Original Article
J Vet Sci 2015, 16(1), 121-126 · http://dx.doi.org/10.4142/jvs.2015.16.1.121

Factors affecting the success of resynchronization protocols with or without progesterone supplementation in dairy cows

Annette Forro[1], Georgios Tsousis[2,*], Nicola Beindorff[1], Ahmad Reza Sharifi[3], Christos Brozos[2], Heinrich Bollwein[1]

[1]*Clinic for Cattle, University of Veterinary Medicine Hanover, 30173 Hanover, Germany*
[2]*Clinic of Farm Animals, Aristotle University of Thessaloniki, Thessaloniki 54627, Greece*
[3]*Institute of Animal Breeding and Genetics, University of Göttingen, 37075 Göttingen, Germany*

The objective of this study was to investigate factors that influence the success of resynchronization protocols for bovines with and without progesterone supplementation. Cow synchronized and not found pregnant were randomly assigned to two resynchronization protocols: ovsynch without progesterone (P4) supplementation (n = 66) or with exogenous P4 administered from Days 0 to 7 (n = 67). Progesterone levels were measured on Days 0 and 7 of these protocols as well as 4 and 5 days post-insemination. Progesterone supplementation raised the P4 levels on Day 7 ($p < 0.05$), but had no overall effect on resynchronization rates (RRs) or pregnancy per artificial insemination (P/AI). However, cows with Body Condition Score (BCS) > 3.5 had increased P/AI values while cows with BCS < 2.75 had decreased P/AI rates after P4 supplementation. Primiparous cows had higher P4 values on Day 7 than pluriparous animals ($p = 0.04$) and tended to have higher RRs ($p = 0.06$). Results of this study indicate that progesterone supplementation in resynchronization protocols has minimal effects on outcomes. Parity had an effect on the levels of circulating progesterone at initiation of the protocol, which in turn influenced the RR.

Keywords: ovsynch, progesterone, resynchronization

Introduction

High circulating progesterone (P4) concentrations before artificial insemination (AI) have been shown to enhance pregnancy rates [4,8] due to improved synchronization of ovulation [8,15] and a higher number of good quality embryos [20]. Moreover, post-insemination uterine function can be negatively affected by low P4 concentrations before AI. [3]. In a study by Cerri *et al.* [3], low P4 concentrations during the development of ovulatory follicles prematurely increased estrogen receptor-α abundance and Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) release by the uterus. Forde *et al.* [13] observed altered expression of multiple endometrial genes with low P4 concentrations.

Resynchronizing non-pregnant cows following early pregnancy diagnosis is an efficient tool for optimizing herd management while reducing culling rates and time until pregnancy. Cows subjected to the ovsynch program for synchronization can benefit when exogenous progesterone is added to the protocol [18] since the risk of high progesterone metabolism or luteal insufficiency might suppress fertility in high yielding dairy cows [21]. A 7 to 10% rise in pregnancy per AI (P/AI) compared to control cows was recently observed in two studies in which supplementary P4 was blindly administered as part of a resynchronization ovsynch protocol [1,9]. However, the positive effects of additional P4 administered with a synchronization protocol are not consistent and can be affected by hormonal manipulations before initiating the actual protocol. In a previous study [11] evaluating P4 supplementation during a synchronization protocol, improved pregnancy rates were achieved. However, this effect could not be reproduced when a presynchronization protocol was implemented, probably due to differing proportions of cyclic cows [11].

It is still largely unknown if previously performed synchronization methods can influence resynchronization protocol outcomes. Depending on the synchronization protocol, different effects on the ovaries can be expected. Moreover, ovarian status of the animals influences the success of a

Received 5 Aug. 2014, Revised 18 Sep. 2014, Accepted 7 Oct. 2014
*Corresponding author: Tel: +30-2310-994466; Fax: +30-2310-994452; E-mail: tsousis@vet.auth.gr
pISSN 1229-845X
eISSN 1976-555X

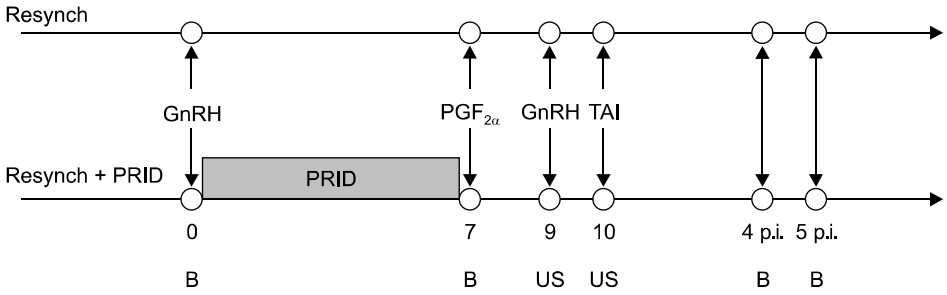

Fig. 1. Resynchronization protocols with or without P4 supplementation. GnRH: gonadotropin-releasing hormone, PGF_2: prostaglandin $F_{2\alpha}$, TAI: timed artificial insemination, PRID: progesterone-releasing intravaginal device, p.i.: post insemination, B: blood samples, US: ultrasonography.

synchronization protocol. It is accepted that ovsynch results in greater pregnancy rates if initiated between Days 5 to 12 of the estrous cycle [23]. In a study by Bisinotto *et al.* [1], cows that started ovsynch with high P4 values (diestrus) had higher P/AI rates than cows with low P4 levels or anovular animals. The main objective of the current study was to determine if P4 supplementation at the initiation of a resynchronization protocol would increase the proportion of resynchronized and pregnant cows after timed AI (TAI). Additionally, factors like hormonal manipulations preceding resynchronization and progesterone levels at different time points were examined to determine their effects on the efficacy of the resynchronization protocol as well as pregnancy rates.

Materials and Methods

Animals and experimental design

Details of the study herd and examination protocols have been previously reported [14] as two consecutive studies were performed. This investigation was approved (research permit no. 22-2684-04-15-102/08) by the independent ethics committee of the Thuringia Federal State Office for Consumer Protection (TLV), Bad Langensalza, (Germany). For the first experiment, an ovsynch protocol was initiated between Days 52 to 63 after calving. Each cow was given Gonadotropin-releasing hormone (GnRH, 100 μg of gonadorelin diacetate tetrahydrate, Ovarelin; CEVA Tiergesundheit, Germany) followed 7 days later by Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, 25 mg of Dinoprost; 33.6 mg Dinoprost-Trometamol, Enzaprost T; CEVA Tiergesundheit). Thereafter, cows were blocked by parity and randomly allocated into two groups: one that underwent the traditional ovsynch protocol with a second dose of GnRH 48 h after PGF_2 (OVS48) administration, and one that was subjected to a modified protocol with a second dose of GnRH 60 h after PGF_2 treatment (OVS60 group). On Day 4 after TAI1, a 50% of the cows were randomly supplemented with PRID alpha 1.55 g (Progesterone 1.55 g; CEVA Tiergesundheit) for 14 days (until 18 days post-insemination). The rest of the cows were untreated.

Pregnancy was diagnosed on Day 33 after TAI1. After a negative pregnancy diagnosis, a total of 145 cows were resynchronized of which 12 were eliminated during the study because of culling (n = 3), loss of the PRID device (n = 5), or a lack of appropriate documentation (n = 4). Thus, 133 cows were eligible for further analysis (Fig. 1). The cows were randomly divided into two groups: one that received the traditional ovsynch protocol as described above (P4− group, n = 66) and one that received additional PRID alpha supplementation from Days 0 to 7 of the resynchronization protocol (P4+ group, n = 67). All cows were bred again by timed artificial insemination (TAI2) 14 to 20 h after the second GnRH administration.

On Days 0 (day of GnRH treatment) and 7 (day of PG treatment) of the resynchronization program and 4 and 5 days after TAI, a 4-mL blood sample from a random 50% of each group (41 from the P4− group and 31 from the P4+ group) was taken from the Vena caudalis mediana after disinfecting the area. The samples were stored on ice until being centrifuged (3,500 × g for 15 min at 4°C) within 60 min after collection. The plasma was recovered and stored at −20°C until hormone analyses. Plasma P4 levels were measured by a standard enzyme immunoassay according to Prakash *et al.* [19]. Briefly, P4 was directly measured in 2 mL of plasma (antibody: P-1922 monoclonal anti-P4; Sigma, USA; enzyme: 4-pregnen-3.20-dione-3-O-carboxymethyloxime horseradish peroxidase; Prof. H. H. D. Meyer, Physiology Weihenstephan, Technische Universitaet Muenchen, Germany). The assay samples were incubated overnight. A standard curve from 0.2 to 12.5 ng/mL was generated, and quality controls (1.0 ng/mL and 3.0 ng/mL) were within the linear range of measurement. Validation of the assay revealed a lower detection limit of 0.5 ng/mL and an upper limit of 4.0 ng/mL. All samples with initially higher concentrations were measured again after dilution. The intra- and inter-assay coefficients of variation were < 10%. In an initial study by our group [14], it was confirmed that blood sampling performed directly after intravaginal application of the PRID without a change of gloves leads to contamination of the samples with P4. Therefore, only samples from P4− cows

were used to measure P4 concentrations on Day 0 of the resynchronization protocol (first GnRH treatment).

Ultrasonography and resynchronization rate

Transrectal ultrasonography of the ovaries was performed with a 5 MHz linear-array transducer (SonoVET 2000; OsteoSys, Korea) on the day of the second GnRH administration and on the day after TAI2 (Fig. 1). Results of the ultrasound examinations were used to classify the cows according to synchronization status. Cows that had a follicle size between 15 and 25 mm on the day of the second GnRH dose that was not observed on the day after TAI were considered resynchronized. Pregnancy was diagnosed on Days 33 to 40 after TAI2. A cow was defined as pregnant if an embryonic heartbeat was detected. Absence of a heartbeat with an otherwise normal pregnancy led to a second examination 10 days later.

Documentation of measurements and exclusion

Documentation and exclusion criteria used in this study have been previously reported [14]. Briefly, all animals with a record of severe or life-threatening illness were excluded from the study. During late puerperium, animals should not have either incomplete uterine involution or purulent/mucopurulent intrauterine fluid as diagnosed by transrectal palpation and vaginoscopy. Body condition score of the cows on the day of resynchronization initiation (BCS100) was graded from 1 to 5 in 0.25 increments as previously described by Edmonson *et al.* [10]. The 100-day milk production (MP100) of each cow was calculated using HERDE (dsp-Agrosoft, Germany) and was included in the statistical analysis as a continuous variable. The season was included in the analysis as a binary variable with the months of April to September defined as summer and those between October and March as winter. Additionally, the cows were divided into two parity groups: primi- and pluriparous animals.

Statistical analysis

Statistical analyses were conducted using the Statistical Analysis System (ver. 9.3; SAS Institute, USA). When a univariate analysis was performed, differences in binary variables were evaluated with the use of a chi-square analysis (PROC FREQ). A set of variables was evaluated in the statistical model to determine their effect on pregnancy and RR: MP100, parity, season, previous synchronization protocol, P4 supplementation after TAI1, BCS100, and resynchronization protocol with or without progesterone. All two-way interactions with the resynchronization protocol were entered into the models. Insignificant variables were removed from the model using Sum of Square Type I and III (Wald-type) tests and *F*-statistics at $p < 0.05$ rather than likelihood ratio tests. The *F*-ratios used for the analysis of variance are identical to the Wald rank (K) *F*-statistics as defined by Littell *et al.* [16]. Finally, statistical analyses for pregnancy and RRs were carried out with linear logistic models and a binary response variable, which is modelled as a binomial random variable (y_i). The data were then analyzed with the GLIMMIX procedure [16]. To analyze the P4 values, general linear models with the use of SAS PROC GLM were implemented. Results are presented as the mean ± standard deviation (SD) unless stated otherwise. Differences were considered to be statistically significant at $p < 0.05$ and as a tendency at $0.05 \leq p < 0.10$.

Results

Resynchronization rate (RR)

The overall RR was 81.2% (108/133). RRs were not influenced by season (summer *vs.* winter), MP100, BCS100, previous or current synchronization schemes, or P4 supplementations (Table 1). Only parity had a strong tendency to affect the RR with cows in the first lactation (n = 42) being resynchronized more efficiently than animals in later (n = 91) lactations (90.5% *vs.* 76.9% respectively, $p = 0.06$).

Table 1. Uncorrected resynchronization rates (RRs) and *p* values for the variables under consideration

Variable and class	Number	Uncorrected RR (%)	*F*-value	Pr > F
Season			0.43	0.51
Summer	52	84.62		
Winter	81	79.01		
MP100			0.05	0.82
Parity			3.61	0.06
1	42	90.48		
> 1	91	76.92		
Ovsynch			0.04	0.84
48	64	81.25		
60	69	81.16		
P4 p.i.			0.66	0.42
Yes	76	82.89		
No	57	78.95		
BCS100			0.80	0.37
Resynch			0.74	0.39
P4+	67	79.10		
P4−	66	83.33		

MP100; 100-day milk production, BCS100; body condition score assessed on the day resynchronization began (90 to 100 days postpartum), Ovsynch; previous synchronization protocol, Resynch; resynchronization, P4 p.i.; previous supplementation with progesterone (P4) post-insemination, P4+; resynchronization with P4 supplementation, P4−; resynchronization without P4 supplementation.

Table 2. Uncorrected PRs and *p* values for the variables under consideration

Variable and class	Number	Uncorrected PR (%)	*F*-value	Pr > F
Season			0.21	0.64
Summer	52	44.23		
Winter	81	39.51		
MP100			0.02	0.88
Parity				
1	42	50.00	0.35	0.55
> 1	91	37.36		
Ovsynch			0.07	0.79
48	64	39.06		
60	69	43.48		
P4 p.i.			0.05	0.81
Yes	76	42.11		
No	57	40.35		
BCS100			2.02	0.15
Resynch			0.94	0.33
P4+	67	37.31		
P4−	66	45.50		

P/AI

The overall P/AI was 41.4% (55/133). This rate was not influenced by season, MP100, previous synchronization protocol, or P4 supplementation post insemination (p.i.). These variables were omitted from the model (Table 2). Although more cows in the first lactation were synchronized and achieved better P/AI rates, this variable was also insignificant in the final model. P/AI values were similar between cows that underwent the two resynchronization protocols (37.1% *vs.* 45.5% for P4+ *vs.* P4−, respectively; $p = 0.33$). BCS100 had no influence on P/AI ($p = 0.15$), but an interaction between BCS100 and P4 supplementation on the resynchronization protocol was apparent ($p = 0.01$). Specifically, animals with low body condition scores (2.25 to 2.75) had better P/AI rates when not supplemented with P4 compared to supplemented cows. The opposite was evident in cows with BCS equal to or above 3.5 (Fig. 2). No other two-way interactions with the resynchronization protocol were significant. The same results were obtained when animals that were not synchronized (n = 25) were excluded from the analysis. Only four out of 25 cows (16%) classified as not being synchronized became pregnant compared to 47.2% of the synchronized cows ($p = 0.004$).

P4 values

When the cows that received P4 supplementation during resynchronization were excluded from the analysis, P4 values (n = 41) on day of resynchronization initiation tended to be influenced by the previous synchronization protocol (OVS48

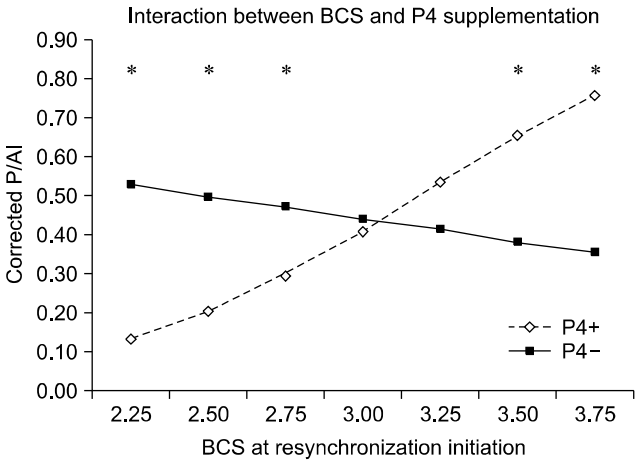

Fig. 2. Effect of P4 supplementation with a resynchronization protocol on different body condition score categories. P/AI: pregnancy per AI. *$p < 0.05$.

4.06 ± 0.72 ng/mL *vs.* OVS60 5.87 ± 0.82 ng/mL, $p = 0.09$). On the day of PG treatment, P4 values where affected only by parity (1st 6.02 ± 1.49 ng/mL *vs.* > 1st 4.67 ± 0.54 ng/mL, $p = 0.04$). No factor influenced progesterone values on Day 4 or 5 p.i. When P4+ animals were added to the analysis, a significant effect of P4 supplementation on P4 values measured on the day of the PG treatment (P4+ 7.57 ± 0.89 *vs.* P4− 4.93 ± 0.52, $p = 0.01$) was observed, but not on Days 4 or 5 p.i. In cows not supplemented with PRID and having P4 values above 1 ng/mL on Day 0, better RRs were observed compared to animals with lower P4 values (88.6% *vs.* 50% respectively, $p = 0.02$). The same was noticed for P/AI rates (54.3% *vs.* 33.3%) although the difference was not significant due to the very small number of animals with low P4 levels on Day 0 (n = 6). For animals that did not receive P4 supplementation, numerical differences for RRs and P/AI rates were found on Day 7 between animals with high (≥ 1 ng/mL) and low P4 concentrations (85.3% *vs.* 71.4% for RR and 55.9% *vs.* 28.6% for P/AI, respectively). Again, these differences were not significant due to very few animals having low P4 levels (n = 7).

Discussion

In the present study, P4 supplementation as part of a resynchronization protocol based on ovsynch did not affect P/AI or RRs. In several recent studies, a positive effect was documented when cows were supplemented with P4 during synch- or resynchronization protocols [4,8,9,18]. In most of these investigations, a modest rise in P/AI of 5 to 10% was achieved. However, the health status of the genital system was not extensively assessed as it was in our experiment. This could be a reason why control animals in the aforementioned studies generally achieved just above 30% (or even as low as 20～25%)

P/AI compared to our control group that had a P/AI rate of 45%. Studies in which the control group achieved satisfactory P/AI often failed to show any positive effect of P4 supplementation [5,11]. It is possible that underlying health problems can lead to luteal insufficiency and adding progesterone can partially compensate for these abnormalities. Moreover, the 305-day milk yield at the farm where the experiment took place was approximately 9,700 kg, which is less than those reported in most US studies. This fact could have resulted in milder progesterone metabolism in our study. It could also be the reason why no overall negative effects of milk yield and/or BCS on P/AI were observed since most animals have a positive energy balance after 100 days of lactation [7].

In addition, Dewey *et al.* [9] attributed improvement resulting from P4 supplementation to better synchrony of the estrous cycle. As concluded by Chebel *et al.* [4], adding P4 during a synchronization protocol seems beneficial because it reduces the incidence of early ovulation before $PGF_{2\alpha}$ treatment. However, in our study the resynchronization rates were equal and efficiently high among P4-supplemented and non-supplemented cows. Dewey *et al.* [9] suggested that it is rather unlikely to use successfully the previous synchronization protocol to predict the ovarian status of the animals at any other time afterwards. Many factors, namely synchronization rate or early embryonic losses, can distort the timing of cyclicity of a dairy cow. Nevertheless, when P4 supplementation is administered after first insemination in a large proportion of the study animals, as in our case, one can expect tighter estrous synchronization of non-pregnant cows [6] and fewer embryonic losses [24]. According to Sartori *et al.* [22] who summarized the findings of many studies, the cycle of modern dairy cows is 23 to 24 days. Thus, most cows in our study were theoretically in an optimal period (Days 9 ~ 10) for synchronization. Actually, there was a limited number of animals not bearing a Corpus luteum (CL) on Day 0 (33 days post-TAI1) or with low P4 values on Day 7. The importance of initiating a synchronization protocol when P4 values are high was evident in our investigation as well as other studies [8,23]. Additionally, in agreement with Martins *et al.* [17] we found that animals with higher P4 levels on Day 7 had better P/AI and RRs, probably due to the fact that induced luteolysis is more efficient when a mature CL is present.

In the previous study by our working group at the same farm and with the same animals, we noticed an interaction between BCS and P4 supplementation p.i. on P/AI [14]. This finding was attributed to the fact that animals with low BCS cannot profit from P4 supplementation as they are not in an optimal physical state for fertilization or pregnancy maintenance. However, in the present study we noticed a similar pattern for cows that received P4 supplementation, but also found relatively steady and high P/AI values for the control group in all BCS categories. It is rather difficult to interpret the very good P/AI rates of thin cows in the P4− group. Since animals probably have a positive energy balance by Day 100, acceptable fertility could be expected even in cows that have still not regained body reserves. On the other hand, the reason for low P/AI rates of animals with a lower BCS only in the supplemented group is unclear as it presupposes a negative action of progesterone. Folman *et al.* [12] have described a negative effect of progesterone on conception rates of cows with an already high P4 concentration. Although we would expect to see no or even a negative effect of low BCS on P4 levels [11,14], contamination of our samples taken on Day 0 did not allow us to perform such an analysis. Since this study was the continuation of a synchronization study, a small sample size (especially regarding two-way interactions) could not be avoided. This fact has led to results that deserve further research in order to draw more concrete conclusions.

Another factor that influenced outcomes in this study was the previous synchronization protocol since cows for which the time interval from $PGF_{2\alpha}$ to the second GnRH treatment was previously extended from 48 to 60 h had greater P4 values by the initiation of resynchronization. Our working group has shown that such a modification promotes luteal blood flow 7 days post-estrus [2] and slightly elevates P4 levels on Day 4 [14] although P/AI is not directly affected. Thus, a carry-over effect of better functioning ovaries cannot be excluded. Moreover, parity influenced many parameters in our study. Cows in the first lactation had greater P4 values on Day 7, which probably resulted in increased RRs and P/AI values. There is also a direct effect of lactation on circulating steroids [22]. Primiparous cows have lower milk production as well as reduced dry matter intake and thus are expected to have lower steroid metabolism than pluriparous cows. In a number of studies, primiparous cows had greater P4 values [1,17] along with higher synchronization [11] and pregnancy rates [1,11,17] than pluriparous cows in accordance with our results.

In conclusion, our data failed to demonstrate any advantage of P4 supplementation as part of a resynchronization protocol in terms of RRs or P/AI. A very large proportion of the animals in the present study was cyclic, and had a corpus luteum and/or elevated P4 values by initiation of the protocol, probably due to hormonal manipulations preceding resynchronization. Based on our results, simple resynchronization protocols initiated 33 days after the first AI can work efficiently in healthy and metabolically unstressed animals. Thus, a more rational use of hormones is indicated.

Acknowledgments

The authors wish to thank CEVA Santé Animale (France) for providing the medicaments and financial support for this study.

Conflict of Interest

There is no conflict of interest.

References

1. **Bisinotto RS, Ribeiro ES, Martins LT, Marsola RS, Greco LF, Favoreto MG, Risco CA, Thatcher WW, Santos JEP.** Effect of interval between induction of ovulation and artificial insemination (AI) and supplemental progesterone for resynchronization on fertility of dairy cows subjected to a 5-d timed AI program. J Dairy Sci 2010, **93**, 5798-5808.
2. **Bollwein H, Prost D, Ulbrich SE, Niemann H, Honnens A.** Effects of a shortened preovulatory follicular phase on genital blood flow and endometrial hormone receptor concentrations in Holstein-Friesian cows. Theriogenology 2010, **73**, 242-249.
3. **Cerri RLA, Chebel RC, Rivera F, Narciso CD, Oliveira RA, Amstalden M, Baez-Sandoval GM, Oliveira LJ, Thatcher WW, Santos JEP.** Concentration of progesterone during the development of the ovulatory follicle: II. Ovarian and uterine responses. J Dairy Sci 2011, **94**, 3352-3365.
4. **Chebel RC, Al-Hassan MJ, Fricke PM, Santos JEP, Lima JR, Martel CA, Stevenson JS, Garcia R, Ax RL.** Supplementation of progesterone via controlled internal drug release inserts during ovulation synchronization protocols in lactating dairy cows. J Dairy Sci 2010, **93**, 922-931.
5. **Chebel RC, Scanavez AA, Silva PRB, Moraes JGN, Mendonça LGD, Lopes G Jr.** Evaluation of presynchronized resynchronization protocols for lactating dairy cows. J Dairy Sci 2013, **96**, 1009-1020.
6. **Chenault JR, Boucher JF, Dame KJ, Meyer JA, Wood-Follis SL.** Intravaginal progesterone insert to synchronize return to estrus of previously inseminated dairy cows. J Dairy Sci 2003, **86**, 2039-2049.
7. **Coffey MP, Simm G, Brotherstone S.** Energy balance profiles for the first three lactations of dairy cows estimated using random regression. J Dairy Sci 2002, **85**, 2669-2678.
8. **Denicol AC, Lopes G Jr, Mendonça LGD, Rivera FA, Guagnini F, Perez RV, Lima JR, Bruno RGS, Santos JEP, Chebel RC.** Low progesterone concentration during the development of the first follicular wave reduces pregnancy per insemination of lactating dairy cows. J Dairy Sci 2012, **95**, 1794-1806.
9. **Dewey ST, Mendonça LGD, Lopes G Jr, Rivera FA, Guagnini F, Chebel RC, Bilby TR.** Resynchronization strategies to improve fertility in lactating dairy cows utilizing a presynchronization injection of GnRH or supplemental progesterone: I. Pregnancy rates and ovarian responses. J Dairy Sci 2010, **93**, 4086-4095.
10. **Edmondson AJ, Lean IJ, Weaver LD, Farver T, Webster G.** A body condition scoring chart for Holstein dairy cows. J Dairy Sci 1989, **72**, 68-78.
11. **El-Zarkouny SZ, Cartmill JA, Hensley BA, Stevenson JS.** Pregnancy in dairy cows after synchronized ovulation regimens with or without presynchronization and progesterone. J Dairy Sci 2004, **87**, 1024-1037.
12. **Folman Y, Kaim M, Herz Z, Rosenberg M.** Comparison of methods for the synchronization of estrous cycles in dairy cows. 2. Effects of progesterone and parity on conception. J Dairy Sci 1990, **73**, 2817-2825.
13. **Forde N, Mehta JP, Minten M, Crowe MA, Roche JF, Spencer TE, Lonergan P.** Effects of low progesterone on the endometrial transcriptome in cattle. Biol Reprod 2012, **87**, 124.
14. **Forro A, Tsousis G, Beindorff N, Sharifi R, Jäkel L, Bollwein H.** Combined use of Ovsynch and progesterone supplementation after artificial insemination in dairy cattle. J Dairy Sci 2012, **95**, 4372-4381.
15. **Galvão KN, Santos JEP, Juchem SO, Cerri RL, Coscioni AC, Villaseñor M.** Effect of addition of a progesterone intravaginal insert to a timed insemination protocol using estradiol cypionate on ovulation rate, pregnancy rate, and late embryonic loss in lactating dairy cows. J Anim Sci 2004, **82**, 3508-3517.
16. **Littell RC, Milliken GA, Stroup WW, Wolfinger RD.** SAS System for Mixed Models. SAS Institute, Cary, 1996.
17. **Martins JPN, Policelli RK, Neuder LM, Raphael W, Pursley JR.** Effects of cloprostenol sodium at final prostaglandin F2α of Ovsynch on complete luteolysis and pregnancy per artificial insemination in lactating dairy cows. J Dairy Sci 2011, **94**, 2815-2824.
18. **Melendez P, Gonzalez G, Aguilar E, Loera O, Risco C, Archbald LF.** Comparison of two estrus-synchronization protocols and timed artificial insemination in dairy cattle. J Dairy Sci 2006, **89**, 4567-4572.
19. **Prakash BS, Meyer HH, Schallenberger E, van de Wiel DF.** Development of a sensitive enzymeimmunoassay (EIA) for progesterone determination in unextracted bovine plasma using the second antibody technique. J Steroid Biochem 1987, **28**, 623-627.
20. **Rivera FA, Mendonça LGD, Lopes G Jr, Santos JEP, Perez RV, Amstalden M, Correa-Calderón A, Chebel RC.** Reduced progesterone concentration during growth of the first follicular wave affects embryo quality but has no effect on embryo survival post transfer in lactating dairy cows. Reproduction 2011, **141**, 333-342.
21. **Sangsritavong S, Combs DK, Sartori R, Armentano LE, Wiltbank MC.** High feed intake increases liver blood flow and metabolism of progesterone and estradiol-17β in dairy cattle. J Dairy Sci 2002, **85**, 2831-2842.
22. **Sartori R, Haughian JM, Shaver RD, Rosa GJM, Wiltbank MC.** Comparison of ovarian function and circulating steroids in estrous cycles of Holstein heifers and lactating cows. J Dairy Sci 2004, **87**, 905-920.
23. **Vasconcelos JLM, Silcox RW, Rosa GJM, Pursley JR, Wiltbank MC.** Synchronization rate, size of the ovulatory follicle, and pregnancy rate after synchronization of ovulation beginning on different days of the estrous cycle in lactating dairy cows. Theriogenology 1999, **52**, 1067-1078.
24. **Villarroel A, Martino A, BonDurant RH, Dèletang F, Sischo WM.** Effect of post-insemination supplementation with PRID on pregnancy in repeat-breeder Holstein cows. Theriogenology 2004, **61**, 1513-1520.