Replacement of the First GnRH Administration in the Ovsynch Protocol by Selecting Cows According to the Stage of Follicular Development

P. HARTMAN, J. ZAJÍC, S. ČECH, Z. JAN, R. DOLEŽEL
University of Veterinary and Pharmaceutical Sciences Brno

Received July 26, 2005
Accepted December 12, 2005

Abstract

Hartman P., J. Zajíc, S. Čech, Z. Jan, R. Doležel: Replacement of the First GnRH Administration in the Ovsynch Protocol by Selecting Cows According to the Stage of Follicular Development. Acta Vet. Brno 2006, 75: 65-72.

The aim of the study was to replace the first GnRH in the Ovsynch protocol by selecting cows bearing corpus luteum as well as follicles in a defined stage of development at $PGF_{2\alpha}$ administration. Additionally, various terms of GnRH administration after $PGF_{2\alpha}$ were tested. Seventy five non-pregnant cows bearing corpus luteum were divided into groups according to the phase of follicular development on D 0 (day of $PGF_{2\alpha}$ administration) – growth (GR, follicles 3.0 – 7.9 mm in diameter), early dominance (ED, dominant follicle 8.0 – 14.9 mm) and late dominance (LD, dominant follicle 15.0 – 23.0 mm). In addition, the cows were divided into groups according to the terms of GnRH administration (24, 48 or 72 h after $PGF_{2\alpha}$). In this way, groups GR 48 (n = 5), GR 72 (n = 6), ED 24 (n = 10), ED 48 (n = 12), ED 72 (n = 12), LD 24 (n = 10), LD 48 (n = 10) and LD 72 (n = 10) were established. Growth of ovulatory follicle, term of ovulation, insemination and conception rates as well as relation of the size of preovulatory follicle (day of ovulation) to the size of following corpus luteum (day 14) were evaluated. The highest level of synchronization of ovulation (100% on D 3) as well as conception rate (50%) was achieved in group ED 48. This protocol gives an opportunity of timing artificial insemination to 18 – 24 hours after GnRH administration, as ovulation occurs with a high probability within 24 - 48 hours after GnRH administration.

Oestrus synchronization, follicle diameter, ovulation, timing of insemination

Synchronization of oestrus in cows with a standard term of ovulation provides a good opportunity for the timing of artificial insemination. It can decrease losses caused by poor oestrus detection and in addition, it can improve the embryo-transfer efficiency. The term of ovulation after administration of $PGF_{2\alpha}$ in the luteal phase of the oestrous cycle is variable because it depends on the stage of follicular development at the time of treatment (King et al. 1982; Macmillan and Henderson 1984; Kastelic et al. 1990; Savio et al. 1990; Kastelic and Ginther 1991). Follicular development can be synchronized by administration of GnRH, followed by removal of the dominant follicle (ovulation or atresia) and recruitment of a new follicular wave (Thatcher et al. 1989; Macmillan and Thatcher 1991; Twagiramungu et al. 1995). This effect of GnRH is applied in the Ovsynch protocol (Pursley et al. 1995) as well as similar methods of oestrus synchronization in cows (Pursley et al. 1997; Pancarci et al. 2002; Thatcher et al. 2002). Nevertheless, the ovulation of a premature follicle after the first GnRH administration (Roy and Twagiramungu 1999; DeJarnette et al. 2001) and an incomplete luteal regression following $PGF_{2\alpha}$ (Burke et al. 1996; Moreira et al. 2000) are described. This is probably associated with the conception failure in the Ovsynch protocol (Kim et al. 2003). In our study we replaced the first GnRH in the Ovsynch protocol by selecting cows bearing corpus luteum as well as follicles in a defined stage of development at $PGF_{2\alpha}$ administration. In addition, the various terms of GnRH administration after $PGF_{2\alpha}$ were tested.

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

Phone: +420 541 562 316
E-mail: dolezelr@vfu.cz
http://www.vfu.cz/acta-vet/actavet.htm

Materials and Methods

Experimental animals

Seventy five lactating cows (crossbreeds Bohemian Pied Cattle and Holstein) bearing corpora lutea (CLs) within 55 and 100 days *post partum* were used in our study. They were housed in three commercial dairy farms, and the average milk production of the herds was between 6 500 and 8 000 kg of milk per lactation. Conception rates after the first insemination in the herds were around 35 - 40% during the period of the study. The cows were divided into groups according to the phase of follicular development in the ultrasonographical image – growth (GR, follicles 3.0 – 7.9 mm in diameter, n = 11), early dominance (ED, dominant follicle 8.0 – 14.9 mm, n = 34) and late dominance (LD, dominant follicle 15.0 – 23.0 mm, n = 30). A follicle ≥ 8 mm in diameter at least 2.0 mm larger than the remaining ones was considered as the dominant follicle. The cows were treated by synthetic analog of $PGF_{2\alpha}$ cloprostenol (Oestrophan, Bioveta, Ivanovice, Czech Rep., 500 μg *pro toto*, i.m.) and following administration of GnRH (lecirelinum, Supergestran, Ferring-Leciva, Jesenice, Czech Rep., 100 μg *pro toto*, i.m.) were performed 24, 48 or 72 h after $PGF_{2\alpha}$. In addition, the cows were divided in groups according to the terms of GnRH administration. In this way groups GR 48 (n = 5), GR 72 (n = 6), ED 24 (n = 10), ED 48 (n = 12), ED 72 (n = 12), LD 24 (n = 10), LD 48 (n = 10) and LD 72 (n = 10) were established. Group GR 24 was not established because of a small number of cows bearing follicles in the growth phase of the follicular wave.

Observation

Transrectal ultrasonographical examinations (Aloca SSD 500 with a 5 MHz rectal probe, Tokyo, Japan) of ovarian structures were performed daily from day 0 (day of $PGF_{2\alpha}$ administration) to day 5 and on day 14. Ultrasonographical images of ovarian structures were taken by a digital video camera (Sony DCR-TRV 235 E) and data were compiled in the studio DV plus Pinnacle® system. Additional measurements of ovarian structures were made in Auto-CAD® 2002. Diameters of ovarian structures were determined as an arithmetical mean of two axes vertical to each other. Peripheral blood samples were obtained on days 0, 2 and 14. The blood was centrifuged and it was stored at – 20 °C until progesterone determination by RIA, using commercial sets (Immunotech, Prague, Czech Republic). The detection of oestrus was performed by herd personnel twice a day and cows in oestrus were inseminated. Pregnancy diagnosis was performed ultrasonographically on day 25 and 39 after insemination (see Fig. 1). Disappearance of the dominant follicle followed by appearance of corpus luteum in the same place of the ovary on day 14 was considered as evidence of ovulation. Oestrus was considered as synchronized when ovulation appeared up to day 5 after $PGF_{2\alpha}$ administration.

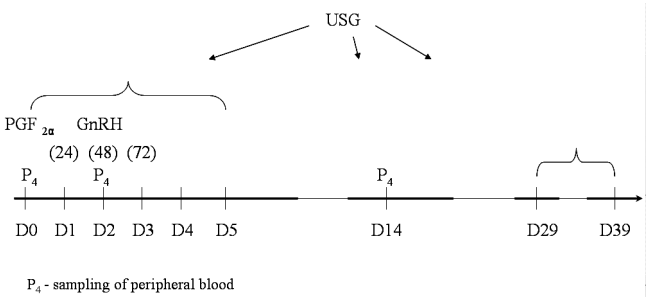

Fig. 1. Scheme of the study

Evaluation

Growth of the ovulatory follicle, the term of ovulation, insemination and conception rates as well as the relation of the size of the preovulatory follicle (day of ovulation) to the size of the following corpus luteum (day 14) were evaluated.

A comparison was made of follicle sizes within an individual group as well as among the groups, using parametric tests. Statistical analysis of ovulation occurrence on day 3 (day 0 – day of $PGF_{2\alpha}$ administration) was done on one hand among common groups divided according to the stage of follicular development at $PGF_{2\alpha}$ administration, regardless of the term of GnRH administration (groups GR, ED and LD); and on the other hand it was done among groups divided according to the term of GnRH administration, regardless of the stage of follicular development at $PGF_{2\alpha}$ administration (groups 24, 48 and 72). Synchronizations of ovulation were evaluated using a Two-way table, which is available at the web site: http://caspar.bgsu.edu/~software/java/1Contingency.html. This table used Chi-square (χ^2) or G-tests (G). Statistically significant deviation is evaluated by the Freeman-Tukey test which determines unexpected higher or lower values in the frame of a null hypothesis. Willam's correction was performed and all statistics were compared by the Chi-square distribution with appropriate degrees of freedom. The relationship of the sizes of preovulatory follicles to the sizes of corpora lutea was evaluated by Kruskal-Wallis or Steel-Dwass tests.

Results

The growth of ovulatory follicles is shown in Tables 1, 2 and 3. Significant differences in follicle diameters were found between follicles on day of $PGF_{2\alpha}$ administration and follicles on day of GnRH administration in GR, LD ($p < 0.05$) as well as ED ($p < 0.01$) groups. The differences were not proved between follicles on the day of GnRH administration and follicles on the day of ovulation. Even follicles became smaller in LD groups during this period.

Table 1. Means (± SEM) of preovulatory follicle diameters in groups GR 48 (n = 3) and GR 72 (n = 1) (mm)

	D0	D1	D2	D3	D4	D5
GR 48	5.73±0.72	8.55±0.84	10.23±1.81	10.96±1.96	0	0
GR 72	7.35	10.25	10.95	13.75	15.5	0

Table 2. Means (±SEM) of preovulatory follicle diameters in groups ED 24 (n = 9), ED 48 (n = 12) and ED 72 (n = 8) (mm)

	D0	D1	D2	D3	D4	D5
ED 24	11.28±2.35	11.37±2.6	12.3±2.81	0	0	0
ED 48	10.45±2.87	11.18±2.26	12.76±2.16	12.34±2.69	0	0
ED 72	10.96±2.8	11.26±4.11	13.7±3.58	15.2±2.04	14.89±2.69	0

Table 3. Means (± SEM) of preovulatory follicle diameters in groups LD 24 (n = 9), LD 48 (n = 5) and LD 72 (n = 7) (mm)

	D0	D1	D2	D3	D4	D5
LD 24	14.9±3.01	15.18±2.68	14.88±2.58	12.78±1.53	0	0
LD 48	16.46±1.64	15.68±2.68	14.90±3.29	15.29±2.37	0	0
LD 72	16.24±1.50	16.98±1.85	16.91±3.43	17.37±2.22	14.50±2.00	0

Ovulation was observed in 72% (54/75) of all experimental cows up to day 5. Ovulations occurred on days 2, 3 and 4. The highest incidence of ovulation was found on day 3. For this reason we evaluated ovulations which occurred on day 3. The highest level of synchronization of ovulation was found in group ED 48 in which 100% (12/12) cows ovulated on day 3 after $PGF_{2\alpha}$ administration on day 0 (Table 4).

Table 4. Occurrence of ovulation in all experimental groups up to day 5 after $PGF_{2\alpha}$ administration on day 0

	Groups							
	GR		ED			LD		
Day	48	72	24	48	72	24	48	72
	(n=5)	(n=6)	(n=10)	(n=12)	(n=12)	(n=10)	(n=10)	(n=10)
D2	0	0	9	0	1	7	0	0
D3	3	0	0	12	3	2	4	6
D4	0	1	0	0	4	0	1	1
D0 – D5	3	1	9	12	8	9	5	7
	60%	17%	90%	100%	67%	90%	50%	70%

In common groups divided according to the stage of follicular development at $PGF_{2\alpha}$administration (regardless to term of GnRH administration) the highest occurrence of ovulation on day 3 (the 3th day after $PGF_{2\alpha}$ administration) was found in group ED and the lowest occurrence was found in group GR ($p < 0.01$), see Fig. 2.

On the other hand, in common groups divided according to the term of GnRH

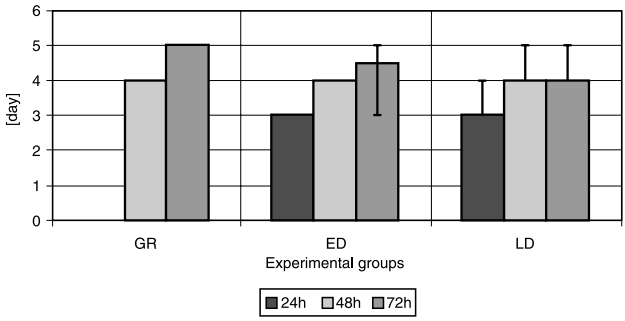

Fig. 2. Terms of ovulation in experimental groups (24, n=18; 48, n=20, 72, n=16) in relationship to phase of follicular development on D 0 (median, max.-min).

administration (regardless of the stage of follicular development at $PGF_{2\alpha}$ administration), the highest occurrence of ovulation on day 3 was found in group 72 and the lowest occurrence was found in group 24 ($p < 0.05$), see Fig. 3.

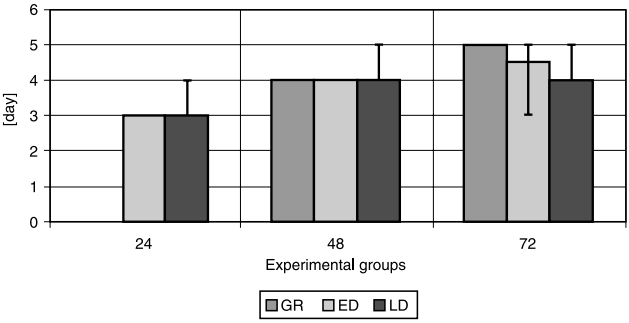

Fig. 3. Term of ovulation in experimental groups (GR, n=4; ED, n=29, LD, n=21) in relationship to term of administration GnRH (median, max.-min).

The highest occurrence of ovulation, within 5 days after $PGF_{2\alpha}$ administration, was found in group 48 ($p < 0.001$).

Insemination was done in 45% (34/75) experimental cows and the average pregnancy rate was only 24%. The highest conception rate (50%) was found in group ED 48. Positive correlations were found between sizes of preovulary follicles on the day of ovulation and sizes of corpora lutea on day 14 in groups ED 24, ED 48, ED 72, LD 24 and LD 72 ($p < 0.001$) as well as in groups GR 48 and LD 48 ($p < 0.01$). Group GR 72 could not be evaluated for a small number of evaluated cows.

Discussion

We could not establish complete groups of cows bearing only small follicles (3.0 – 7.9 mm in diameter) on ovaries. Low incidence of these cows was probably caused by frequent persistence of a morphologically dominant follicle during the growth phase of a new follicular wave. Persistence of morphological dominance after the loss of functional dominance in follicles during follicular waves in cows has been described (Fortune 1993; Savio et al. 1990). For that reason the growth phase of a follicular wave cannot be exactly differentiated from late dominance in cows on the basis of ultrasonographical examination.

Thus some cows bearing a follicle 15.0 – 23.0 mm in diameter could be in fact in the growth phase of a new follicular wave in our study. There is a chance to induce a growth phase of a new follicular wave artificially, by puncturing follicles under ultrasonographical guidance (the ovum pick up method - OPU). However, we did not use it, as the OPU may negatively influence the next oestrous cycle. Smaller corpora lutea with lower progesterone concentrations in peripheral blood and a low conception rate after OPU were described (Vasconcelos et al. 2001).

We found high growth potency of ovulatory follicles after $PGF_{2\alpha}$ up to GnRH administration in all experimental groups. Low growth potency after GnRH up to ovulation especially in groups LD is partly in agreement with authors DiZerega and Hodgen (1980) who found in cows treated by GnRH an outstandingly higher growth potency of small growing follicles before selection, compared to large dominant follicles. Reduction of size in some preovulatory follicles can be caused by a decrease of follicle tension after a temporal decrease of FSH concentration (Ginther 2000).

The term of ovulation after single administration of $PGF_{2\alpha}$ in cows bearing corpus luteum is variable, because it depends on the stage of follicular development at the time of treatment (Macmillan and Henderson 1984; Savio et al. 1990; Kastelic et al. 1990; Turzillo and Fortune 1993). The highest dispersion of ovulation after $PGF_{2\alpha}$ was described when treatment was performed in the middle of the oestrous cycle (Tanabe and Hann 1984). Nevertheless, most cows ovulate on day 3 after treatment (Stellflung et al. 1975; Král et al. 1981; Larson and Ball 1992). In our study, 72% of cows ovulated up to day 5 after $PGF_{2\alpha}$ administration regardless of the stage of follicular development at $PGF_{2\alpha}$ treatment as well as the term of GnRH administration. In agreement with the above stated authors, most cows (55.6%) ovulated on day 3 after treatment. The highest level of synchronization of ovulation after $PGF_{2\alpha}$ was found when treatment was performed on cows bearing corpus luteum as well as an early dominant follicle, and GnRH was administered 48 hours after $PGF_{2\alpha}$ (group ED 48). Ovulation occurred in 100% of these cows on day 3 after $PGF_{2\alpha}$ treatment.

Although a relatively standard occurrence of ovulation after $PGF_{2\alpha}$ treatment was found in our study, a few cows (45%) were inseminated and the total conception rate was very low (24%). Ten cows were inseminated despite ovulation not having occurred. On the other hand, 30 cows were not inseminated despite ovulation having occurred. These data show low efficiency of oestrus detection in the herds. A poor rate of oestrus detection in herds of milk cows is also described by many other authors (Noakes et al. 2001; Arney et al. 1994; Kerbrat a Disenhaus 2004). The authors mention the level of incorrect detection around 61%. An especially low rate of oestrus detection was found after $PGF_{2\alpha}$ treatment (Slenning and Farver 1990). For this reason, the low conception rate was partly caused by poor oestrus detection allowing insemination of unovulatory cows in our study. On the basis of these data, a higher conception rate after insemination could be expected regardless of oestrus detection. A low conception rate is often associated with abnormal corpus luteum with short and low production of progesterone after insemination (Rosenberg et al. 1990; Gyawu et al. 1991; Morell et al. 1991; Xu et al. 1997). Nevertheless, the quality of corpus luteum depends on the quality of the preovulatory follicle (Smith 1986; Vasconcelos et al. 2001). Likewise, the length as well as the level of higher concentration of progesterone before ovulation influences the quality of preovulatory follicles as well as the quality of the subsequent corpus luteum (White et al. 1985; Smith 1986; Vasconcelos et al. 2001). For this reason low conception rate could be partly caused by the short luteal phase before ovulation in our study. Generally, environmental factors significantly influence the conception rate. But the conception rate after the first insemination in the herds involved in the study was higher (35 – 40%) at the time of the experiment. These data show that a low

conception rate is probably not caused by environmental conditions. Frequent manipulation with uterus and ovaries within the transrectal ultrasonographical examination could negatively influence conception, too. Roelofs et al. (2004) reported that frequent ultrasonographical examination did not have a negative effect on oestrus behaviour nor the rate of oestrus detection. But higher variability of the term of ovulation was noted. Nevertheless, the relatively high conception rate (50%) in group ED 48 shows that the main factors influencing conception rate in our study are probably the protocol of hormonal treatment as well as the quality of ovarian structures. Lower conception rate (31.1%) was found after the Ovsynch protocol (Peters and Pursley 2003).

Our results show that $PGF_{2\alpha}$ treatment in cows bearing corpus luteum as well as the early dominant follicle with following administration of GnRH 48 hours after $PGF_{2\alpha}$ represents an efficient protocol of oestrus synchronization. This protocol gives an opportunity for timing artificial insemination to 18 – 24 hours after GnRH administration because ovulation occurs with a high probability within 24 - 48 hours after GnRH administration.

Nahrazení první aplikace GnRH v Ovsynch metodě výběrem krav podle fáze folikulárního vývoje

Cílem studie bylo nahradit první aplikaci GnRH v Ovsynch metodě synchronizace říje výběrem krav se žlutým tělískem a folikuly v definovaném stádiu vývoje při aplikaci $PGF_{2\alpha}$. Navíc byl testován různý termín následné aplikace GnRH. Sedmdesát pět jalových krav vykazujících žluté tělísko bylo rozděleno do skupin podle stádia folikulárního vývoje v D 0 (den aplikace $PGF_{2\alpha}$) na skupiny – růstu (GR, folikuly 3,0 – 7,9 mm v průměru), časné dominance (ED, dominantní folikul 8,0 – 14,9 mm) a pozdní dominance (LD, dominantní folikul 15,0 – 23,0 mm). Následně byly skupiny rozděleny podle doby aplikace GnRH (24, 48 nebo 72 hodin po $PGF_{2\alpha}$. Tímto způsobem byly vytvořeny skupiny GR 48 (n = 5), GR 72 (n = 6), ED 24 (n = 10), ED 48 (n = 12), ED 72 (n = 12), LD 24 (n = 10), LD 48 (n = 10) a LD 72 (n = 10). Ultrasonografické vyšetření bylo prováděno denně od D 0 do D 5 a v D 14 a vzorky periferní krve ke stanovení progesteronu RIA metodou byly odebírány v D 0, D 2 a D 14. Říjící se krávy byly inseminovány. Nejvyšší úroveň synchronizace ovulace byla zaznamenána ve skupině ED 48, kde ovulovalo 100 % (12/12) krav v D 3. Rovněž nejvyšší úroveň zabřezávání (50 %) byla zjištěna v této skupině. Výsledky naší studie ukazují, že aplikace $PGF_{2\alpha}$ u krav vykazujících žluté tělísko a časně dominantní folikul s následnou aplikací GnRH 48 hodin po $PGF_{2\alpha}$ představuje účinnou metodu synchronizace říje. Uvedený postup umožňuje časování inseminace na interval 18 – 24 hodin po aplikaci GnRH, poněvadž ovulaci lze s velkou pravděpodobností očekávat v intervalu 24 – 48 hodin po aplikaci GnRH.

Acknowledgements

This study was supported by grant SMS 6215712403. We thank dairy farmers (Agrodružstvo Brťov-Lipůvka, AGRIA, a. s. Nížkovice and GENAGRO Říčany) which made research possible on their farms. In addition we thank Dr. M. Vyskočil, PhD. for statistical analysis.

References

ARNEY DR, KITWOOD SE, PHILIPS CJC 1994: The increase in activity during oestrus in dairy cows. Appl Anim Behav Sci. **40**: 211-218

BURKE JM, SAL SOTA RL, DE LA RISCO CA, STAPLES CR, SCHMITT EJ, THATCHER WW 1996: Evaluation of timed insemination using a gonadotropin-releasing hormone agonist in lactating dairy cows. J Dairy Sci **79**: 1385-1393

DEJARNETTE JM, SALVERSON RR, MARSHALL CE 2001: Incidence of premature estrus in lactating cows and conception Mates to standing estrus or fixed-time inseminations after synchronization usány GnRH and $PGF_{2\alpha}$. Anim Reprod Sci **67**: 27-35

DI ZEREGA GS, HODGEN GD 1980: The primate ovarian cycle: suppression of human menopausal gonadotropin-induced follicular growth in the presence of the dominant follicle. J Clin Endocrinol Metabol **50**: 819-825

FORTUNE JE 1993: Follicular dynamics during the bovine estrous cycle: A limiting factor in improvement of fertility? Anim Reprod Sci **33**: 111-125
GINTHER OJ 2000: Selection of the dominant follicle in cattle and horses. Anim Reprod Sci: **60/61**: 61-79
GYAWU P, DUCKER MJ, POPE GS, SAUNDERS RW, WILSON GDA 1991: The value of progesterone, oestradiol benzoate and cloprostenol in controlling the timing of oestrus in dairy cows and allowing successful fixed-time insemination. Br Vet J **147**: 171-182
KASTELIC JP, GINTHER OJ 1991: Factors affecting the origin of the ovulatory follicle in heifers with induced luteolysis. Anim Reprod Sci **26**: 13-24
KASTELIC JP, KNOPF L, GINTER OJ 1990: Effect of day of prostaglandin $F_{2\alpha}$ treatment on selection and development of the follicle in heifers. Anim Reprod Sci **23**: 169-180
KERBRAT S, DISENHAUS C. 2004: A proposition for an updated behavioural characterization of the oestrus period in dairy cows. Appl Anim Behav Sci **87**: 223-238
KIM IH, SUH GH, SON DS 2003: A progesterone-based timed AI protocol more effectively prevents premature estrus and incomplete luteal regression than an Ovsynch protocol in lactating Holstein cows. Theriogenology **60**: 809-817
KING ME, KIRACOFE GH, STEVENSON JS, SCHALLES RR 1982: Effect of stage of the estrous cycle on interval to estrus after $PGF_{2\alpha}$ in beef cattle. Theriogenology **18**: 191-200
KRÁL J, BÍLEK P, ORTELOVÁ J, DVOŘÁK M, ROUTA V, ŠEVČÍK B 1981: Sledování biologické účinnosti Oestrophanu inj. (Spofa) na jalovicích. Biol Chem Vet **17**: 17-27
LARSON LL, BALL PJH 1992: Regulation of estrous cycles in dairy cattle: a review. Theriogenology **38**: 255-267
MACMILLAN KL, HENDERSON HV 1984: Analyses of the variation in the interval from an injection of prostaglandin $F_{2\alpha}$ to oestrus as a method of studying patterns of follicle development during dioestrus in dairy cows. Anim Reprod Sci **6**: 245-254
MACMILLAN KL, THATCHER WW 1991: Effects of an agonist of gonadotropin-releasing hormone on ovarian follicles in cattle. Biol Reprod **45**: 883-889
MOREIRA F, DE LA SOTA RL, DIAZ T, THATCHER WW 2000: Effect of day of the estrous cycle at the initiation of a timed artificial insemination protocol on reproductive responses in dairy heifers. J Anim Sci **78**: 1568-1576
MORRELL JM, NOAKES DE, ZINTZARAS E, DRESSER DW 1991: Apparent decline in fertility in heifers after repeated estrus synchronization with cloprostenol. Vet Rec **128**: 404-407
NOAKES DE, PARKINSON TJ, ENGLAND GCW 2001: Artur's Veterinary Reproduction and Obstetrics (8^{th} ed.). W. B. Saunders, London.
PANCARCI SM, JORDAN ER, RISCO CA, SCHOUTEN MJ, LOPEZ FL, MOREIRA F, THATCHER WW 2002: Use of estradiol cypionate in a presynchronized timed artificial insemination program for lactating dairy cattle. J Dairy Sci **85**: 122-131
PETERS MW, PURSLEY JR 2003: Timing of final GnRH of the Ovsynch protocol affects ovulatory follicle size, subsequent luteal function, and fertility in dairy cows. Theriogenology **60**: 1197-1204
PURSLEY JR, MEE MO, WILTBANK MC 1995: Synchronization of ovulation in dairy cows using $PGF_{2\alpha}$ and GnRH. Theriogenology **44**: 915-923
PURSLEY JR, WILTBANK MC, STEVENSON JS, OTTOBRE JS, GAVERICK HA, ANDERSON LL 1997: Pregnancy rates per artificial insemination for cows and heifers inseminated at a synchronized ovulation or synchronized estrus. J Dairy Sci **80**: 295-300
ROELOFS JB, BOUWMAN EG, DIELEMAN SJ, VAN EERDENBURG FJCM, KAAL-LANSBERGEN LMTE, SOEDE NM, KEMP B 2004: Influence of repeated rectal ultrasound examinations on hormone profiles and behaviour around oestrus and ovulation in dairy cattle. Theriogenology **62**: 1337-1352
ROSENBERG M, KAIM M, HERZ Z, FOLMAN Y 1990: Comparison of methods for the synchronization of estrous cycles in dairy cows. 1. Effects on plasma progesterone and manifestation of estrus. J Dairy Sci **73**: 2807-2816
ROY GL, TWAGIRAMUNGU H 1999: Time interval between GnRH and prostaglandin injections influences the precision of estrus in synchronized cattle. Theriogenology **51**: 413 p.
SAVIO JD, BOLAND MP, HYNES N, MATTIACCI MR, ROCHE JF 1990: Will the first dominant follicle of the estrous cycle of heifers ovulate following luteolysis on day 7? Theriogenology **33**: 677-387
SLENNING BD, FARVER TB 1990: Oestrus detection efficiency and the effects of using prostaglandin F2 in a commercial dairy herd. Prev Vet Med **8**: 269-282
SMITH MF 1986: Recent advances in corpus luteum physiology. J Dairy Sci **69**: 911-926
STELLFLUG JN, HAFS HD, LUUIS TM, SEGUIN BE 1975: Luteolysis, estrus and ovulation, and blood prostaglandin F after intramuscular administration of 15, 30 or 60 mg prostaglandin F2alpha. Prostaglandins **9**: 609-615
TANABE TY, HANN RC 1984: Synchronized estrus and subsequent conception in dairy cattle heifers treated with prostaglandin F 2α 1. Influence of stage of cycle at treatment. J Anim Sci **58**: 805-811
THATCHER WW, MACMILLAN KL, HANSEN PJ, DROST M 1989: Concepts for regulation of corpus luteum function by the conceptus and ovarian follicles to improve fertility. Theriogenology **31**: 1-282

THATCHER WW, MOREIRA F, PANCARCI SM, BARTOLOME JA, SANTOS JEP 2002: Strategies to optimize reproductive efficiency by regulation of ovarian function. Domestic Animal Endocrinology **23**: 243-254

TURZILLO AM, FORTUNE JE 1993: Effects of suppressing plasma FSH on ovarian follicular dominance in cattle. J Reprod Fert **98**: 113-119

VASCONCELOS JL, SARTORI R, OLIVEIRA HN, GUENTHER JG, WILTBANK MC 2001: Reduction in size of the ovulatory follicle reduces subsequent luteal size and pregnancy rate. Theriogenology **56**: 307-314

WHITE LM, KEISLER DH, DAILEY RA, INSKEEP EK 1985: Characterization of preovulatory follicle destined to form „subfunctional" corpora lutea. Biol Reprod **32** (Suppl. 1): 43-56

XU ZZ, BURTON LJ, MACMILLAN KJ 1997: Reproductive performance of lactating dairy cows following estrus synchronization regimes with $PGF_{2\alpha}$ and progesterone. Theriogenology **47**: 687-701