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Short communication

Evaluation of efficacy of selected oestrous synchronization programs depending on the functional state of the ovaries, heifers and cows of meat breed

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ABSTRACT. The objective of this study was to assess the effect of progesterone concentration (P4) and ovarian structure models before initiation of different synchronization programmes on pregnancy outcomes per artificial insemination (AI) (P/AI) in beef cattle. Cattle were randomly assigned to one of three different synchronization programes (8-d PRID+7PGF&TAI, PGF 7-d Select Synch+PRID&TAI and 7-d PRID+6PGF&TAI); blood sampling and ultrasound examinations were performed before (day −7) and at initiation (day 0) of synchronizations, and during the experiment. The models were designed based on the turnover in P4 and ovarian structures at day −7 and day 0: groups of Model 1 were based on P4 (<1, 1–3.99 and ≥4 ng/ml); groups of Model 2 were based on P4 (<1 and ≥1 ng/ml) and referred to the phase of the oestrus cycle; groups of Model 3 were based on follicle (F) diameter (absent, <10 and ≥10 mm); groups of Model 4 were based on corpus luteum (CL) diameter (absent, <10, 10–20 and ≥21 mm). It was indicated that P/AI for heifers and cows were 54.6 and 55.3% in the 1st, 61.5 and 60.6% in the 2nd and 36.1% (only cows) in the 3rd synchronizations ($P < 0.05$), respectively. Significant effect on P/AI of Model 1 in the LH, HL, HM, HH groups ($P < 0.05$) was found in 2nd and of Model 4 in MA, MM, HH, LA in 1st ($P < 0.05$) synchronizations. So, the presence of higher P4 concentration (Model 1) and CL (Model 4) before initiation of synchronizations improves P/AI in beef cattle.

Introduction

Nowadays, improving genetic progress is possible by changing traditional biotechnologies to artificial insemination and synchronization of the oestrus cycle in beef cattle herds.

Progesterone (P4) for over 30 years has been used to monitor ovarian activity and it is well accepted that blood serum P4 ≥1 ng/ml indicates a functional corpus luteum (CL), whereas concentrations <1 ng/ml the lack of a functional CL or non-cyclicity (Colazo et al., 2008; Stevenson et al., 2015). In terms of the oestrus cycle phase, cows in *dioestrus* had higher mean P4 concentrations (6.0 ± 0.05 ng/ml) than those in *oestrus* or *anoestrus* (0.8 ± 0.01 and 0.6 ± 0.01 ng/ml), in *prooestrus*, *oestrus* and *metoestrus* usually will have either a small or no visible CL, and concentration will be below 1.0 ng/ml (Colazo et al., 2008; Santos et al., 2016). It is clear that cows that develop the ovulatory follicle

(>10 mm) with low P4 concentrations have marked reductions in pregnancies per artificial insemination (P/AI) (Santos et al., 2016).

Prostaglandins ($PGF_{2\alpha}$) as a pre-synchronization in females with a mature CL will usually exhibit heat 2–5 days later and up to 40% of any group of randomly cyclic animals will not respond to a single administration (Diskin et al., 2001; Bridges et al., 2016). In addition, treatment with $PGF_{2\alpha}$ in the early stages of the oestrous cycle (first 5 days) was found to be ineffective in causing a luteolytic response in cattle (Dirandeh et al., 2015). The use of exogenous progesterone inserts (for 7–9 days) during a synchronization program increases blood P4 in suckled beef cows, increases the proportion of cows detected in *oestrus* and increases P/AI of cows subjected to timed AI (Garcia-Ispierto and Lopez-Gatius, 2014; Santos et al., 2016; Stevenson and Lamb, 2016). The observation that the presence of CL and not oestrus cyclic status has the greatest impact on P/AI is critical considering that approximately 25% of the cows receiving the first AI *postpartum* and 22 to 46% of those receiving resynchronized AI lack a CL when the synchronization protocol is initiated (Santos et al., 2016).

So, the aim of the study was to assess the effect of different P4 and ovarian structure models on P/AI prior to synchronization in beef cattle. Moreover, it is difficult to measure P4 in real-time or in farm conditions, so we hypothesized from a practical point of view, that understanding the turnover of ovarian structures would facilitate a decision on the further choice of synchronization program when the composition of the cows' group is distinct (cyclic vs non-cyclic, nulliparous vs multiparous).

Material and methods

All procedures were carried out in accordance with the guidelines of the State Law of Veterinary Activities, the Welfare and Protection of Animals of the Republic of Lithuania, and in compliance with EU Directive 2010/63/EU.

Angus, limousin and simmental beef heifers and cows were included in this study (n = 226). The study included clinically and reproductively healthy beef cattle and the experimental groups were housed under the same barn conditions with *ad libitum* access to water and fed a common ration formulated to meet the beef cattle feeding recommendation. Heifers (n = 35) were selected as: not bred before, the average age: 23 months, weight: 449 ± 97.2 kg and reproductive tract scoring not less than 3, and postpartum beef cows (n = 191) – primiparous and multiparous, at least 45 days *postpartum*, not bred in this period, weight: 597 ± 93.6 kg.

In total, 678 ultrasound examinations and samplings were performed. The ovarian examination was performed by rectal ultrasonography (using a 7.5 MHz linear probe, iScan, Draminski, Olsztyn, Poland) to measure maximum diameter of the follicles (F) and CL (mm) before (day −7) and at initiation (day 0; first sampled then synchronization application) of the synchronization programs, and at AI.

A blood sample was aseptically collected by coccygeal venipuncture immediately after each ultrasound examination in the same intervals (day −7, day 0 and AI) and was delivered to the laboratory in ambient temperature within 4 h. Serum samples were decanted and stored at −20 °C until assay. Progesterone was determined with the IBL International Progesterone ELISA determination kit (Hamburg, Germany). The range of the assay was between 0–40 ng/ml and the intra- and inter-assay CV and the sensitivity were 6.4%, 6.6% and 0.03 ng/ml, respectively.

Statistical analysis was performed using the SPSS Statistics 18 software package (SPSS Inc., Chicago, IL, USA) and was considered statistically significant when $P < 0.001$, $P < 0.01$ and $P < 0.05$.

Experimental design

The models were designed based on the turnover in P4 and ovarian structures at day −7 and day 0. Two models of P4 were replicated from Stevenson et al. (2015) study and two models of ovarian structures were concluded.

Model 1 was assigned by P4 with low (L; <1 ng/ml), medium (M; 1–3.99 ng/ml) and high (H; ≥4 ng/ml) value and the following groups were formed: LL (<1 to <1), LM (<1 to 1–3.99), LH (<1 to ≥4), ML (1–3.99 to <1), MM (1–3.99 to 1–3.99), MH (1–3.99 to ≥4), HL (≥4 to <1), HM (≥4 to 1–3.99) and HH (≥4 to ≥4); **Model 2** was assigned based on low <1 and high ≥1 ng/ml P4 with reference to the phase of the oestrus cycle: early *dioestrus* (<1 to ≥1), late *dioestrus* (≥1 to ≥1), *prooestrus-oestrus-metoestrus* (≥1 to <1) or *anoestrus* (<1 to <1); **Model 3** was assigned based on F diameter (absent (A), low <10 (L) and high ≥10 mm (H)) and the following groups were formed: AA, AL, AH, LA, LL, LH, HA, HL and HH; **Model 4** was assigned based on CL diameter (absent (A), low <10 (L), medium 10–20 (M) and high ≥21 mm (H)) and the following groups were formed: AA, AL, AM, AH, LA, LL, LM, LH, MA, ML, MM, MH, HA, HL, HM and HH.

Cows were assigned to one of the three synchronization programmes.

1st synchronization programme (8-d PRID+ 7PGF&TAI). Heifers and cows of angus, limousin and simmental breed (n = 98) were included in this program. All animals received an 8-day P4-releasing intravaginal device (PRID) (PRID Delta, containing 1.55 g of progesterone; Ceva Santé Animale, Libourne, France). An injection of $PGF_{2\alpha}$ (Dinolytic, containing 5 mg/ml of dinoprost trometamol; Zoetis, Louvain-La-Neuve, Belgium) was given 24 h before PRID removal. An injection of equine chorionic gonadotropin (eCG) (Folligon, containing 1000 IU of eCG; Intervet, Boxmeer, Netherlands) was made at the time of PRID removal for heifers 400 IU and for cows 500 IU, and were subjected to timed AT (TAI) 56 h after PRID removal.

2nd synchronization programme (PGF 7-d Select Synch+PRID&TAI). Heifers and cows of angus and limousin breed (n = 89) were included. At the pre-synchronization period an injection of $PGF_{2\alpha}$ (Dinolytic, containing 5 mg/ml of dinoprost trometamol; Zoetis, Louvain-La-Neuve, Belgium) was given on day 0, and heat detection and AI were till day 4. Afterward, on day 4 all non-inseminated cows received a 7-day PRID Delta (containing 1.55 g of progesterone; Ceva Santé Animale, Libourne, France) and an injection of GnRH (Receptal, containing 0.004 ng/ml of buserelin acetate; Intervet, Boxmeer, Netherlands). An injection of $PGF_{2\alpha}$ was given at the time of PRID removal. Cows were subjected to TAI at 72 h with an injection of GnRH (Receptal, containing 0.004 ng/ml of buserelin acetate; Intervet, Boxmeer, Netherlands).

3rd synchronization programme (7-d PRID+ 6PGF&TAI). Cows of angus and limousin breed (n = 39) were included. Cows received an 7-day PRID Delta (containing 1.55 g of progesterone; Ceva Santé Animale, Libourne, France). An injection of $PGF_{2\alpha}$ (Dinolytic, containing 5 mg/ml of dinoprost trometamol; Zoetis, Louvain-La-Neuve, Belgium) was given 24 h before PRID removal. An injection of GnRH (Receptal, containing 0.004 ng/ml of buserelin acetate; Intervet, Boxmeer, Netherlands) was made at the time of PRID removal and subjected to TAI 56 h after PRID removal.

Pregnancy was diagnosed *via* ultrasonography at 32 and 38 days post-AI.

Results

In total, 213 beef heifers and cows were inseminated. The P/AI within 1st, 2nd and 3rd synchronizations in cows and heifers was 55.3 and 54.6%, 60.6 and 61.5%, and 36.1% (only cows), respectively ($P < 0.05$). During pre-synchronization in the 2nd synchronization, only cows responded (31.6%) with P/AI of 62.5%.

A comparison of P4 and F at day −7, day 0 and AI in pregnant heifers and cows between the different synchronizations is presented in Table 1. P4 concentrations in pregnant cows at day −7 and day 0 were the highest in the 2nd and the lowest in the 3rd synchronization. P4 level in pregnant at day −7 and day 0 was higher in heifers than in cows, whereas it was similar in heifers and cows during insemination in 1st and 2nd synchronizations and higher in 3rd synchronization. During the AI, the F in heifer was slightly smaller in comparison to cows.

Not all groups of models were found in synchronizations. Significance effect on P/AI of the Model 1 was found in the 2nd synchronization ($P < 0.05$) with the highest number of pregnant cows in LH (88.9%), HH (72.7%), HM (66.7%) and HL (100%) groups. The comparison of these data is presented in Table 2. Whereas no significant effect on P/AI of Model 1 was found in 1st and 3rd synchronizations, and of Model 2 and Model 3 – in any of the synchronizations.

Table 1. Comparison of data average of the level of progesterone (P4) (ng/ml), follicle size (F) (mm) at 7 days before synchronization (day −7), day of synchronization initiation (day 0) and at insemination (AI) in pregnant heifers and cows from different synchronization programmes

Groups of P4 and F diameter	Synchronization programme		
	8-d PRID + 7PGF&TAI	PGF 7-d Select Synch + PRID&TAI	7-d PRID + 6PGF&TAI
P4 of pregnant heifers at day −7	6.46 ± 5.64	9.45 ± 4.89*	–
P4 of pregnant cows at day −7	2.40 ± 3.19	3.22 ± 3.72	1.33 ± 2.09
P4 of pregnant heifers at day 0	6.97 ± 6.72	9.41 ± 7.33	–
P4 of pregnant cows at day 0	2.85 ± 3.63	4.60 ± 3.98	0.65 ± 0.37
P4 of pregnant heifers at AI	0.37 ± 0.27	0.49 ± 0.31	–
P4 of pregnant cows at AI	0.38 ± 0.47	0.37 ± 0.27	0.68 ± 0.90
F diameter of pregnant heifers at AI	12.55 ± 1.13	12.25 ± 1.28	–
F diameter of pregnant cows at AI	12.82 ± 2.19	12.72 ± 1.40	12.08 ± 2.66

* the asterisk indicates the significance level at $P < 0.05$

Table 2. Comparison of the level of progesterone (P4) (ng/ml), follicle size (F) (mm) and corpus luteum (CL) (mm) of Model 1 groups in 2nd synchronization programme

Groups of Model 1	Synchronization period											
	day −7			day 0			AI_1		AI_2		AI_3	
	P4*	F	CL*	P4*	F	CL	P4	F	P4	F	P4	F
LH	0.52	10.86	13.50	5.84	12.67	20.33	2.17	13.50	0.23	9.00	0.35	13.00
HL	6.64	11.50	24.00	0.17	11.00	13.00	-	-	-	-	0.26	12.00
HM	14.27	11.33	20.33	2.23	12.00	16.00	3.66	10.50	-	-	0.82	13.00
HH	6.55	10.94	23.73	6.67	11.15	20.55	0.53	12.17	0.58	12.00	0.51	13.40

day −7 – the period before synchronization, day 0 – the period of synchronization initiation, AI_1 – first insemination in presynchronization period, AI_2 – second insemination in presynchronization period, AI_3 – insemination at the end of synchronization; * – the asterisk indicates the significance level at $P < 0.001$

Table 3. Comparison of the level of progesterone (P4) (ng/ml), follicle size (F) (mm) and corpus luteum (CL) (mm) of Model 4 groups in 1st synchronization programme

Groups of Model 4	Synchronization period							
	day −7			day 0			AI	
	P4	F	CL*	P4**	F	CL*	P4	F
LA	0.16	11.50	8.67	0.60	10.00	-	0.24	-
MA	6.13	13.20	16.00	1.41	11.38	-	0.38	13.33
HH	4.68	8.80	24.00	7.98	12.25	27.40	0.21	13.00
MM	4.96	10.77	15.57	4.50	9.90	15.29	0.47	11.88

day −7 – period before synchronization, day 0 – period of synchronization initiation, AI – insemination at the end of synchronization; * – the asterisk indicates the significance level at $P < 0.001$, ** – asterisks indicate the significance level at $P < 0.01$

Significance on P/AI of Model 4 was found in the 1st synchronization with the highest pregnancy in groups MM (78.6%), MA (55.6%), LA (100%) and HH (80%, $P < 0.05$) (Table 3). The significance of Model 4 was not determined in the 2nd and 3rd synchronizations.

Discussion

The objective of the current study was to assess the effect on P/AI of P4 and ovarian structure models designed at day −7 and day 0 prior to synchronization in beef cattle.

The P/AI varied between programs but efficiency within synchronization between heifers and cows was similar. Stevenson et al. (2015) indicated that when progesterone status was high (≥4.0 ng/ml) 10 days before the onset of the TAI program and was elevated at controlled internal drug release (CIDR) insertion, pregnancy outcome was suppressed (Stevenson and Lamb, 2016). In this study it was indicated that when P4 was high (HM, HL and HH) or increasing (LH) on day −7 and high (LH and HH) on day 0 the P/AI was significantly improved.

It was noted that 54.17% of all cows enrolled in the study at day −7 and 48.61% at day 0 have the concentration of P4 up to 1 ng/ml in the period prior to synchronization. In the 1st synchronization the concentration of P4 up to 1 ng/ml in cows at day −7 and day 0 was 52.04 and 45.92%, and in the 2nd synchronization was 53.03 and 41.51%, and in the 3rd synchronization 64.29 and 71.43%, respectively. Synchronization based on using intravaginal P4 can reduce animal numbers with low P4.

Treatment of anoestrus cows with a CIDR insert for 7 days and subsequent injection of $PGF_{2\alpha}$ at CIDR removal increased the proportion of cows in oestrus and increased pregnancy rate in comparison to anoestrus cows treated with $PGF_{2\alpha}$ alone (Lucy et al., 2001). Applying pre-synchronization in a group with varying level of P4 can increase P/AI in distinct groups. Dirandeh et al. (2015) indicated that combining $PGF_{2\alpha}$ and GnRH for pre-synchronization might be beneficial in acyclic cows. Injection of GnRH at CIDR insertion has been shown to increase pregnancy of anoestrus cows in comparison to the administration of GnRH with $PGF_{2\alpha}$ or $PGF_{2\alpha}$ alone (Stevenson et al., 2003). Anovulatory cows and those oestrus cyclic initiating the TAI protocol under low P4 have increased risk of pregnancy loss and have marked reductions in P/AI (Bisinotto et al., 2010; Wiltbank et al., 2014).

P4 level was low in both heifers and cows during AI, which may suggest effective luteolysis. Cows with >0.4 ng/ml of P4 in the blood near the time of AI had much lower fertility than cows with P4 <0.4 ng/ml (Joseph et al., 2017).

There was no effect on the P/AI of Model 3 in any of the synchronizations. According to our data, there was a relation on F diameter at the AI of Model 3 in the 3rd synchronization which varied within the highest pregnancy groups from 9.6 to 13.0 mm ($P < 0.05$). Cows induced to ovulate small dominant follicles have reduced pregnancy rate in comparison to cows that ovulated large follicles due to the development of smaller CL (Vasconcelos et al., 2001; Atkins et al., 2010). Noteworthy, P4 at day −7 and day 0 was rather higher in group of heifers than of cows; however ovulatory follicle during AI was lower in heifers.

The effect on P/AI of Model 4 was detected in the 1st synchronization where the diameter of CL was the same in all group both at day −7 and day 0. The benefit of treating cows with P4 during the TAI programs was greater in cows without CL than those in *dioestrus* (Santos et al., 2016). The mean size of CL at day 10 after ovulation was similar after synchronized (13.2 ± 1.5 mm) and spontaneous *oestrous* (13.5 ± 1.7 mm) (Quezada-Casasola et al., 2015).

Conclusions

Progesterone (P4) levels and ovarian structures are usually not considered as a practical aid in assessing the effectiveness of synchronization during its initiation. In summary, in pregnant cows group, P4 from high to high (≥4.0 ng/ml) or increasing (≥1 ng/ml) level has a greater effect on pregnancy per artificial insemination (P/AI) prior synchronizations in beef cattle. As well as, the presence of a corpus luteum prior to synchronization has a positive effect on levels P/AI. A pre-synchronization strategy with varying P4 at the beginning of synchronization should be considered.

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