Oestrus Synchronization by $PGF_{2\alpha}$ and GnRH in Intervals according to Stage of Follicular Development at Time of Initial Treatment in Cows

R. DOLEŽEL, S. ČECH, J. ZAJÍC, V. HAVLÍČEK

Department of Animal Reproduction, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic

Received November 20, 2001
Accepted February 13, 2002

Abstract

Doležel R. , S. Čech, J. Zajíc, V. Havlíček: *Oestrus Synchronization by $PGF_{2\alpha}$ and GnRH in Intervals according to Stage of Follicular Development at Time of Initial Treatment in Cows.* Acta Vet. Brno 2002, 71: 101-108.

The objective of this study was to evaluate the efficiency of oestrus/ovulation synchronization using $PGF_{2\alpha}$ and GnRH at intervals according to the sizes of ovarian follicles at the time of initial treatment. Oestrus synchronization was performed in 65 cows from 3 commercial dairy farms. Synthetic analogue of $PGF_{2\alpha}$ cloprostenol was administered in cows bearing corpus luteum between days 50 to 100 post partum. The cows were divided into 3 groups according to diameters of ovarian follicles in ultrasonographical image: Group 1 – small follicles (3-7 mm, n = 10), Group 2 – small and medium follicles (3–7 and 8–14 mm, n = 35) and Group 3 – small, medium and large follicles (3-7 mm, 8-14 mm and 15–23 mm, n = 20). GnRH was administered 72 hours (Group 1), 48 h (Group 2) and 24 h (Group 3) after $PGF_{2\alpha}$. Ultrasonographical examinations of ovarian structures were performed on days 0 (day of $PGF_{2\alpha}$ administration) 2, 4, 6, and 12. Blood samples for progesterone determination by RIA were obtained in 50 cows (10 cows in Group 1, 25 cows in Group 2 and 15 cows in Group 3) on days 0 and 12. Detection of oestrus was performed twice daily and cows in oestrus were inseminated. Term of oestrus and ovulation, progesterone concentration and conception rate were evaluated. Oestrus was detected and insemination was performed in 8 of 10 (80%), 33 of 35 (94%) and 12 of 20 (60%) cows in experimental Groups 1, 2 and 3, respectively, within 5 days after $PGF_{2\alpha}$ administration. Ovulation occurred in all inseminated cows in Groups 1 and 2 within 2 days after insemination. In spite of oestrus occurring and insemination being performed only in 12 cows in Group 3 ovulation occurred in 15 cows in this Group within 5 days after $PGF_{2\alpha}$. Corpora lutea were found in all ovulated cows (except 1 cow in Group 2) on day 12. Conception rates in inseminated cows were 50%, 64% and 42% in Groups 1, 2, and 3, respectively. The highest efficiency of oestrus synchronization was found in Group 2 in which oestrus occurred in 29 of 35 (83%) observed cows on day 3 after $PGF_{2\alpha}$ and conception rate was 66% in these cows.

$PGF_{2\alpha}$, GnRH, corpus luteum, follicle diameter, oestrus, ovulation, conception rate

An efficient method of oestrus synchronization with precised timing of ovulation in cows is needed because the rate of oestrus detection in dairy herds is generally low. Synchronized cows could be inseminated in predicted time without the requirement for consistent oestrus detection. One of the traditional methods of oestrus synchronization represents artificial luteolysis by $PGF_{2\alpha}$ in cows bearing corpus luteum. Administration of $PGF_{2\alpha}$ or its analogues during luteal phase of the sexual cycle is usually followed by ovulation between days 3 and 5 after treatment (Stellflug et al. 1975; Sirois and Fortune 1988; Larson et al. 1996; Martinez et al. 2000b). However variability in the interval from treatment to ovulation does not make possible insemination in predicted time. The term of ovulation depends on the stage of the sexual cycle and follicular development at the time of treatment (King et al. 1982; Macmillan and Henderson 1984; Kastelic et al. 1990; Kastelic and Ginther 1991; Kanitz et al. 1996; Larson et al. 1996; Xu et al. 1997; Lamb et al. 2000). In addition oestrus detection rate after $PGF_{2\alpha}$ is low as well (Archbald et al. 1993; Momcilovic et al. 1998). Ovulation can be synchronized more precisely by a previous and/or additional

Address for correspondence:
Doc. MVDr. Radovan Doležel, CSc.
Dept. of Animal Reproduction
University of Veterinary and Pharmaceutical Sciences
Palackého 1-3, 612 42 Brno, Czech Republic

Phone: + 420 5 4156 2316
E-mail: dolezelr@vfu.cz
http://www.vfu.cz/acta-vet/actavet.htm

synchronization of follicular development by administration of GnRH. It is followed by atresia of the dominant follicle or ovulation during luteal phase of the sexual cycle or it stimulates follicular maturation and ovulation of a dominant follicle during follicular phase and the recruitment of a new follicular wave occurs up to the day 4 after treatment (Pursley et al. 1995; Twagiramungu et al. 1995; Cruz et al. 1997; Martinez et al. 1999; Martinez et al. 2000a). On the basis of these data various protocols of oestrus synchronization with synchronization of follicular development using GnRH ($PGF_{2\alpha}$ – GnRH, GnRH – $PGF_{2\alpha}$, GnRH – $PGF_{2\alpha}$ - GnRH) were established. An improved reproductive performance (higher pregnancy rate, shorter interval from calving to conception) was demonstrated in cows inseminated in a predicted time at a synchronized ovulation by GnRH rather than in those inseminated at a detected synchronized oestrus period using single or double administration of $PGF_{2\alpha}$ (Vascencelos et al. 1999). However, the effectiveness of GnRH is also affected by the stage of sexual cycle and follicular development at the time of treatment, namely GnRH is effective in the presence of functional dominant follicle during its growth and the early-static phases (Prescot et al. 1992; Silcox et al. 1993; Pursley et al. 1995; Martinez et al. 1999). Therefore we focused our attention on follicular population at the time of $PGF_{2\alpha}$ treatment for a well-timed administration of GnRH. Evaluation of efficiency of oestrus synchronization using $PGF_{2\alpha}$ and GnRH in intervals according to the sizes of ovarian follicles at the time of initial treatment represents the subject of our study.

Materials and Methods

Lactating cows (crossbreds Bohemian Pied Cattle and Holstein) housed in 3 commercial dairy farms were used in the study. The average milk production of the herds where somewhere between 6 000 and 6 800 kg milk per lactation. Days open and conception rate after the 1st insemination where 100 – 118 and 39 – 43% in these herds during the study. Sixty five cows bearing corpora lutea between days 50 to 100 *post partum* were treated by synthetic analogue of $PGF_{2\alpha}$ - cloprostenol (Oestrophan, Léčiva Prague, Czech Rep., 500 µg pro toto, im) in combination with synthetic GnRH - lecirelinum (Supergestran, Ferring-Léčiva Prague, Czech Rep., 50 µg pro toto, im).

The cows were divided into 3 groups according to diameters of follicles on ovaries in ultrasonographical image at the time of $PGF_{2\alpha}$ administration: Group 1 – small follicles (3-7 mm, n = 10), Group 2 – small and medium follicles (3–7 and 8–14 mm, n = 35) and Group 3 – small, medium and large follicles (3-7 mm, 8-14 mm and 15–23 mm, n = 20). GnRH was administered 72 hours (Group 1), 48 h (Group 2) and 24 h (Group 3) after $PGF_{2\alpha}$.

Ovarian structures (presence of corpus luteum, numbers and sizes of follicles) were observed by ultrasound using Aloka SSD 500 (Tokyo, Japan) scanner with 5 MHz linear transrectal probe on days 0 (day of $PGF_{2\alpha}$ administration) 2, 4, 6, and 12. In addition, samples of peripheral blood for progesterone determination by RIA (commercial set, Immunotech, Prague, Czech Rep.) were obtained in 50 cows (10 cows in Group 1, 25 cows in Group 2 and 15 cows in Group 3) on days 0 and 12. Detection of oestrus by herd personnel was performed twice daily and cows in oestrus were inseminated. Only cows with oestrus/insemination and/or ovulation up to day 5 after $PGF_{2\alpha}$ were considered as synchronized. Twelve cows did not show any symptoms of oestrus and they were not inseminated until day 5. Term of oestrus and ovulation (disappearance of a dominant follicle > 15 mm in diameter), quality of luteal phase of the sexual cycle (corpus luteum and concentration of progesterone in peripheral blood on day 12) and conception rate (presence of embryonal vesicle in ultrasound image within days 23 and 37 after insemination) were evaluated in synchronized cows.

Statistical analysis

Numbers of cows showed oestrus and ovulation up to day 5 after initial treatment as well as numbers of cows bearing corpora lutea on day 12 and numbers of pregnant cows in the experimental groups (Table 1) were compared using the Chi-square test or Fisher's exact test (when the first test was not valid due to low numbers of evaluated cows). Comparisons of progesterone concentrations on days 0 and 12 (Table 2) in the frame of the experimental groups and among the groups were performed by Student's *t*-test and regard to variability of individual values we used Kruskal-Wallis test as well.

Results

Oestrus was detected and insemination was performed in 8 of 10 (80%), 33 of 35 (94%) and 12 of 20 (60%) cows in experimental Groups 1, 2 and 3, respectively, within 5 days after $PGF_{2\alpha}$ administration. The highest level of synchronization was found in Group 2. Oestrus occurred and insemination was performed in 29 of 35 (83%) cows in this group on day 3. Ovulation

Table 1
Number of cows showing oestrus, ovulation, corpus luteum and pregnancy after oestrus synchronization by $PGF_{2\alpha}$ and GnRH

	Group 1 (n = 10)	Group 2 (n = 35)	Group 3 (n = 20)
Oestrus/insemination			
D 2	0	1^{a}	6^{a}
D 3	4^{a}	$29^{a,b}$	6^{b}
D 4	$4^{a,b}$	3^{a}	0^{b}
Ovulation			
D 0-1	0	0	2
D 2-3	4^{a}	27^{a}	12
D 4-5	4^{a}	6	1^{a}
Corpus luteum			
D 12	8	32	15
Pregnancy	4	21^{a}	5^{a}

a,b – ($p < 0.05$), Other significant differences ($p < 0.05$)
oestrus/insemination: D2 + D3 Groups 1 : 2, 2 : 3; D2 + D3 + D4 Groups 2 : 3
ovulation: D0-1 + D2-3 Groups 1 : 2

occurred in all inseminated cows in Groups 1 and 2 within 2 days after insemination. In spite of oestrus occurring and insemination being performed only in 12 cows in Group 3 ovulation occurred in 15 cows in this Group within 5 days after $PGF_{2\alpha}$. Corpora lutea were found in all ovulated cows in group 1 and 3. Corpus luteum was not found in 1 cow in Group 2 after the disappearance of a dominant follicle. Total conception rates/conception rates in inseminated cows were 40/50%, 60/64% and 25/42% in Groups 1, 2, and 3, respectively (Table 1, Fig. 1).

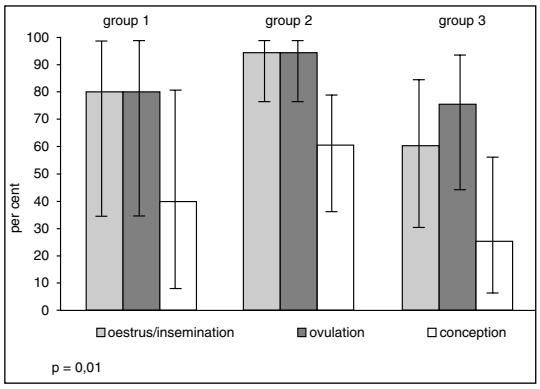

Fig. 1. Occurrence of oestrus and ovulation up to day 5 after initial treatment and conception rate after synchronization of oestrus by $PGF_{2\alpha}$ and GnRH

Ovarian cysts were found in 1 non-inseminated cow in the Group 1 as well as in 2 non-inseminated and 1 inseminated cows in the Group 3 after treatment. In addition endometritis was diagnosed in 1 non-inseminated cystic cow in the Group 3. Other non-inseminated cows showed oestrus after day 5 or they did not show any symptoms of oestrus during observation.

Table 2
Progesterone concentrations on days 0 and 12 after oestrus synchronization by $PGF_{2\alpha}$ and GnRH (nmol/L)

	Group 1 (n = 10)	Group 2 (n = 25)	Group 3 (n = 15)
D 0	6.7 ± 2.80	13.7 ± 9.76	0.8 ± 6.81
D 12	11.5 ± 8.87	10.5 ± 7.09	10.2 ± 9.41

Mean ± SD

Average values of progesterone concentration on days 0 and 12 in all groups were similar but large variability in the individual values was found (Table 2). Values of progesterone concentration did not show any significant differences.

Discussion

Diagnostics of early pregnancy using ultrasonography is becoming part of periodical examination in cows in the frame of reproduction managing in large dairy herds. Ultrasonographic examination makes it possible to evaluate not only pregnancy but ovarian structures namely follicular population as well. This is therefore a chance to use this method for determining the stage of follicular development to increase the accuracy of oestrus/ovulation synchronization using $PGF_{2\alpha}$ and GnRH in non pregnant cows bearing corpus luteum.

Follicular development on ovaries in cows is characterized by follicular waves (Pierson and Ginther 1987; Pierson and Ginther 1988; Sirois and Fortune 1988; Ginther et al. 1989; Ginther et al. 1997; Ginther 2000). On the basis of these data we determined 3 phases of the follicular development according to diameters of ovarian follicles – growing phase (follicles 3-7 mm, Group 1), phase of early dominance (follicles 3-7 mm and 8-14 mm, Group 2) and phase of late dominance with preovulatory/preatretic follicles (follicles 4-7 mm, 8-14 mm and 15-23 mm, Group 3). Past research showed that growing dominant follicles (greater than 10 mm in diameter) usually ovulate after GnRH treatment (Prescot et al. 1992; Pursley et al. 1995; Silcox et al. 1995). But GnRH is effective only in the presence of functional dominant follicle during its growing and the 1st part of the static phase (Silcox et al. 1993) when follicle granulosa cells content maximum number of LH receptors (Xu et al. 1995; Bodensteiner et al. 1996b). Diameters of this follicle are usually more than 14 mm (Savio et al. 1988; Bodensteiner et al. 1996a). Follicle LH receptors decrease as the dominant follicle achieves a static phase (Rolloson et al. 1994; Bodensteiner et al. 1996b). We hypothesized that GnRH administration would be performed in all experimental cows by the end of the growing phase or at the beginning of the static phase of the dominant follicle when GnRH would be administered 72, 48 and 24 h after $PGF_{2\alpha}$ in cows bearing small, medium and large follicles at the time of initial treatment. In our study most of the cows bearing corpus luteum showed small (3-7 mm) as well as medium (8-14 mm) follicles at the initial examination. The number of cows bearing only small follicles was very low. It can be caused by survival of morphological dominant but non-functional follicle on ovaries in the growing phase of the follicular wave. For this reason determination of the stage of follicular development could be incorrect in some cows in Groups 1 and 3.

In spite of absence of oestrus symptoms up to day 2 after $PGF_{2\alpha}$ in all observed cows, ovulation occurred in two cows (Group 3) during this interval. Preovulatory follicles on ovaries probably were present in these cows at the time of initial treatment so that follicles ovulated very early apart from administration of GnRH. We considered only oestrus and ovulation up to day 5 after initial treatment as manifestation of successful synchronization. We found higher oestrus/insemination and ovulation rate within 5 days after $PGF_{2\alpha}$ in Group 2 in comparison with other groups. Nevertheless, similar ovulation rates were found in all experimental groups (80% Group 1, 77% Group 2 and 76% Group 3) within 48 hours after GnRH. Ovulation rate after GnRH is influenced by the stage of follicular development at the time of treatment during luteal phase as well as follicular phase of the sexual cycle. Martinez et al. (1999) found ovulation rates 89%, 56% and 22% in heifers within 36 hours after GnRH administration on days 3 (expected phase of growing dominant follicle), 6 (expected phase of early static dominant follicle) and 9 (expected phase of late static dominant follicle) of the sexual cycle, respectively. Similarly, ovulation occurred only in

2 of 6 observed cows which were treated by GnRH during expected static phase of the dominant follicle development between days 15 and 18 of the oestrus cycle (Kohram et al. 1998) and a lower ovulation rate after GnRH was reported between days 1 and 4, 10 and 16 than between days 5 and 9, 17 and 21 of the sexual cycle (Vasconcelos et al. 1999). In comparison with these data, similar ovulation rates in all goups of this study could be evidence that GnRH was administered in a similar stage of follicular development (probably in growing or early-static phase of the dominant follicle). Ovulation occurred in all inseminated cows in Groups 1 and 2 within 2 days after insemination. In spite of oestrus occurring and insemination being performed only in 12 cows in Group 3, ovulation occurred in 15 cows in this Group within 5 days after $PGF_{2\alpha}$. In another study GnRH was administered 24 (A), 48 (B) and 72 (C) hours after $PGF_{2\alpha}$ apart from follicular development and ovulations occurred between the 2nd and 3rd day (A) and 3rd and 4th day (B,C) after $PGF_{2\alpha}$ and between 24 and 48 hours (A) and within 24 hours (B,C) after GnRH (Taponen et al. 1999). Disharmony in occurrence of oestrus and ovulation and variability in the term of ovulation in Group 3 were probably caused by various qualities of large (15-23 mm) follicles at the time of $PGF_{2\alpha}$ administration in this study. The follicles could be in preovulatory, preatretic or atretic stage of their development. Even preatretic follicles (static-phase dominant follicles) are capable of further growth after induced luteolysis (Oxender et al. 1974; Pierson and Ginther 1987).

It is impossible to evaluate precisely the quality of the luteal phase of the synchronized sexual cycle on the basis of our results because we did not observe dynamic morphological (volume of the luteal tissue) and functional (progesterone concentration) changes of corpora lutea. In spite of having a typical view of corpora lutea in ultrasonographical images, variability in progesterone concentrations probably shows variable quality of the corpora lutea at the time of treatment also on day 12 after $PGF_{2\alpha}$ in all experimental groups. Variability in progesterone concentrations at the time of initial treatment corresponds to incidental selection of experimental cows bearing corpora lutea. These cows were in variable stages of luteal phase of the sexual cycle. Induction of ovulation by GnRH can shorten the subsequent luteal phase of the sexual cycle (Stevens et al. 1993; Taponen et al. 1999). Ovulation of a premature dominant follicle can be followed by a lower quality of corpora lutea or it can be followed by a shorter lifespan of initially normal corpora lutea because quality of the corpora lutea depends on the quality of the preovulatory follicle (White et al. 1985; Smith 1986). This may have been caused by progesterone production being lower or luteolysis starting in some experimental cows before day 12 in our study, even though ultrasonographical image of corpora lutea had not changed yet. Since growth of the corpora lutea and rise of progesterone concentrations early after GnRH induction of ovulation is comparable with normal sexual cycles in untreated cows (Taponen et al. 1999) earlier luteolysis is more likely. Therefore absence of corpus luteum in one cow from Group 2 on day 12 does not eliminate the possibility of ovulation. We found neither significant differences among progesterone concentrations nor relations of progesterone concentrations to synchronization as well as conception rates. Progesterone concentration in time of $PGF_{2\alpha}$ treatment can influence follicular development because higher concentration reduces pulses of LH, possibly leading to loss of function in the dominant follicle and it decreases follicle responsibility to GnRH (Robertson et al. 1989; Savio et al. 1993).

Pregnancy rates in inseminated cows were higher (64%) in Group 2 compared to Group 1 (50%) and Group 3 (42%). The results are partly in accordance with data showing lower pregnancy rate after timed artificial insemination following GnRH administered 24 h after $PGF_{2\alpha}$ compared to insemination at detected oestrus after $PGF_{2\alpha}$, although there were no differences when GnRH was administered 48 h after prostaglandin (Schmitt et al. 1996). Greater pregnancy rates were observed in cows bearing smaller ovulatory follicles after

oestrus synchronization using GnRH - $PGF_{2\alpha}$ - GnRH (Ovsynch) protocol (Vasconcelos et al. 1999). We did not evaluate precisely the sizes of the preovulatoty follicles in our study. But the average diameters of the largest follicles in our last examination before ovulation was similar or smaller in Groups 1 and 2 compared to Group 3. In spite of different pregnancy rates in experimental groups of our study, the values are similar or higher in comparison with other synchronization protocols using GnRH (Pursley et al. 1995; Twagiramungu et al. 1995; Burke et al. 1996; Roy and Twagiramungu 1997; Britt and Gaska 1998; Momcilovic et al. 1998; Vasconcelos et al. 1999; Martinez et al. 2000b). In view of the pregnancy rate, the optimal time intervals after $PGF_{2\alpha}$ for GnRH and for fixed artificial insemination are proposed at 42 to 50 h and 53 to 61 h in heifers and 52 to 60 h and 63 to 71 hours in cows, respectively (Roy and Twagiramungu 1997). Variability of progesterone concentrations on day 12 as well as differences between high rates of synchronized ovulation and lower conception rates in all experimental groups (see Fig. 1) show that the quality of the luteal phase of the synchronized sexual cycle could be more important in the limitation of pregnancy rather than the term of ovulation. The differences show that various qualities of corpora lutea as a result of various qualities of ovulatory follicles negatively influenced the pregnacy rate more strongly in Groups 3 and 1 in comparison with Group 2.

Results of this study show high efficiency of oestrus synchronization using GnRH administered 48 h after $PGF_{2\alpha}$ in cows bearing corpus luteum and small (3–7 mm) as well as medium (8–14 mm) follicles on ovaries at the time of initial treatment. Oestrus occurred in 29 of 35 (83%) observed cows on day 3 after $PGF_{2\alpha}$ and the conception rate was 66% in these cows. Probably inaccurate determination of the stage of follicular development in cows bearing only small or large follicles caused lower efficiency of oestrus synchronization in these cows.

Synchronizace říje prostaglandinem $PGF_{2\alpha}$ a GnRH v intervalech podle stadia folikulárního vývoje v době úvodního ošetření u krav

Předmětem studie bylo zhodnotit účinnost synchronizace říje/ovulace aplikací $PGF_{2\alpha}$ a GnRH v intervalech podle velikosti ovariálních folikulů v době úvodního ošetření. Synchronizace říje byla provedena u 65 krav ze 3 komerčních mléčných chovů. Syntetický analog $PGF_{2\alpha}$ cloprostenol byl aplikován kravám se žlutým tělískem v době 50–100 dnů po porodu. Krávy byly rozděleny do 3 skupin podle průměrů ovariálních folikulů v ultrazvukovém zobrazení: skupina 1 – malé folikuly (3-7 mm, n = 10), skupina 2 – malé a střední folikuly (3-7 mm a 8-14 mm, n = 35) a skupina 3 – malé, střední a velké folikuly (3-7 mm, 8-14 mm a 15-23 mm, n = 20). GnRH byl aplikován 72 h (skupina 1), 48 h (skupina 2) a 24 h (skupina 3) po $PGF_{2\alpha}$. Ultrazvukové vyšetření ovariálních folikulů bylo provedeno ve dnech 0 (den aplikace $PGF_{2\alpha}$), 2, 4, 6 a 12. U 50 krav v den 0 a 12 byly odebrány vzorky krve pro stanovení progesteronu metodou RIA (u 10 krav ve skupině 1, u 25 krav ve skupině 2 a u 15 krav ve skupině 3). Detekce říje byla provedena dvakrát denně a říjející se krávy byly inseminovány. Hodnotili jsme termín říje a ovulace, koncentraci progesteronu a úroveň koncepce. Do 5. dne po aplikaci $PGF_{2\alpha}$ byla zjištěna říje a inseminace byla provedena u 8 z 10 (80 %), 33 z 35 (94 %) a u 12 z 20 (60 %) krav ve skupinách 1, 2 a 3. Ovulace proběhla u všech inseminovaných krav ve skupinách 1 a 2 během 2 dní po inseminaci. Přesto, že ve skupině 3 do 5. dne po $PGF_{2\alpha}$ se říjelo a bylo inseminováno 12 krav, ovulace proběhla v tomto období u 15 krav. Žlutá tělíska v den 12 byla prokázána u všech ovulovaných krav (kromě jedné ve skupině 2). Úroveň koncepce u inseminovaných krav byla 50 %, 64 % a 42 % ve skupinách 1, 2 a 3. Nejvyšší účinnost synchronizace říje byla zjištěna ve skupině 2, ve které říje proběhla u 29 z 35 (83 %) krav 3. den po $PGF_{2\alpha}$ a úroveň koncepce u těchto krav činila 66 %.

Acknowledgments

This study was supported by Ministry of Education, Youth and Physical Training of the Czech Republic (project No. 161700002). We thank dairy farmers (Agrodružstvo Brťov-Lipůvka, AGRIA a.s. Nížkovice and GENAGRO Říčany) which make research possible to provide in their farms. In addition we thank Dr. M. Dvořák for veterinary assistance and Dr. M.Vyskočil, PhD for statistical analysis.

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