovsynch protocol with a double dose of $PGF_{2\alpha}$; 2PGF = an Ovsynch protocol with a second $PGF_{2\alpha}$ treatment 24 h after the first one.

[3] Blood progesterone ≥1 ng/mL at enrolment; CL = corpus luteum.

Table 2. Comparison of progesterone (P4, ng/mL ± SEM) concentrations among treatments[1]

Item[2]	Treatment[3]			*P*-value[4]			
	Control	**DoubleDose**	**2PGF**	**Trt**	**C1**	**C2**	**C3**
P4 at G1, ng/mL	2.31 ± 0.11	2.38 ± 0.11	2.24 ± 0.11	0.630	0.612	0.649	0.340
P4 at PG, ng/mL	3.65 ± 0.14	3.54 ± 0.14	3.33 ± 0.15	0.282	0.586	0.115	0.305
P4 at G2, ng/mL	0.35 ± 0.02	0.29 ± 0.02	0.30 ± 0.02	0.037	0.023	0.043	0.753

[1] Lactating Holstein cows were randomly assigned to receive (1) control: d 0, 100 µg of GnRH + progesterone-releasing intravaginal device (PRID); d 7, 25 mg of dinoprost; d 8, PRID removal; d 9, 100 µg of GnRH; (2) DoubleDose: d 0, 100 µg of GnRH + PRID; d 7, 50 mg of dinoprost; d 8 PRID removal; d 9, 100 µg of GnRH; (3) 2PGF: d 0, 100 µg of GnRH + PRID; d 7, 25 mg of dinoprost; d 8, 25 mg of dinoprost and PRID removal; d 9, 100 µg of GnRH; all cows received timed AI approximately 16 h after the second GnRH treatment (G2).

[2] Progesterone (P4) concentrations were derived from the GENLINMIXED model including treatment, corpus luteum at d 0 of the protocol (G1), parity, sampling time, AI number, their interactions with sampling time and the interaction between treatment and corpus luteum at G1; PG = d 7 of the protocol.

[3] DoubleDose = an Ovsynch protocol with a double dose of $PGF_{2\alpha}$; 2PGF = an Ovsynch protocol with a second $PGF_{2\alpha}$ treatment 24 h after the first one.

[4] Trt = treatment; C1 = pairwise comparison between the control group and DoubleDose group; C2 = pairwise comparison between the control group and 2PGF group; C3 = pairwise comparison between the 2PGF group and DoubleDose group.

Table 3. Comparison of pregnancy per AI [P/AI, % (number of pregnant cows, n/number of all cows, N)] among treatments within luteal status at d 0[1]

Treatment	Luteal status[2]						P-value[3]			
	No CL, %			CL, %						
	Control	DoubleDose	2PGF	Control	DoubleDose	2PGF	Trt	C1	C2	C3
P/AI at d 38 ± 3	36.1	32.7	47.9	44.5	43.0	45.2	0.119	0.548	0.144	0.045
(n/N)	(37/103)	(29/88)	(39/84)	(111/253)	(113/265)	(118/263)				
P/AI at d 80 ± 7	35.5	30. Sep	45.8	40.0	40.7	42.4	0.134	0.620	0.139	0.055
(n/N)	(36/102)	(27/87)	(37/84)	(98/250)	(105/261)	(110/262)				

[1] Lactating Holstein cows were randomly assigned to receive (1) control: d 0, 100 µg of GnRH + progesterone-releasing intravaginal device (PRID); d 7, 25 mg of dinoprost; d 8 PRID removal; d 9, 100 µg of GnRH; (2) DoubleDose: d 0, 100 µg of GnRH + PRID; d 7, 50 mg of dinoprost; d 8, PRID removal; d 9, 100 µg of GnRH; (3) 2PGF: d 0, 100 µg of GnRH + PRID; d 7, 25 mg of dinoprost; d 8, 25 mg of dinoprost and PRID removal; d 9, 100 µg of GnRH. All cows received timed AI approximately 16 h after the second GnRH treatment.

[2] Percentages for P/AI were derived from the GENLINMIXED model including treatment, parity, CL at first GnRH treatment (G1), and the interaction of CL at G1 × treatment; no CL = lack of a corpus luteum at d 0 based on a progesterone threshold of 1 ng/mL at d 0; CL = presence of a corpus luteum at d 0 based on a progesterone threshold of 1 ng/mL at d 0; DoubleDose = an Ovsynch protocol with a double dose of $PGF_{2\alpha}$; 2PGF = an Ovsynch protocol with a second $PGF_{2\alpha}$ treatment 24 h after the first one.

[3] Trt = treatment; C1 = pairwise comparison between the control group and DoubleDose group; C2 = pairwise comparison between the control group and 2PGF group; C3 = pairwise comparison between the 2PGF group and DoubleDose group.

2.6 Discussion

Outcomes from this study provide additional evidence on the effect of different $PGF_{2\alpha}$ modifications in reproductive programs, using dinoprost tromethamine and the inclusion of a PRID. Failure of complete luteal regression (P4 above 0.3–0.5 ng/mL at G2) is a major problem that occurs in 12 to 21% of cows in TAI protocols with a single $PGF_{2\alpha}$ treatment (Brusveen et al., 2009; Heidari et al., 2017; Barletta et al., 2018). This has a deleterious effect on P/AI (Borchardt et al., 2018). Especially cows without a CL at the initiation of the protocol are prone to incomplete luteolysis. Cows, experiencing a low P4 environment, have a greater amplitude of LH surge induced by the G1 (Giordano et al., 2012a), leading to a higher ovulatory response (Giordano et al., 2013; Carvalho et al., 2015b) and a newly formed CL. The latter needs at least 5 d to be susceptible to a $PGF_{2\alpha}$ dose for induction of luteolysis (Pursley et al., 1995; Tsai and Wiltbank, 1998; Levy et al., 2000). The young CL remains reluctant to regression, although $PGF_{2\alpha}$ receptors with high $PGF_{2\alpha}$ affinity already exist within d 2 after ovulation (Pursley et al., 1995; Wiltbank et al., 1995). It is assumed that pulsatile release rather than the amplitude of $PGF_{2\alpha}$ contributes to luteolysis, which may explain the greater P/AI results in cows that received 2 consecutive treatments of $PGF_{2\alpha}$ (McCracken et al., 1999; Ginther et al., 2009). This finding was supported by the pattern of $PGF_{2\alpha}$ -induced gene expression being more distinct for luteolysis after multiple treatments compared with 1 single application (Atli et al., 2012).

In our study, cows benefitted most of the Ovsynch modification with a second $PGF_{2\alpha}$ treatment. The positive effect on reproductive performance can especially be seen in cows without a CL at G1, with an 11.8 percentage-points increase in P/AI compared with the control group. This supports the notion that a CL at the beginning of an Ovsynch protocol does influence treatment effects. However, these results need to be assessed cautiously because of the small subgroup of cows without a CL (n = 275) at the beginning of the protocol. Barletta et al. (2018) provided similar results for cows without a CL at initiation of a resynch protocol. These authors reported an increase of 19 percentage-points (P = 0.09) in cows treated with 2 consecutive doses of dinoprost tromethamine and also acknowledged that the lack of statistical significance might be due to the relatively small subgroup of cows without a CL at G1 (n = 66). In a recent study, Tippenhauer et al. (2021) compared a standard Ovsynch protocol including 1 $PGF_{2\alpha}$ treatment (0.5 mg cloprostenol) on d 7 with a double $PGF_{2\alpha}$ dose (1 mg) and 2 treatments (0.5 mg cloprostenol) 24 h apart on 8 different dairy farms. The results showed

that 2 consecutive cloprostenol sodium treatments increased P/AI by 8.5 percentage-points compared with the control group, whereas a higher $PGF_{2\alpha}$ dose increased P/AI by 1.6 percentage-points, indicating the inferiority of the latter. On the contrary, Minela et al. (2021) showed that both modifications achieve similar results when using cloprostenol sodium, considering the different metabolization leading to an enhanced half-life of approximately 3 h for cloprostenol sodium compared with approximately 8 to 9 min for dinoprost tromethamine (Reeves, 1978; Veronese et al., 2019). These cows were, however, synchronized using a Double-Ovsynch or a GnRH administration 7 d before Ovsynch for subsequent AI. Regarding the addition of a second $PGF_{2\alpha}$ treatment in a 7-d Ovsynch protocol, both types of prostaglandin have been shown to be effective (Barletta et al., 2018; Tippenhauer et al., 2021).

Different presynchronization protocols might explain differences between studies as fertility protocols including GnRH (e.g., Double-Ovsynch, G6G) increase the proportion of cows with a functional CL at the beginning of the protocol and with an accessory CL at the time of $PGF_{2\alpha}$ treatment. Although incomplete luteolysis is an issue related to this accessory CL, the simultaneous presence of an older CL, associated with higher P4 concentrations at protocol initiation or at the time of $PGF_{2\alpha}$ induced luteolysis (Martins et al., 2011), seems to have a beneficial effect on luteal regression in the accessory CL (Carvalho et al., 2018). In contrast to that, using a presynchronization with $PGF_{2\alpha}$ (i.e., Presynch-Ovsynch) and AI after spontaneous estrus (i.e., cherry picking) might lead to a greater proportion of cows without an active CL at G1. We observed no difference in the proportion of cows with an active CL at G1 for cows receiving first AI (26.9%) compared with cows receiving subsequent AI (25.4%). However, we found a difference in the average P4 concentration at G1 for cows receiving first AI (1.94 ± 0.08 ng/mL) compared with cows receiving subsequent AI (2.68 ± 0.08 ng/mL). We did not observe an interaction of treatment by service number regarding fertility. Therefore, it remains questionable whether the treatment effect of a $PGF_{2\alpha}$ modification is different among various presynchronization protocols.

In the literature, the effect of parity on P/AI is inconsistent when comparing a single $PGF_{2\alpha}$ dose at d 7 with a modified $PGF_{2\alpha}$ schedule. Although primiparous cows may have greater luteal regression compared with multiparous cows when submitted to an Ovsynch protocol (Martins et al., 2011; Giordano et al., 2012b), P/AI results of various studies differ. A recent meta-analysis by Borchardt et al. (2021) showed that in primiparous and multiparous

cows, an additional dose of $PGF_{2\alpha}$ on d 8 of the protocol yielded a 4.24 (95% CI = 0.31–8.17) and 5.31 (95% CI = 2.75–7.87) risk difference in P/AI, respectively. In our study, reproductive performance tended to be improved in primiparous cows. However, we found a potential confounding through BCS, with multiparous cows having overall lower BCS and more cows having a BCS below 2.5. A study by Atanasov et al. (2021) showed that thinner (≤2.75) cows were less successful in luteal regression and had lower P/AI compared with cows having a BCS above 2.75, whereas no difference was observed between treatments. On the contrary, Stevenson and Phatak (2010) stated that cows with a BCS below 2.5 had significantly greater luteolysis.

Progesterone concentrations below 1.0 ng/mL during the follicle growth phase result in lower P/AI (Carvalho et al., 2018) and greater risk for double ovulation (Carvalho et al., 2019). In this study, the relatively low pregnancy losses could be explained by the addition of the PRID device to the protocol. The insert provides P4 during the follicular growth phase in cows without a CL at G1, which has a beneficial effect on oocyte quality due to a decreased LH pulse frequency that would otherwise cause overstimulation (Kinder et al., 1996; Santos et al., 2016a). This assumption is supported by the outcomes of previous studies (Bisinotto et al., 2015a) observing decreased pregnancy loss or greater P/AI after TAI protocols with intravaginal P4 inserts. Herlihy et al. (2011) demonstrated that P4 inserts in combination with an Ovsynch protocol can increase conception rates when the P4 insert was applied from d 0 to d 8 of the protocol as implemented in our study. Furthermore, Lauber et al. (2021) observed a decrease of early estrus expression when delaying the removal of the P4 insert from d 5 until d 6 in a modified 5-d Cosynch protocol in dairy heifers. Whether the extended time of PRID insertion has an influence on fertility and cyclic synchronization, ultimately affecting reproductive performance and oocyte quality, needs to be addressed in further studies.

It has been shown in a recent meta-analysis (Bisinotto et al., 2015a) that the benefit of P4 supplementation was observed primarily in cows without CL at the initiation of the synchronization protocol. Therefore, the accuracy of detecting a functional CL is critical to maximize the effect of P4 supplementation. In our study, we observed a sensitivity of 89.6% and a specificity of 80%. The sensitivity is in the range with 2 other studies (Bicalho et al., 2008; Sauls-Hiesterman et al., 2020). Specificity seems a bit higher than what has been observed in these studies. Sauls-Hiesterman et al. (2020) described a discrepancy between different AI technicians in terms of specificity, ranging from 20 to 70% with an average of 39.8%, and

Bicalho et al. (2008) reported a specificity of 45.7%. The accuracy of a technician to identify cows with a functional CL needs to be taken into account to maximize the potential of P4 supplementation in cows with a CL.

Whereas high P4 levels during the follicle development can be advantageous, slight elevations of P4 (Giordano et al., 2012b; Carvalho et al., 2015b) near AI can cause a dramatic reduction in fertility (Brusveen et al., 2009; Wiltbank et al., 2012; Borchardt et al., 2018). This effect might be mediated through altered oviductal and uterine contractility (Hunter, 2005), reduced endometrial thickness (Souza et al., 2011), and a reduced magnitude of the LH surge after G2 (Stevenson and Pulley, 2016). Considering this, complete luteal regression in TAI protocols is crucial for fertility in dairy cows. Research by Barletta et al. (2018) described a tendency for cows with complete luteolysis when treated with 2 separate $PGF_{2\alpha}$ doses (94%), which also resulted in a tendency for greater P/AI results, where the increased dose of $PGF_{2\alpha}$ did not show any differences in luteal regression (88%) or P/AI compared with a control group (88%). Our results confirm those findings as cows in the 2PGF group had significantly greater risk for luteolysis (92.6%) compared with control cows (84.8%). The DoubleDose cows, however, also had greater risk for luteal regression, but this was not accompanied by an improvement in fertility.

An interesting observation was the association between VS at PRID removal and P/AI. To the best of our knowledge, this is the first study, where we observed greater P4 levels at d 7 of the protocol in cows with a greater VS (Figure 4). The higher P4 levels observed in those cows with more local inflammation, based on mechanical irritation (Ahmadi et al., 2007), may be due to higher vaginal blood flow and thereby an increased hormonal absorption. In addition to that, however, the results observed in our study may be influenced by service number and parity. Several studies did not show a negative or positive influence of VS after vaginal P4 insert removal on reproductive performance and P4 concentrations. Walsh et al. (2008) could not find an association between vaginal discharge caused by intravaginal devices and systemic inflammation. This indicates that inflammation caused by a P4 insert is locally limited to the vagina. Considering the results of the aforementioned authors, we conclude that intravaginal P4 inserts have no negative effect on P/AI. In our study, this local inflammation in the vagina was actually associated with greater P4 levels leading to greater P/AI.

The study was limited by the total number of cows enrolled, creating relatively small subgroups. The beneficial effect of constantly high P4 levels during the follicular growth phase, regarding pregnancy loss, remains hypothetical, as it was not the scope of the study to validate an effect of P4 on pregnancy loss.

2.7 Conclusions

A second treatment of dinoprost at d 8 of the Ovsynch protocol combined with a PRID increased overall P/AI by 6.2 percentage-points. An increased dinoprost dose did not result in better fertility. Particularly, cows without a CL at G1 benefitted from a second treatment with dinoprost, whereas cows with a CL at G1 did not show improved fertility. Two consecutive dinoprost treatments decreased P4 levels near TAI as well as doubling the dose of dinoprost. Vaginal discharge score at d 8 of the protocol, after PRID removal, was associated with higher P4 concentrations at d 7 and greater P/AI.

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3 PUBLICATION II

Effect of a progesterone-releasing intravaginal device (PRID) for 8 days during a modified Ovsynch protocol on pregnancy outcomes in lactating Holstein cows.

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3.1 Abstract

Our objective was to evaluate the effect of a progesterone-releasing intravaginal device (PRID) in a 7-d Ovsynch protocol on pregnancy per artificial insemination (P/AI) and pregnancy loss, compared with a standard 7-d Ovsynch protocol without progesterone supplementation. We hypothesized that progesterone supplementation during an Ovsynch protocol would increase P/AI and decrease pregnancy loss. Data were collected on lactating Holstein cows (n = 716) that either received a 7-d Ovsynch protocol (control: d 0, 100 μg of GnRH; d 7, 500 μg of cloprostenol; d 9, μg of GnRH; n = 360) or a modified Ovsynch protocol with addition of a PRID (PRIDsynch; d 0, 100 μg of GnRH + PRID; d 7, 25 mg of dinoprost; d 8, PRID removal; d 9, 100 μg of GnRH; n = 356). All cows received timed artificial insemination (TAI) approximately 16 h after the second GnRH treatment. Pregnancy diagnosis was performed via ultrasonography on d 38 ± 3 after TAI and rechecked on d 80 ± 7 after TAI. Reproductive performance differed between treatments, with PRIDsynch cows having greater (38.9%) P/AI compared with control cows (31.7%) at d 38 ± 3 and also at d 80 ± 7 (34.6% vs. 28.9%, for PRIDsynch and control cows, respectively). Pregnancy loss did not differ among treatments.

3.2 Summary

The objective of this study was to compare the effect of a progesterone-releasing intravaginal device (PRID) in a 7-day Ovsynch protocol on pregnancy per artificial insemination and pregnancy loss to a standard 7-day Ovsynch protocol without progesterone supplementation. Progesterone supplementation increased pregnancy per artificial insemination by 7.2 percentage points.

3.3 Highlights

Insertion of a PRID from day 0 until day 8 of the protocol increased pregnancy per artificial inseminationin dairy cows submitted to a 7-day Ovsynch protocol. Pregnancy loss was not affected by progesterone supplementation. We observed no interaction between treatment and season, parity, or number of artificial inseminations.

3.4 Short Communication

Improving fertility outcomes in protocols for timed artificial insemination (TAI) has been a central part of reproductive research in recent years (Stevenson and Britt, 2017). The initial Ovsynch protocol (Pursley et al., 1997), allowing for AI without the necessity of heat detection, consisted of a GnRH treatment, followed by $PGF_{2\alpha}$ at d 7 of the protocol and a final GnRH treatment at d 9, approximately 16 h before TAI. Different presynchronization regimens [e.g., Presynch-Ovsynch (Moreira et al., 2001), Double-Ovsynch (Souza et al., 2008), or G6G (Bello et al., 2006)] and modifications of timing (Brusveen et al., 2009) and dose (Giordano et al., 2013) of $PGF_{2\alpha}$ treatments were developed to optimize the hormonal environment at initiation and during an Ovsynch protocol. Another approach to improve fertility in TAI protocols is the supplementation of progesterone (P4; El-Zarkouny and Stevenson, 2004) by insertion of a progesterone-releasing intravaginal device (PRID). Lactating dairy cows without a mature corpus luteum (CL) at the start of an Ovsynch protocol (i.e., at the first GnRH treatment, G1) experience insufficient concentrations of P4 during follicular development, which causes reduced reproductive performance (Stevenson et al., 2006; Wiltbank et al., 2014). Approximately 30% of first AI cows submitted to TAI protocols (Stevenson et al., 2008) and 22 to 46% of cows resynchronized for AI (Fricke et al., 2003) do not bear a functional CL at protocol initiation, leading to low P4 concentrations at G1. This compromises oocyte quality (Santos et al., 2016) due to suboptimal follicular development (Cerri et al., 2011), which results in reduced P/AI and greater pregnancy losses (Wiltbank et al., 2011; Bisinotto et al., 2015b). Additionally, cows suffering from low preovulatory P4 concentrations are more susceptible to double ovulations and greater twinning rates (Carvalho et al., 2019). Insufficient P4 levels may also affect fertility in high-producing dairy cows with a CL at protocol initiation, due to an increased hepatic P4 metabolism (Wiltbank et al., 2006). Several studies have addressed the positive effects of progesterone during the first 7 d of an Ovsynch protocol (Bisinotto et al., 2015b); however, the effect of an extended P4 supplementation for 8 d during an Ovsynch protocol on reproductive performance is only occasionally described (Xu and Burton, 2000).

Therefore, the objective of this study was to compare the standard 7-d Ovsynch protocol with a modified protocol including a PRID device. We hypothesized that P4 supplementation by insertion of a PRID device for 8 d would increase P/AI and decrease pregnancy loss in lactating Holstein cows submitted to an Ovsynch protocol.

All experimental procedures were approved by the Institutional Animal Care and Use Committee of the Freie Universität Berlin (reference number 2347-A-3–2-2020).

The experiment was conducted from October 2020 until July 2021. Lactating Holstein cows (n = 716; 236 primiparous and 480 multiparous cows) from one commercial dairy farm in Northeast Germany were used in this study. The farm comprised 1,600 Holstein Friesian cows with an average 305-d ECM yield of 9,809 kg. Lactating cows were housed in a freestall barn with slatted floors and beds equipped with rubber mats. Group composition was dynamic, with cows entering and leaving the study barn depending on their calving dates. Cows were fed a TMR twice a day in a 12-h interval with free access to feed and water. The rations were formulated to meet or exceed the requirements according to the NRC (2001). Lactating cows were milked 2 times daily, starting at 07:30 and 20:00 h.

The farm's reproductive management consisted of a Presynch-Ovsynch protocol for first AI, with the possibility of AI either at detected estrus after the first $PGF_{2\alpha}$ (50 ± 3 DIM) or the second $PGF_{2\alpha}$ treatment (64 ± 3 DIM). Cows not detected in estrus during this presynchronization received TAI at the end of the following Ovsynch protocol (86 ± 3 DIM; Figure 1). Cows were assigned to treatment for first AI after presynchronization. After nonpregnancy diagnosis, open cows were also assigned to treatment. Lactating Holstein cows received an Ovsynch protocol with addition of a PRID from d 0 until removal at d 8 as part of a larger study (Hölper et al., 2023) conducted by our research team. All cows not enrolled in that previous study were treated with a standard 7-d Ovsynch protocol by the AI technician as part of the farm's reproductive procedure. The scope of the present study was a comparison of an 8-d P4-based Ovsynch protocol (PRIDsynch: d 0, 100 µg of GnRH + PRID; d 7, 25 mg of dinoprost; d 8, PRID removal; d 9, 100 µg of GnRH) with a standard 7-d Ovsynch protocol (control: d 0, 100 µg of GnRH; d 7, 500 µg of cloprostenol; d 9, 100 µg of GnRH). Cows that lost the PRID before scheduled removal (n = 20), cows that left the herd before pregnancy diagnosis (n = 16), and cows that were not treated or inseminated according to the protocol (n = 14) were excluded. For the PRID group (n = 356), GnRH (gonadorelin diacetate tetrahydrate; Ovarelin 50 µg/mL), $PGF_{2\alpha}$ (dinoprost trometamol; Enzaprost T 5 mg/mL), and progesterone devices (progesterone; PRID Delta 1.55 g) were from Ceva Santé Animale. For the control group (n = 360), GnRH (gonadorelin acetate; Gonavet Veyx Forte 50 µg/mL) and $PGF_{2\alpha}$ analog (cloprostenol sodium; PGF Veyx Forte 250 µg/mL) were from Veyx Pharma

GmbH. Approximately 16 h after the second GnRH treatment, all cows received TAI by a single trained AI technician.

Pregnancy diagnosis was performed by transrectal ultrasound examination d 38 ± 3 after AI and reconfirmed d 80 ± 7 after AI by a veterinarian using a portable ultrasound device (Easi-Scan:GO, IMV Imaging) equipped with a 7.5-MHz linear-array transducer. A positive pregnancy diagnosis was based on visualization of an embryo with a heartbeat. Nonpregnancy diagnosis was based on absence of pregnancy at the day of examination or a rebreeding to an estrus before pregnancy diagnosis. Pregnancy loss included cows that experienced pregnancy loss from d 38 to 80.

The results of pregnancy diagnosis and additional breeding information were obtained from the herd management software (HerdeW, dsp-Agrosoft GmbH) and transferred to Excel (Office 2016, Microsoft Deutschland Ltd.). All data were analyzed using SPSS for Windows (version 25.0, IBM Corp.). Logistic regression models for P/AI and pregnancy loss analysis were built using the GENLINMIXED procedure of SPSS. Cow was the experimental unit. Model building was conducted as recommended by Dohoo et al. (2009), where each parameter was first analyzed separately in an univariable model. Only parameters resulting in univariable models with $P \leq 0.10$ were included in the final mixed model. Selection of the model that best fit the data was performed by using a backward stepwise elimination procedure that removed all variables with $P > 0.10$ from the model. The initial model included the following explanatory variables as fixed effects: treatment (control vs. PRIDsynch), parity (primiparous vs. multiparous), season of AI (winter from December 1 to February 28, spring from March 1 to May 31, summer from June 1 to July 31, and autumn from October 1 to November 30), and AI number (first TAI vs. subsequent TAI), as well as interactions between these variables. Treatment and parity were forced to stay in the final model, regardless of their significance level. The final model consisted of treatment, parity, and season with no interaction between these variables.

A Bonferroni adjustment was used to account for multiple comparisons. Variables were declared to be significant when $P < 0.05$. A statistical tendency was declared when $P \geq 0.05$ and $P \leq 0.10$.

Descriptive data are summarized in Table 1. Parity distribution differed (P = 0.003) among treatments [control (101/360) vs. PRIDsynch (135/356)]. Average milk production did not differ among treatments (P = 0.390; PRIDsynch: 34.9 ± 7.7 kg; control: 35.5 ± 7.0 kg). The proportion of cows receiving first postpartum AI did not differ (P = 0.347) among treatments. The proportion of cows receiving second AI and greater did not differ (P = 0.289) among treatments. Days in milk at enrolment did not differ among treatments (P = 0.411).

Pregnancy per AI at d 38 ± 3 differed among treatments (P = 0.014). Cows receiving PRIDsynch had greater P/AI (38.9%) at d 38 ± 3 after AI (Table 1), compared with the control (31.7%). Pregnancy per AI at d 38 ± 3 after AI did not differ between primiparous and multiparous cows (P = 0.171). There was no treatment by parity interaction (P = 0.989).

Pregnancy per AI differed (P = 0.004) among treatments at d 80 ± 7 after AI, with PRIDsynch cows having greater (36.3%) P/AI compared with cows in the control group (28.9%). Pregnancy per AI differed (P = 0.044) among parity at d 80 ± 7 after AI, with primiparous cows having greater (34.6%) P/AI compared with multiparous cows (30.4%). There was, however, no interaction between treatment and parity (P = 0.670). Season had a significant influence on P/AI at d 38 ± 3 (P = 0.001) and d 80 ± 7 (P = 0.001). Lowest P/AI at d 38 ± 3 and d 80 ± 7 post AI was observed in summer (19.9% and 18.6%, respectively). Cows had the greatest P/AI at d 38 ± 3 and d 80 ± 7 post AI in spring and winter (43% and 39.7%, respectively). In August and September, no cows were enrolled in this study. Pregnancy loss did not differ (P = 0.279) among treatments (8.8% vs. 6.2% for control and PRIDsynch cows, respectively).

Results from this study support the use of P4 devices in Ovsynch protocols from d 0 until removal at d 8 in lactating Holstein cows. Our results show that inserting a PRID at the beginning of an Ovsynch protocol increased P/AI regardless of parity or number of AI.

Insufficient P4 concentrations during the follicular development are a major concern in lactating dairy cows submitted to TAI protocols (Bisinotto et al., 2014; Wiltbank et al., 2014). Lower P4 concentrations lead to an increased pulse frequency of LH (Endo et al., 2012) and premature meiotic resumption, ultimately decreasing oocyte quality (Revah and Butler, 1996). Moreover, decreased preovulatory blood P4 concentrations can alter endometrial morphology and secretory functions during early gestation (Shaham-Albalancy et al., 1997) and cause an increased release of $PGF_{2\alpha}$ in response to oxytocin in the subsequent cycle after

AI (Cerri et al., 2011). Interestingly, previous research showed that interferon tau (IFN-τ) secretion on d 17 of gestation, associated with pregnancy establishment and maintenance, was not affected by follicular wave dynamics and therefore P4 concentrations during follicle growth (Carvalho et al., 2019; Bisinotto et al., 2022). These and our findings indicate that further research on the effects of progesterone regarding embryonic development and pregnancy establishment needs to be conducted.

Nonetheless, P4 concentrations of at least 2.0 to 3.0 ng/mL were described as beneficial regarding P/AI and pregnancy loss, restoring fertility of cows with no CL at the beginning of the protocol to the level of those cows initiated at diestrus (Bisinotto et al., 2013). In the above-mentioned studies, however, 2 controlled internal drug release (CIDR) devices with a P4 content of 1.38 g each were used to elevate progesterone concentrations. Using a single PRID device with a P4 content of 1.55 g from d 0 until d 8 of the protocol resulted in mean P4 concentrations of 2.68 and 4.34 ng/mL at d 7 of the protocol for cows without and with a CL at G1, respectively (Hölper et al., 2023). These findings are supported by previous studies on P4 supplementation with PRID devices, describing overall greater P4 concentrations and P/AI (van Werven et al., 2013) and a greater initial increase in P4 (Silva et al., 2021), compared with other intravaginal devices for P4 supplementation. Further research is warranted to investigate the influence of the surface area or material of the outer layer on P4 absorption.

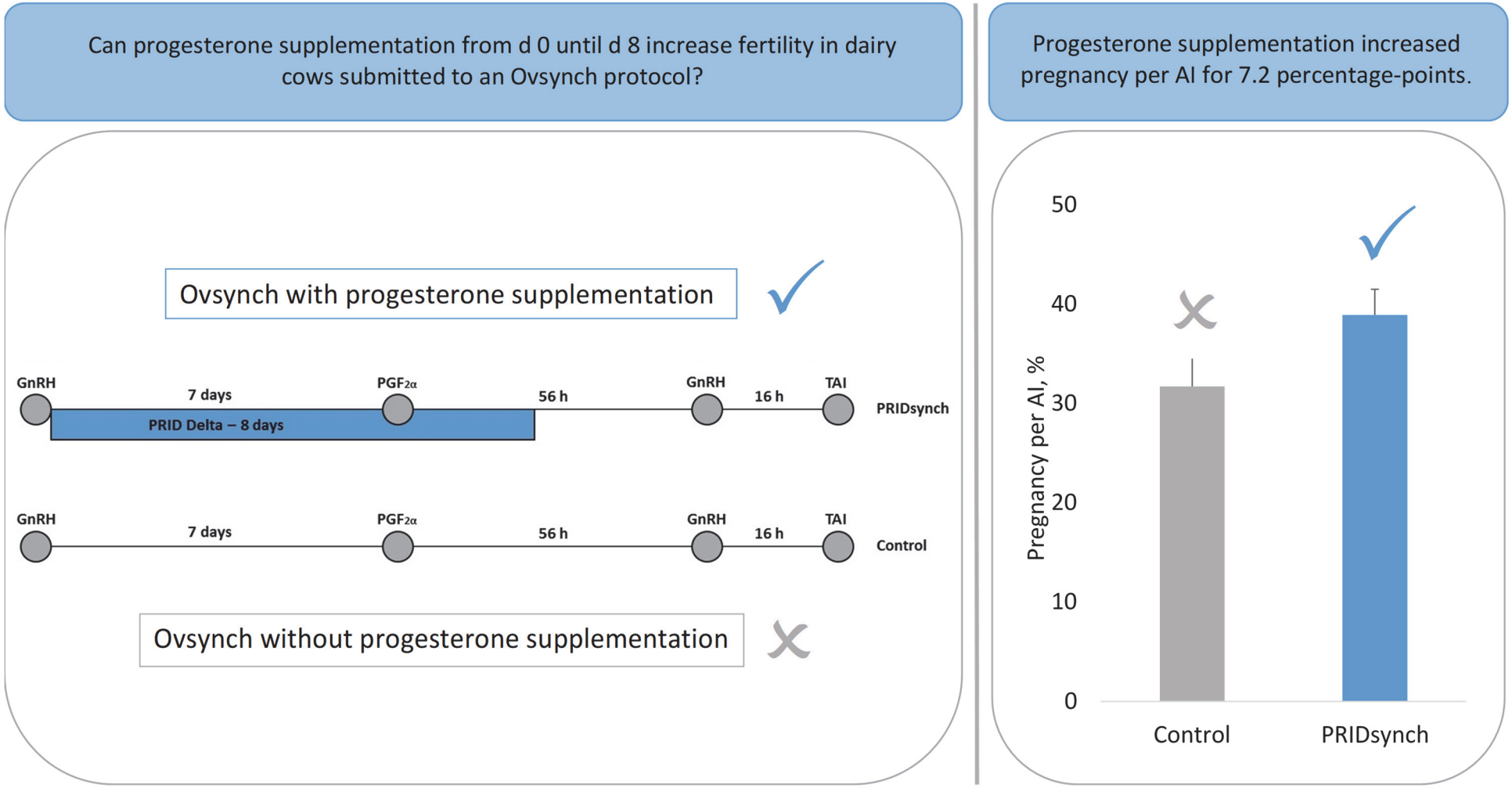
In a meta-analysis by Bisinotto et al. (2015b), it was described that the insertion of a single P4 device can benefit reproductive performance, but mainly in cows without a CL on d 0 of the TAI protocol. The meta-analysis described an overall increase in P/AI of 3.4 percentage points on d 32 after AI, but a tendency for a decrease in pregnancy loss. In our study, an overall P/AI increase of 7.2 percentage points was achieved, whereas pregnancy loss was not affected by treatment. A direct comparison with our study seems difficult as only 3 out of 25 studies included in this analysis used a PRID device for progesterone supplementation and most of the studies in the meta-analysis removed the CIDR or PRID after 7 d compared with 8 d in our study.

Estrus expression on the day of TAI is generally beneficial for fertility (Santos et al., 2010). The extended time of P4 supplementation may contribute to greater fertility by reducing the proportion of cows with premature ovulation (Colazo et al., 2013) and estrus

expression before TAI. This occurs in 5 to 7% of cows submitted to TAI protocols (Bisinotto et al., 2015a). An improved precision in the onset of estrus was observed by Xu and Burton (2000) when using a P4 device for 8 d. Research by Herlihy et al. (2013) confirms this statement and additionally shows a beneficial effect of P4 supplementation for 8 d during TAI protocols on P/AI in high-producing cows, independent of luteal status at protocol initiation, thus supporting our initial hypothesis. Furthermore, P4 supplementation increases the proportion of cows with a functional CL at d 11 to 14 after TAI (Chebel et al., 2010) indicating improved synchrony of ovulation. Stevenson et al. (2008) reported a decrease in ovulatory response to the first GnRH treatment when P4 was supplemented at the same time, thus impeding fertility by extending the period of follicle dominance. However, various studies did not show an association between the addition of P4 devices and a lack of ovulatory response after G1 (Bisinotto et al., 2010; Dewey et al., 2010). Although Stevenson (2016) described an association between greater P4 concentrations at G1 and a decreased ovulatory response after the first GnRH treatment, he also observed greater P/AI in cows with P4 concentrations ≥3 ng/mL at protocol initiation. This author concludes that greater P4 concentrations during follicular development may have a greater impact on fertility compared with the ovulatory response after G1.

One limitation of the study is the unequal percentage of primiparous cows in the 2 groups. Bamber et al. (2009) described a greater proportion of anovular cows within the first lactation. On the other hand, primiparous cows have better fertility as shown in multiple studies (Tenhagen et al., 2004; Fricke et al., 2014). Therefore, parity was forced to remain in the model. Considering this, the results of this study may be somewhat influenced by the greater proportion of primiparous cows in the PRIDsynch group. In addition, different types of prostaglandin were used in between the 2 groups. Although several studies did not show an effect of different products on fertility (Stevenson and Phatak, 2010; Baryczka et al., 2018), other studies described that the subtle differences between those variations may lead to a greater reproductive performance when using cloprostenol sodium (Pursley et al., 2012), which should be considered when interpreting the results of the present study.

Taking that into account, supplementation of progesterone by means of a PRID device from d 0 until d 8 in a 7-d Ovsynch protocol seemingly improved P/AI without effect on pregnancy loss. Further research needs to be conducted regarding the effect of prolonged P4 supplementation in Ovsynch protocols on reproductive performance and P4 concentrations. Furthermore, the effect of a CL at protocol initiation and estrus expression before TAI throughout the protocol needs to be addressed in future studies.



Graphical Abstract.

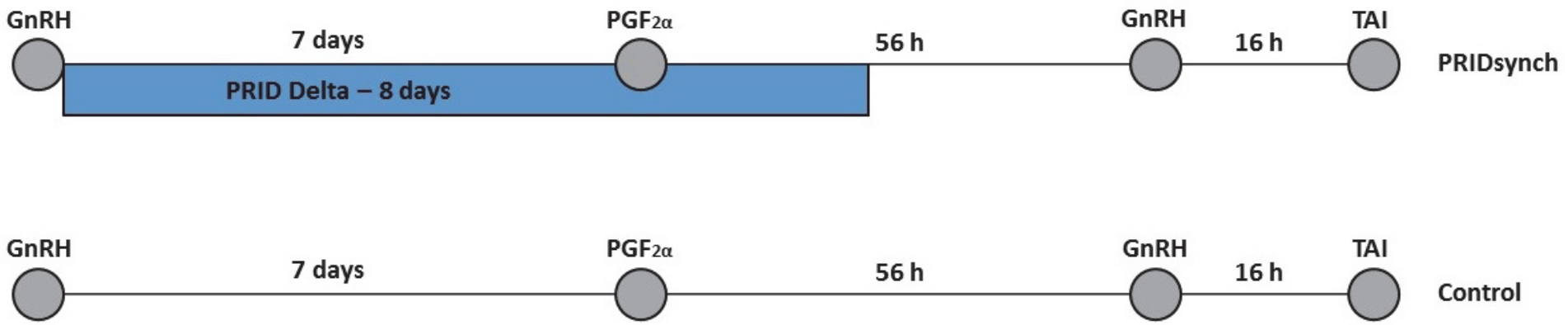


Figure 1. Schematic representation of the study design. Control cows (n = 360) received a standard 7-d Ovsynch protocol (d 0, 100 µg of GnRH; d 7, 500 µg of cloprostenol; d 9, 100 µg of GnRH). PRIDsynch cows (n = 356) received an Ovsynch protocol with an addition of a progesterone-releasing intravaginal device (PRID) from d 0 until removal at d 8 (d 0, 100 µg of GnRH + PRID; d 7, 25 mg of dinoprost; d 8, PRID removal; d 9, 100 µg of GnRH). All cows received TAI approximately 16 h after the second GnRH treatment. $PGF_{2\alpha}$ = prostaglandin $F_{2\alpha}$; TAI = timed artificial insemination.

Table 1. Comparison of descriptive data for cows enrolled in this experiment and comparison of pregnancy per artificial insemination [P/AI, % (number of pregnant cows/number of all cows)] and pregnancy loss between treatments

Item	**Treatment[1]**		***P*-value**
	Control	**PRIDsynch**	
DIM at enrolment (± SD)	145.9 ± 82.7	141.6 ± 80	0.411
Primiparous cows, % (n/N)	28.1 (101/360)	37.9 (135/356)	0.003
First service, % (n/N)	45.3 (197/360)	43.5 (201/356)	0.347
P/AI, % at d 38 ± 3 (n/N)	31.7 (115/360)	38.9 (148/356)	0.014
P/AI, % at d 80 ± 7 (n/N)	28.9 (103/358)	36.3 (137/354)	0.004
Pregnancy loss, % (n/N)	8.8 (10/113)	6.2 (9/146)	0.279

[1]Lactating Holstein cows were randomly assigned to receive an Ovsynch protocol with addition of a progesterone-releasing intravaginal device (PRID) from d 0 until removal at d 8 (PRIDsynch; d 0, 100 µg of GnRH + PRID; d 7, 25 mg of dinoprost; d 8, PRID removal; d 9, 100 µg of GnRH) or a standard 7-d Ovsynch protocol (control: d 0, 100 µg of GnRH; d 7, 500 µg of cloprostenol; d 9, 100 µg of GnRH). All cows received timed artificial insemination (TAI) approximately 16 h after the second GnRH treatment. Percentages for P/AI were derived from the GENLINMIXED model including treatment, parity, and season, with no interaction between these variables.

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4 DISCUSSION

The overall objectives of this thesis were 1) to compare the effect of an additional $PGF_{2\alpha}$ treatment and an increased dosage of $PGF_{2\alpha}$ on d 7 during an Ovsynch protocol with an untreated control group, regarding luteolysis and reproductive performance; 2) to determine the effect of P4 supplementation by means of a PRID insert for 8 days during an Ovsynch protocol on P/AI and pregnancy loss; 3) to evaluate vaginal discharge at device removal on fertility, and 4) to evaluate the diagnostic performance of transrectal ultrasound to detect a functional CL based on circulating P4 levels.

Results from both studies underline the importance and opportunities using modified protocols for TAI in order to improve fertility compared to a standard Ovsynch protocol.

With the invention of the Ovsynch protocol (Pursley et al., 1995), the researchers created a successful strategy for CL and follicle manipulation in order to allow for synchronized TAI. Although service rate could be improved, results for P/AI were not optimized (Pursley et al., 1997). However, a greater knowledge and understanding of the physiology underlying TAI protocols resulted in the development of various modifications, improving pivotal aspects of follicular development and luteal regression.

For instance, presynchronization with a $PGF_{2\alpha}$ based Presynch-Ovsynch resulted in increased P/AI after TAI and also granted the flexibility to inseminate cows observed in estrus (Chebel and Santos, 2010; Fricke et al., 2014), possibly explaining the wide adoption in US dairy farms for first service (Ferguson and Skidmore, 2013). Despite being a useful strategy for increasing reproductive performance in dairy herds, a major limitation of Presynch-Ovsynch protocols is the inability to improve P/AI in anovular cows, ranging from 7.3 – 41.1 % at about 60 DIM (Bamber et al., 2009). More refined fertility programs such as G6G or the Double-Ovsynch protocol, utilizing GnRH additionally to $PGF_{2\alpha}$, were introduced to overcome those limitations (Herlihy et al., 2012). A meta-analysis by Borchardt et al. (2017), considering 25 articles including 21,046 TAI services, showed that primiparous cows submitted to a Double-Ovsynch protocol had increased P/AI compared with cows receiving only $PGF_{2\alpha}$ in a Presynch-Ovsynch protocol (51.4 % vs. 43.4 %). There was, however, no difference observed in multiparous cows, presumably because of other limiting factors like incomplete luteal

regression after a single $PGF_{2\alpha}$ treatment during the breeding Ovsynch, especially occurring in multiparous cows (Martins et al., 2011; Giordano et al., 2012).

Lack of complete luteal regression after a single $PGF_{2\alpha}$ treatment on d 7 of the Ovsynch protocol, resulting in impaired fertility (Carvalho et al., 2018), lead to incorporation of different $PGF_{2\alpha}$ regimen, altering the dose (Giordano et al., 2013) or frequency of $PGF_{2\alpha}$ treatments (Wiltbank et al., 2015). In comparing these modifications directly with each other and a control group, the results of our study provided additional evidence to this topic, as they demonstrate the advantage of a second $PGF_{2\alpha}$ treatment 24 h after the first one compared with doubling the dose of $PGF_{2\alpha}$ on d 7. Various studies have outlined the positive effect on fertility of adding a second $PGF_{2\alpha}$ treatment on d 8, culminating in another meta-analysis by Borchardt et al. (2021), summarizing the results of 11 manuscripts and 9,735 cows. Interestingly, this analysis showed that both multiparous and primiparous cows had increased P/AI and greater revenue associated with the improvement in reproductive performance in 95 % of all scenarios. Although more profitable in multiparous cows (primiparous: €1.99/cow; 5th percentile = −€3.08; 95th percentile = €22.52 vs. multiparous: €7.76/cow; 5th percentile = €0.01; 95th percentile = €29.40), the benefit of adding a second $PGF_{2\alpha}$ treatment remains also in primiparous cows, justifying the associated labor and cost of the hormones. A study conducted by Minela et al. (2021) re-evaluated this justification by using a double dose of 1.0 mg cloprostenol sodium on day 7 compared with two treatments of 0.5 mg cloprostenol sodium each on day 7 and 8 of an Ovsynch protocol, arguing that both modifications result in the same enhancement of reproductive performance. Although the results may support their hypothesis in some way, as both modifications did not differ regarding P/AI, both did not increase reproductive performance compared with the control group as well. This effect may be explained by the presynchronization schedule used for first service in their study. Cows received a Double-Ovsynch with an additional $PGF_{2\alpha}$ treatment on d 8 during the first Ovsynch, creating arguably most favorable conditions for initiation of the breeding Ovsynch. This, however, compromises the objective of labor and cost reduction by omitting the second $PGF_{2\alpha}$ treatment in the breeding Ovsynch when utilizing a second $PGF_{2\alpha}$ treatment in the first Ovsynch, creating an even more complex presynchronization regimen. In contrast, a study conducted by Tippenhauer et al. (2021b) with a comparable approach was able to demonstrate a significant increase in P/AI for cows receiving an additional $PGF_{2\alpha}$ treatment (40.3 %) compared with cows receiving a double dose of cloprostenol (33.4 %) and control

cows (31.8 %) when the Ovsynch protocol was initiated during a random stage of the estrus cycle. Additionally, the researchers determined the impact of the different $PGF_{2\alpha}$ modifications on P4 concentrations at G2, with two $PGF_{2\alpha}$ treatments significantly decreasing P4 (0.49 ± 0.16 ng/mL) and a double dose of $PGF_{2\alpha}$ tending to decrease P4 (0.73 ± 0.15 ng/mL) compared with cows in the control group (1.22 ± 0.16 ng/mL). This indicates more complete luteolysis in cows treated with $PGF_{2\alpha}$ on d 7 and 8. However, the study had some limitations as blood sampling was only performed in a subgroup of cows at G2. Furthermore, an evaluation of a functional CL was accomplished by transrectal ultrasonography without verification via serum P4 levels (P4 ≥ 1 ng/mL). This might explain why cows without CL at protocol initiation, more prone to incomplete luteolysis based on the refractory state of the young CL formed after G1 (Nascimento et al., 2014), were not affected by $PGF_{2\alpha}$ modifications regarding reproductive performance in this study. Based upon these insights, our study complements the work of these researchers by adding a closer analysis of P4 concentrations to further validate the luteal status at G1, G2 and at the time of the first $PGF_{2\alpha}$ treatment. This approach allowed for a more precise determination of CL status and therefore luteolysis in cows within each group. Cows in the 2PGF group had the greatest increase in luteal regression (control 84.8 %; DoubleDose 91 %; 2PGF 92.6 %), unaffected by parity. There was, however, a CL x treatment interaction when cows had no CL at protocol initiation (control 88.3 %; DoubleDose 88.4 %; 2PGF 89 % vs. control 81.3 %; DoubleDose 93.1 %; 2PGF 95.4 %, for cows with and without a CL, respectively). Similar to that, 2PGF cows showed the greatest increase in pregnancy outcomes when no CL was present at G1 (control: 36.1 % vs. DoubleDose: 32.7 % vs. 2PGF: 47.9 %), indicating that luteal status and $PGF_{2\alpha}$ modifications indeed have an effect on P/AI in cows submitted to Ovsynch protocols. Results from a study conducted by Barletta et al. (2018) support our observations, as they also demonstrate the greatest increase in P/AI and complete luteal regression in cows submitted to the altered Ovsynch with two $PGF_{2\alpha}$ treatments, compared with control cows. As only a subset of cows were analyzed for blood P4 in order to determine luteal status, the authors emphasized that even a 19 percentage-points increase in P/AI, observed in cows without a CL at protocol initiation, may only result in a tendency because of the limited number of cows in that particular subgroup.

Overall, although some studies are lacking statistical significance due to a limited number of cows without a functional CL on d 0 of the protocol, the second $PGF_{2\alpha}$ treatment on d 8 can be seen as a viable addition to the protocol for enhancement of luteolysis and reproductive performance.

Cows not bearing a functional CL at protocol initiation do not only suffer more often from incomplete luteal regression, but also low concentrations of blood P4 during the development of the preovulatory follicle. Several studies highlighted the importance of sufficient circulating P4 during this stage of follicular development, as it supports proper follicle growth and uterine morphology (Shaham-Albalancy et al., 1997), decreases LH pulse frequency (Kinder et al., 1996), optimizes follicle diameter (Cerri et al., 2011a), prohibits double ovulations with concurrently lower twinning rates (Carvalho et al., 2019) and increases follicular fluid IGF-1 concentration as well (Cerri et al., 2011b). A method to beneficially influence the serum P4 level in cows without a CL or with high metabolic steroid catabolism is the supplementation of P4 by means of an intravaginal P4 device. A study by Bisinotto et al. (2013) depicted an increased risk of pregnancy on d 62 after AI by 58% (Adjusted risk ratio = 1.58; 95% CI = 1.30 to 1.93) in P4 supplemented cows compared with a control group, elevating P/AI to a level similar to that of cows submitted to the protocol in diestrus. However, the cows enrolled in this study received a 5-day Cosynch protocol with two simultaneously inserted CIDR devices with a P4 content of 1.38 g each. Although supported by the outcomes of Bisinotto et al. (2015a), achieving similar results in cows receiving a 7-d Ovsynch protocol, the authors agree that insertion of two CIDR devices is necessary to elevate circulating P4 to a minimum of approximately 2.0 ng/mL during the growth of the preovulatory follicle in order to sustain high fertility in dairy cows without a CL. As the simultaneous insertion of multiple devices is neither practical nor approved, our studies focused on a different approach by utilizing a single PRID device for 8 days during a 7-d Ovsynch protocol. As P4 concentrations (2.68 ng/mL on average) on d 7 of the protocol exceeded the 2.0 ng/mL threshold in the majority of cows (i.e., 66.2 %) without a CL at protocol initiation, insertion of a single PRID device seems to be efficient in increasing P4 to a level described as beneficial for P/AI and pregnancy loss. As already discussed by van Werven et al. (2013), various factors such as P4 content (CIDR = 1.38 g vs. PRID= 1.55 g), surface area (CIDR $\sim$ 120 cm^2 vs. PRID $\sim$ 155 cm^2), shape (CIDR = T-shape vs. PRID = triangle-like) and outer layer material (CIDR = silicone vs. PRID = ethyl vinyl acetate) may contribute to the greater P4 levels observed in cows treated

with a PRID compared with other devices. Although the exact reason is not further elucidated, observations from other studies are in agreement with the results depicted by van Werven et al. (2013). For instance, Silva et al. (2021) compared multiple P4 devices with different P4 contents and attributes in terms of P4 absorption and maintenance over 10 days, leading to significantly greater circulating P4 in cows treated with a PRID compared with the other devices. However, results from this study were collected from nonlactating multiparous cows, making it difficult to draw a direct comparison to our studies, as lactating primiparous and multiparous cows were included in the analysis. Nonetheless, beneath the extended duration of P4 supplementation, possibly creating an optimized hormonal milieu and cycle synchronization, adequate P4 absorption may be a factor contributing to the increase in reproductive performance observed in cows within our second study. When using a single PRID insert during an Ovsynch protocol from d 0 until removal on d 8, an overall improvement in P/AI of 7.2 percentage-points could be observed, however, there was no influence on pregnancy loss. Additionally, a direct comparison of different P4 devices utilized in an extended 8-d P4 supplemented Ovsynch protocol (Frenkel et al., 2023) did result in a significant improvement in P/AI (+12 percentage-units) in cows without a CL when treated with a PRID compared to control cows, whereas no difference was observed in cows treated with a different P4 device containing 1 g of P4. Furthermore, a treatment effect on pregnancy loss could not be observed, thus supporting our outcomes. Also, a large meta-analysis conducted by Bisinotto et al. (2015b) described an overall increase of only 3.4 percentage-points on d 32 after AI when using a single P4 device. Again, a direct comparison with our study seems difficult as only 3 out of 25 studies included in this analysis used a PRID device for P4 supplementation and all of the studies in the meta-analysis removed the P4 insert before d 8 of the protocol. Additionally, the authors emphasized on a more distinct effect in cows without a CL, but also acknowledged a positive effect on pregnancy loss regardless of luteal status. Another interesting observation of the meta-analysis was the decrease of treatment effect when cows were able to be inseminated in estrus during the protocol. Unfortunately, luteal status as well as estrus expression during the protocol was not assessed during the second study, leaving room for speculation regarding their impact on reproductive performance. Data of the aforementioned experiment with a similar 8-d P4 supplementation approach conducted by Frenkel et al. (2023), however, was able to show a significant decrease in estrous cows (1/357) during the PRID added protocol, indicating optimized synchronization,

ultimately leading to greater fertility in cows submitted to TAI protocols (Stevenson et al., 2006).

A side effect frequently observed in cows treated with an intravaginal device is vaginal discharge. Understandably, the question arises whether this discharge, as a sign of local vaginal inflammation, negatively influences fertility similar to purulent vaginal discharge often associated with clinical endometritis (LeBlanc, 2023). The extent of vaginal discharge observed in various studies is inconsistent. Walsh et al. (2007) observed that almost half of the cows treated with a PRID device did not show any signs of vaginitis. However, results acquired by Fischer-Tenhagen et al. (2012) depicted that all cows treated with a vaginal insert showed signs of inflammation by means of vaginal discharge to some degree. A study conducted by Santos et al. (2016) showed somewhat similar proportions of VS throughout the treated cows compared with our study, with the greatest amount of cows showing discharge declared as VS 1. Despite these discrepancies, taking the observations of Krueger and Heuwieser (2011) into account, the authors agree that vaginal discharge after P4 device insertion does not have a negative impact on reproductive performance or general health of the cows (Chenault et al., 2003). Unfortunately, these authors did not focus on a potential correlation between different degrees of vaginal inflammation and circulating P4 concentrations, leaving our study to be the first one to describe such a phenomenon. In fact, the data collected from our first study even indicated that a greater local inflammatory response is associated with improved serum P4 levels and therefore greater P/AI. The exact mechanism why VS 2 cows have the highest P4 concentrations and greatest results in P/AI, however, have yet to be discovered. Although this effect could not be reproduced in a recent study (Frenkel et al., 2023), observations by Wagener et al. (2021) lead to the conclusion that a certain level of inflammation at molecular and cellular levels at AI, accompanied by upregulation of several factors and chemokines, might be favorable for fertility in cows, thus supporting our initial outcomes and also creating room for speculations, underlining the need for further detailed studies in this regard.

As the decision for appropriate modifications of the Ovsynch protocol may depend heavily on the presence of a CL at protocol initiation, a standardized regimen for CL detection needs to be created for practitioners in the field. Our first study contributes to that, as the CL diameter observed during ultrasound examinations of the ovaries was compared simultaneously with blood P4 concentrations. Our findings depict a highly accurate correlation between circulating P4 levels, indicative for the presence of a functional CL (P4 ≥ 1 ng/mL),

and a CL visualized during the transrectal ultrasonographic examination when the cutoff value was set to a 20 mm diameter. Although an interobserver bias needs to be taken into account, as especially the ability to detect the absence of a functional CL can differ significantly between multiple observers (Sauls-Hiesterman et al., 2020), the cutoff value of 20 mm seems to be efficient in raising specificity when utilizing ultrasound for ovarian examinations. Results by Bicalho et al. (2008) support our observations, as they depict a major increase in specificity when increasing the CL diameter cutoff value from 15 mm to 20 mm. It should be mentioned at this point that these suggestions are only applicable for lactating dairy cows, as circulating progesterone concentrations in heifers with a comparably smaller CL can exceed those of lactating cows with a greater CL (Sartori et al., 2004).

With new strategies and technologies emerging, the dairy industry is permanently changing and improving in terms of reproductive management. Whereas more complex and refined fertility programs for TAI can lead to considerable fertility, even exceeding reproductive performance of cows inseminated to estrus (Fricke and Wiltbank, 2022), the additional labor and increased hormonal applications may be deterrent, particularly when looking at the consumer's perception. Results from a survey of 1,646 participants in Germany indicate that, despite a lack of basic knowledge concerning milk production, most people (65 %) perceive hormonal applications to improve fertility in dairy cows as negative and unwelcome (Pieper et al., 2016). Therefore, alternative solutions and novel approaches need to be considered, yielding satisfactory reproductive outcomes without the necessity for extensive hormonal applications, but rather a precise treatment based upon the specific needs of cows diagnosed to be in a low fertility cohort or identified to be a subgroup in need for a specific reproductive management strategy [targeted reproductive management (**TRM**); Giordano et al. (2022)]. While dairy farms are adopting modern technology like automated activity monitoring (**AAM**) and automated camera systems, a plethora of data can be acquired in order to predict reproductive performance based on several characteristics like transition cow health, estrus expression and intensity within the voluntary waiting period (Tippenhauer et al., 2021a; Bretzinger et al., 2023), changes in BCS during the transition period (Pinedo et al., 2022; Lauber and Fricke, 2023) and many more. Although researchers already outlined promising results regarding fertility when submitting cows to TRM programs combined with modified TAI protocols (Rial et al., 2022), extensive work is still required before comprehensive implementation by commercial dairy farms.

Results from the present thesis contribute to this transition in outlining the potential benefits of precisely tailored alterations and modifications of well-established protocols in the dairy industry. As TRM strategies starting to evolve, taking numerous factors into account, these hormonal optimizations of Ovsynch protocols should be considered at some point in order to improve fertility in cows with an accurately diagnosed specific condition. As Chebel et al. (2023) summarized aptly, combining TRM with tailored hormonal protocols represent an opportunity for fewer animal interventions and a more prudent hormonal use, ultimately leading to more optimized reproductive management schedules beneficial for farmers and dairy cows, and additionally may lead to improved public acceptance as well.

5 SUMMARY

Optimization of Ovsynch protocols in lactating dairy cows using modified prostaglandin treatments and progesterone intravaginal devices

The overall objectives of this thesis were 1) to compare the effect of an additional prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) treatment and an increased dosage of $PGF_{2\alpha}$ on d 7 during an Ovsynch protocol with an untreated control group, regarding luteolysis and reproductive performance; 2) to determine the effect of progesterone (P4) supplementation by means of a P4 releasing intravaginal device (PRID) insert for 8 days during an Ovsynch protocol on pregnancy per artificial insemination (P/AI) and pregnancy loss; 3) to evaluate vaginal discharge at device removal on fertility, and 4) to evaluate the diagnostic performance of transrectal ultrasound to detect a functional corpus luteum (CL) based on circulating P4 levels.

In the first study, the aim was to compare the effect of two $PGF_{2\alpha}$ treatments 24 h apart (25 mg of dinoprost at d 7 and 8) and the treatment of a double $PGF_{2\alpha}$ dose (50 mg of dinoprost at d 7) with a control group (25 mg of dinoprost at d 7) during a 7-d Ovsynch protocol with inclusion of a PRID device for 8 days on luteal regression and P/AI in lactating Holstein cows. Furthermore, we wanted to evaluate the potential impact of vaginal discharge caused be the P4 insert on fertility and whether serum P4 levels correspond with ultrasound CL diagnostic.

The experiment was conducted on a single commercial farm in Northeast Germany. A total of 1,056 cows were randomly assigned within parity to receive 1 of 3 Ovsynch protocols on a weekly basis: 1) a control group in which cows received an Ovsynch protocol with a single $PGF_{2\alpha}$ treatment (control: d 0, 100 µg of GnRH + PRID; d 7, 25 mg of dinoprost; d 8, PRID removal; d 9, 100 µg of GnRH), 2) an Ovsynch protocol with a double dose of $PGF_{2\alpha}$ (DoubleDose: d 0, 100 µg of GnRH + PRID; d 7, 50 mg of dinoprost; d 8, PRID removal; d 9, 100 µg of GnRH) and 3) an Ovsynch protocol with an additional $PGF_{2\alpha}$ treatment 24 h after the first one (2PGF: d 0, 100 µg of GnRH + PRID; d 7, 25 mg of dinoprost; d 8, 25 mg of dinoprost and PRID removal; d 9, 100 µg of GnRH). All cows received a PRID from d 0 until removal on d 8, on which vaginal discharge was evaluated and scored [vaginal score (VS) 0 = no debris, VS 1 = small flecks of purulent debris, VS 2 = abundant amount of purulent debris]. To facilitate first post partum timed AI (TAI), the farm used a Presynch-Ovsynch protocol ($PGF_{2\alpha}$, 14 d later

$PGF_{2\alpha}$, 12 d later start of an Ovsynch protocol). All cows were inseminated approximately 16 h after the second GnRH treatment (G2) of the Ovsynch protocol. Ovarian ultrasonography was performed on d 0 (G1) to determine abence or presence of a CL and its size. Blood samples were collected on d 0, 7 and 9 in order to asses P4 concentrations. Pregnancy diagnosis was performed using transrectal ultrasound d 38 ± 3 after AI and reconfirmed d 80 ± 7.

Treatment affected P4 concentration at G2. Mean P4 concentrations at G2 differed (*P* = 0.037) among treatments (control 0.35 ± 0.02 ng/mL; DoubleDose 0.29 ± 0.02 ng/mL; 2PGF 0.30 ± 0.02 ng/mL) and were lower for 2PGF cows (*P* = 0.043) and DoubleDose cows (*P* = 0.023) compared with cows in the control group. There was an interaction between treatment and CL at G1 (*P* = 0.087). Cows without a CL at initiation of the protocol had decreased (*P* = 0.014) P4 concentrations when treated with a double dose of $PGF_{2\alpha}$ and tended (*P* = 0.062) to have a decreased P4 concentration at G2 when receiving 2 consecutive treatments 24 h apart. Proportion of cows with luteolysis differed among treatments (*P* = 0.025; control 84.8%; DoubleDose 91%; 2PGF 92.6%) considering only cows with P4 concentrations above 1.0 ng/mL (indicative of presence of a CL) at first $PGF_{2\alpha}$ (863/1011) and 0.5 ng/mL at G2 (indicative of complete luteolysis).

Cows that received an additional treatment 24 h apart had greater P/AI (46.5%) compared with DoubleDose cows (37.7%; *P* = 0.045), but did not differ compared with cows in the control group (40.3%; *P* = 0.144). Considering luteal status at G1, cows within the 2PGF group had greater (47.9%; *P* = 0.043) P/AI when compared with DoubleDose cows (32.7%), but did not differ compared with control cows (36.1%; *P* = 0.109) when no CL was present at protocol initiation.

The overall accuracy was 87.2% to identify a functional CL using transrectal ultrasound at the beginning of the protocol. The area under the curve (AUC) was 0.901 and can be considered highly accurate. The optimum cutoff was a 20-mm diameter of the CL.

Vaginal discharge score had a significant association (*P* = 0.001) with P4 concentrations at the first $PGF_{2\alpha}$ treatment. Cows assigned to a VS of 2 had greater (4.3 ± 0.2 ng/mL) serum P4 levels than cows assigned to score 0 (3.0 ± 0.3 ng/mL; *P* = 0.001) and 1 (3.7 ± 0.1 ng/mL; *P* = 0.002). Progesterone concentration at d 7 was greater (*P* = 0.027) for cows assigned to VS 1 compared with cows assigned to VS 0. Vaginal discharge score at PRID removal tended to have an association with P/AI at d 38 after AI (*P* = 0.072). Pregnancy per AI did not differ (*P* = 0.459)

among cows scored with a VS of 0 and 1 [36.5% (25/69); 41.3% (320/780)], but was significantly greater [49.7% (102/207)] for cows having a VS of 2 compared with VS 1 (*P* = 0.039), and tended to be greater compared with VS 0 (*P* = 0.066).

Outcomes from this study demonstrate that both $PGF_{2\alpha}$ modifications can increase luteolysis in P4 supplemented Ovsynch protocols compared with a standard $PGF_{2\alpha}$ treatment on d 7 of the protocol. Interestingly, only cows receiving an additional $PGF_{2\alpha}$ treatment had a numerical increase in P/AI (6.2 %), especially in cows without a CL at G1 (11.8 %), whereas an increased dose of $PGF_{2\alpha}$ did not lead to any improvement in reproductive performance. Vaginal discharge at d 8 of the protocol, after PRID removal, was associated with higher P4 concentrations at d 7 and greater P/AI. Ultrasound can be deemed as a valuable tool for CL detection.

The second study aimed to elucidate the effect of P4 supplemention for 8 days, by means of a PRID insert, during an Ovsynch protocol on P/AI and pregnancy loss compared with a standard 7-d Ovsynch protocol.

A total of 716 lactating Holstein cows from one commercial dairy farm in Northeast Germany were enrolled in this study. The treatment group was part of the aforementioned study and received an 8-d P4-based Ovsynch protocol (PRIDsynch: d 0, 100 µg of GnRH + PRID; d 7, 25 mg of dinoprost; d 8, PRID removal; d 9, 100 µg of GnRH), whereas the control group received a standard 7-d Ovsynch protocol as part of the farms reproductive schedule (control: d 0, 100 µg of GnRH; d 7, 500 µg of cloprostenol; d 9, 100 µg of GnRH). All cows were inseminated approximately 16 h after G2. Pregnancy diagnosis was performed by transrectal ultrasound examination d 38 ± 3 after AI and reconfirmed d 80 ± 7. Pregnancy loss included cows that experienced pregnancy loss from d 38 to 80.

Results from this study demonstrate the overall beneficial effect of an elongated P4, 8 d supplementation with a PRID insert in an Ovsynch protocol on reproductive performance. Pregnancy per AI was significantly increased in PRIDsynch cows [*P* = 0.014; 38.9 % (115/360)] compared with untreated control cows [31.7 % (115/360)] on d 38 ± 3 after AI. However, pregnancy loss was not affected by treatment [*P* = 0.279; control: 8.8 (10/113) vs. PRIDsynch: 6.2 (9/146)].

Overall, this thesis shows that 1) the proportion of cows with complete luteal regression near TAI was increased by both $PGF_{2\alpha}$ modifications, but only the second treatment 24 h apart resulted in greater P/AI compared with the increased $PGF_{2\alpha}$ dose and a numerical increase in P/AI compared with control cows, especially in cows lacking a CL at protocol initiation, 2) the addition of a PRID from d 0 until removal on d 8 of the TAI protocol increased reproductive performance but did not influence pregnancy loss, 3) vaginal discharge caused by P4 inserts has no negative impact on fertility and 4) ovarian ultrasound examinations are suitable for detection of a functional CL. Taken together, these results have a potential to improve reproductive performance on dairy farms using TAI protocols.

6 ZUSAMMENFASSUNG

Optimierung von Ovsynch Protokollen durch modifizierte Prostaglandin Behandlungen und intravaginale Progesteron-Freisetzungssysteme bei laktierenden Milchkühen

Die Ziele dieser Arbeit waren 1) der Vergleich einer zusätzlichen Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)-Behandlung und einer erhöhten Dosierung von $PGF_{2\alpha}$ an Tag 7 während eines Ovsynch-Protokolls mit einer unbehandelten Kontrollgruppe in Bezug auf Luteolyse und Reproduktionsleistung; 2) das Herausstellen der Wirkung einer Progesteron (P4)-Supplementierung mittels einer P4 freisetzenden Intravaginalspange (PRID) über 8 Tage während eines Ovsynch-Protokolls auf den Besamungserfolg (P/AI) und die Trächtigkeitsverluste; 3) die Auswirkungen des vaginalen Ausfluss auf die Fruchtbarkeit zu bewerten und 4) die diagnostische Leistung des transrektalen Ultraschalls zur Erkennung eines funktionellen Gelbkörpers (CL) auf der Grundlage der zirkulierenden P4-Spiegel zu bewerten.

In der ersten Studie sollte die Wirkung von zwei $PGF_{2\alpha}$-Behandlungen im Abstand von 24 Stunden (25 mg Dinoprost an Tag 7 und 8) und der Behandlung mit einer doppelten $PGF_{2\alpha}$-Dosis (50 mg Dinoprost an Tag 7) mit einer Kontrollgruppe (25 mg Dinoprost an Tag 7) während eines 7-tägigen Ovsynch-Protokolls unter Einbeziehung einer PRID-Intravaginalspange für 8 Tage auf die Luteolyse und den Besamungserfolg bei laktierenden Holstein-Kühen verglichen werden. Darüber hinaus wollten wir die möglichen Auswirkungen des durch die P4-Spangen verursachten Vaginalausflusses auf die Fruchtbarkeit bewerten und feststellen, ob die P4-Serumspiegel mit der Ultraschall-CL-Diagnose übereinstimmen. Der Versuch wurde in einem kommerziellen Milchviehbetrieb im Nordosten Deutschlands durchgeführt. Insgesamt 1.056 Kühe wurden randomisiert innerhalb einer Parität einem von 3 Ovsynch-Protokollen wöchentlich zugewiesen: 1) eine Kontrollgruppe, in der die Kühe ein Ovsynch-Protokoll mit einer einzigen $PGF_{2\alpha}$-Behandlung erhielten (Kontrolle: d 0, 100 µg GnRH + PRID; d 7, 25 mg Dinoprost; d 8, PRID-Entfernung; d 9, 100 µg GnRH), 2) ein Ovsynch-Protokoll mit einer doppelten Dosis von $PGF_{2\alpha}$ (DoubleDose: d 0, 100 µg GnRH + PRID; d 7, 50 mg Dinoprost; d 8, PRID-Entfernung; d 9, 100 µg GnRH) und 3) ein Ovsynch-Protokoll mit einer zusätzlichen $PGF_{2\alpha}$-Behandlung 24 h nach der Ersten (2PGF: d 0, 100 µg GnRH + PRID; d 7, 25 mg Dinoprost; d 8, 25 mg Dinoprost und PRID-Entfernung; d 9, 100 µg GnRH). Alle Kühe erhielten von Tag 0 bis zur Entfernung am 8. Tag eine PRID, woraufhin der Vaginalausfluss beurteilt und bewertet wurde [Vaginal Score (VS) 0 = kein Ausfluss, VS 1 = kleine Flecken

eitrigen Ausflusses, VS 2 = reichlich eitriger Ausfluss]. Zur Erstbesamung nach der Geburt verwendete der Betrieb ein Presynch-Ovsynch-Protokoll ($PGF_{2\alpha}$, 14 Tage später $PGF_{2\alpha}$, 12 Tage später Beginn eines Ovsynch-Protokolls). Alle Kühe wurden etwa 16 Stunden nach der zweiten GnRH-Behandlung (G2) des Ovsynch-Protokolls besamt. Am Tag 0 (G1) wurde eine Ultraschalluntersuchung der Eierstöcke durchgeführt, um das Vorhandensein von Gelbkörpern und deren Größe festzustellen. Blutproben wurden an den Tagen 0, 7 und 9 entnommen, um die P4-Konzentration zu bestimmen. Die Trächtigkeit wurde mittels transrektalem Ultraschall 38 ± 3 Tage nach der terminorientierten künstlichen Besamung (TAI) diagnostiziert und 80 ± 7 Tage später erneut bestätigt.

Die Behandlung beeinflusste die P4-Konzentration bei G2. Die mittleren P4-Konzentrationen bei G2 unterschieden sich signifikant (P = 0,037) zwischen den Behandlungen (Kontrolle 0,35 ± 0,02 ng/mL; DoubleDose 0,29 ± 0,02 ng/mL; 2PGF 0,30 ± 0,02 ng/mL) und waren bei 2PGF-Kühen (P = 0,043) und DoubleDose-Kühen (P = 0,023) im Vergleich zu den Kühen der Kontrollgruppe niedriger. Der Effekt der modifizierten Behandlungen war beinflusst von der Präsenz eines CL an G1 (P = 0,087). Bei Kühen, die zu Beginn des Protokolls keinen CL aufwiesen, waren die P4-Konzentrationen bei der Behandlung mit einer doppelten Dosis $PGF_{2\alpha}$ verringert (P = 0,014), und bei zwei aufeinanderfolgenden Behandlungen im Abstand von 24 Stunden war die P4-Konzentration bei G2 tendenziell verringert (P = 0,062). Der Anteil an Kühen mit vollständiger Luteolyse unterschied sich zwischen den Behandlungsgruppen (P = 0,025; Kontrolle 84,8 %; DoubleDose 91 %; 2PGF 92,6 %), wobei nur Kühe mit P4-Konzentrationen über 1,0 ng/mL (was auf das Vorhandensein eines CL hinweist) bei der ersten $PGF_{2\alpha}$-Behandlung (863/1011) und 0,5 ng/mL bei G2 (was auf eine vollständige Luteolyse hinweist) berücksichtigt wurden. Kühe, die im Abstand von 24 Stunden eine zusätzliche Behandlung erhielten, hatten einen höheren Besamungserfolg (46,5 %) im Vergleich zu DoubleDose-Kühen (37,7 %; P = 0,045), unterschieden sich aber nicht von Kühen in der Kontrollgruppe (40,3 %; P = 0,144). Wenn zu Beginn des Protokolls keine CL vorhanden waren, wiesen die Kühe der 2PGF-Gruppe einen höheren Besamungserfolg auf (47,9 %; P = 0,043) als die Kühe der DoubleDose-Gruppe (32,7 %), unterschieden sich jedoch nicht von den Kühen der Kontrollgruppe (36,1 %; P = 0,109).

Die Genauigkeit zur Identifizierung eines funktionellen CL mittels transrektalem Ultraschall zu Beginn des Protokolls betrug 87,2 %. Bei der ROC-Analyse betrug die Fläche

unterhalb der Kurve (AUC) 0,901 und kann als sehr genau angesehen werden. Der optimale Grenzwert lag bei einem CL Durchmesser von 20 mm.

Die Bewertung des Scheidenausflusses stand in signifikantem Zusammenhang (*P* = 0,001) mit den P4-Konzentrationen bei der ersten $PGF_{2\alpha}$-Behandlung. Kühe mit einem VS von 2 hatten höhere P4-Serumspiegel (4,3 ± 0,2 ng/mL) als Kühe mit einem VS von 0 (3,0 ± 0,3 ng/mL; *P* = 0,001) und 1 (3,7 ± 0,1 ng/mL; *P* = 0,002). Die Progesteronkonzentration am Tag 7 war bei Kühen, denen VS 1 zugewiesen wurde, höher (*P* = 0,027) als bei Kühen, denen VS 0 zugewiesen wurde. Das Ergebnis des vaginalen Ausflusses bei der PRID-Entfernung stand tendenziell in Zusammenhang mit dem Besamungserfolg an Tag 38 ± 3 nach der künstlichen Besamung (*P* = 0,072). Der Besamungserfolg unterschied sich nicht (*P* = 0,459) zwischen Kühen mit einem VS von 0 und 1 [36,5 % (25/69); 41,3 % (320/780)], war aber signifikant größer [49,7 % (102/207)] für Kühe mit einem VS von 2, verglichen mit VS 1 (*P* = 0,039), und tendierte dazu, größer zu sein als bei VS 0 (*P* = 0,066).

Die Ergebnisse dieser Studie zeigen, dass beide $PGF_{2\alpha}$-Modifikationen die Luteolyse in P4 gestützten Ovsynch-Protokollen im Vergleich zu einer Standard-$PGF_{2\alpha}$-Behandlung an Tag 7 des Protokolls erhöhen können. Interessanterweise hatten nur Kühe, die eine zusätzliche $PGF_{2\alpha}$-Behandlung erhielten, einen numerischen Anstieg des Besamungserfolges (6,2 %), insbesondere bei Kühen ohne CL bei G1 (11,8 %), während eine erhöhte Dosis von $PGF_{2\alpha}$ zu keiner Verbesserung der Reproduktionsleistung führte.

Vaginaler Ausfluss an Tag 8 des Protokolls, nach Entfernung der PRID, war mit höheren P4-Konzentrationen an Tag 7 und einem höheren Besamungserfolg verbunden. Die Ultraschalluntersuchung kann als wertvolles Instrument zur Erkennung von CL angesehen werden.

Die zweite Studie hatte zum Ziel, die Auswirkungen einer 8-tägigen P4-Supplementierung mittels einer PRID-Spange während eines Ovsynch-Protokolls auf P/AI und Trächtigkeitsverluste im Vergleich zu einem 7-tägigen Standard-Ovsynch-Protokoll zu erforschen. Insgesamt wurden 716 laktierende Holstein-Kühe aus einem kommerziellen Milchviehbetrieb im Nordosten Deutschlands in diese Studie aufgenommen. Die Behandlungsgruppe war Teil der oben erwähnten Studie und erhielt ein 8-tägiges P4-basiertes Ovsynch-Protokoll (PRIDsynch: d 0, 100 µg GnRH + PRID; d 7, 25 mg Dinoprost; d 8, PRID-Entfernung; d 9, 100 µg GnRH), während die Kontrollgruppe ein standardmäßiges 7-d

Ovsynch-Protokoll als Teil des Reproduktionsplans des Betriebs erhielt (Kontrolle: d 0, 100 µg GnRH; d 7, 500 µg Cloprostenol; d 9, 100 µg GnRH). Alle Kühe wurden etwa 16 Stunden nach G2 besamt. Die Trächtigkeitsdiagnose wurde mittels transrektaler Ultraschalluntersuchung 38 ± 3 Tage nach der künstlichen Besamung durchgeführt und 80 ± 7 Tage nach der Besamung erneut bestätigt.

Die Ergebnisse dieser Studie zeigen die insgesamt positive Wirkung einer verlängerten P4 Supplementierung über 8 Tage mit einer PRID-Spange in einem Ovsynch-Protokoll auf die Reproduktionsleistung. Die Besamungserfolge waren bei PRIDsynch-Kühen signifikant erhöht [P = 0,014; 38,9 % (115/360)] im Vergleich zu unbehandelten Kontrollkühen [31,7 % (115/360)] an Tag 38 ± 3 nach künstlicher Besamung. Die Trächtigkeitsverluste wurden jedoch durch die Behandlung nicht beeinflusst [P = 0,279; Kontrolle: 8,8 (10/113) vs. PRIDsynch: 6,2 (9/146)].

Insgesamt zeigt diese Arbeit, dass 1) der Anteil der Kühe mit vollständiger Luteolyse nahe der Besamung durch beide $PGF_{2\alpha}$-Modifikationen erhöht wurde, aber nur die zweite Behandlung im Abstand von 24 Stunden zu einem größeren Besamungserfolg führte, verglichen mit der erhöhten $PGF_{2\alpha}$-Dosis, und zu einem numerischen Anstieg des Besamungserfolges im Vergleich zu Kontrollkühen, insbesondere bei Kühen, die zu Beginn des Protokolls keine CL hatten, 2) die Ergänzung einer PRID von Tag 0 bis zur Entfernung an Tag 8 des Protokolls die Reproduktionsleistung erhöhte, aber keinen Einfluss auf den Trächtigkeitsverlust hatte, 3) vaginaler Ausfluss, der durch P4-Spangen verursacht wird, keinen negativen Einfluss auf die Fruchtbarkeit hat und 4) Ultraschalluntersuchungen der Ovarien für den Nachweis eines funktionellen CL geeignet sind. Zusammengenommen haben diese Ergebnisse das Potenzial, die Reproduktionsleistung in Milchviehbetrieben mit Protokollen zur terminorientierten Besamung zu verbessern.

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8 PUBLICATIONS

Research articles

Hölper M., Bretzinger L., Randi F., Heuwieser W., Borchardt S. (2023):
Effect of dose and frequency of prostaglandin F2α treatments during a 7-day Ovsynch protocol with an intravaginal progesterone releasing device on luteal regression and pregnancy outcomes in lactating Holstein cows.
Journal of Dairy Science, 106:755–768. doi.org/10.3168/jds.2022-22245.

Hölper M., Bretzinger L., Randi F., Heuwieser W., Borchardt S. (2023):
Effect of a progesterone-releasing intravaginal device (PRID) for 8 days during a modified Ovsynch protocol on pregnancy outcomes in lactating Holstein cows.
JDS Communications, 4:303–307. doi.org/10.3168/jdsc.2022-0314.

Oral presentations

Hölper M. (2023):
Aktuelle Studien zum Einsatz der PRID Delta in Ovsynch-Protokollen.
5. reprodAction ExpertenForum Berlin, 23.08.2023.

Poster presentations

Hölper M., Bretzinger L., Randi F., Heuwieser W., Borchardt S. (2023):
Effect of dose and frequency of prostaglandin F2α treatments during a 7-day Ovsynch protocol with an intravaginal progesterone releasing device on luteal regression and pregnancy outcomes in lactating Holstein cows.
European Buiatrics Congress and ECBHM Jubilee Symposium Berlin, Germany, August 24 – 26, 2023.

Hölper M., Bretzinger L., Randi F., Heuwieser W., Borchardt S. (2024):

Impact of vaginal discharge on dairy cows' fertility subjected to an ovsynch protocol with an intravaginal progesterone-releasing device.

57th Annual Conference of Physiology and Pathology of Reproduction and 49th Joint Conference of Veterinary and Human Reproductive Medicine, Berlin, Germany, February 28 – March 01, 2024.

In: Reproduction in Domestic Animals, 59:10. doi.org/10.1111/rda.14531.

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11 CONFLICT OF INTEREST

Die Hormone wurden bereitgestellt von Ceva Santé Animale. Darüber hinaus bestehen keine Interessenskonflikte durch Zuwendung Dritter.

12 DECLARATION OF INDEPENDENCE

Hiermit erkläre ich, Marvin Hölper, dass ich alle Studien selbstständig durchgeführt und die vorliegende Arbeit selbstständig angefertigt habe. Ich versichere, dass ich ausschließlich die angegebenen Quellen und Hilfen in Anspruch genommen habe.

Tabelle 1. Eigener Anteil[1] an den Forschungsprojekten der vorliegenden Dissertation

	Studie 1[a]	Studie 2[b]
Studienplanung	++	+++
Datenerhebung	+++	+++
Datenanalyse	++	+++
Verfassen des Manuskripts	+++	+++
Editieren des Manuskripts	++	+++

[1]Legende: +++: > 70%
++: 50-70%
+: < 50%

[a]Effect of dose and frequency of prostaglandin F2α treatments during a 7-d Ovsynch protocol with an intravaginal progesterone releasing device on luteal regression and pregnancy outcomes in lactating Holstein cows.

[b]Effect of a progesterone-releasing intravaginal device (PRID) for 8 days during a modified Ovsynch protocol on pregnancy outcomes in lactating Holstein cows.

Berlin, den 31.05.2024

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