

POSTPARTUM OVARIAN FOLLICULAR DYNAMICS AND ESTRUS ACTIVITY USING PROSTAGLANDIN F_{2A} IN DAIRY COWS

Arūnas Rutkauskas, Henrikas Žilinskas

Lithuanian Veterinary Academy, Department of Noninfectious Diseases, Laboratory of Animal Reproduction, Tilžės st. 18, LT – 47181, Kaunas; tel.: 36 33 18. E – mail: arunas@lva.lt

Summary. The objective of performed study was to characterize postpartum follicular dynamics of corpus luteum persistens using prostaglandins F_{2α} in dairy cows. Twenty Lithuanian Black and White breed 3-7 year old cows were divided into three groups: two experimental – Group 1 (n=13) and Group 2 (n=4), and control – Group 3 (n=3). Before and after treatment with two different prostaglandin F_{2α} commercial formations – Dalmazin® (Group 1) and Estron® (Group 2) ovaries, corpus luteum and follicles were examined by ultrasonography and blood level of progesterone was determined. It was estimated that after treatment with Dalmazin (Group 1) follicle have grown 2.03 mm per day and estrus signs was shown by 92.3 % of animals. In Group 1 follicle were on 2.35 mm larger, estrus signs increased by 17.3 % cows, artificial insemination rates were on 16.6 % higher compared animals in the Group 2 treated with *Estron* (P<0.05). In conclusion, Dalmazin have shown significantly higher efficiency for treatment of corpus luteum persistens compared to *Estron*.

Keywords: cow, corpus luteum, follicles, prostaglandin F_{2α}.

FOLIKULŲ AUGIMO KIAUŠIDĖSE DINAMIKA IR PIENINIŲ VEISLIŲ KARVIŲ RUJOS PASIREIŠKIMAS DUODANT PROSTAGLANDINUS F_{2A}

Santrauka. Tyrimo tikslas – nustatyti pieninių veislių karvių folikulų augimo dinamikos ypatumus prostaglandiniais gydant susilaikiusius geltonkūnius. Ultragarsu atlikti karvių kiaušidžių, geltonkūnių ir folikulų dydžio matavimai. Progesterono koncentracijai kraujyje nustatyti paimti kraujo mėginiai. Gydyta preparatais *Dalmazin* ir *Estron* – skirtingais kloprostenolio dariniais. Dvidešimt Lietuvos juodmargių veislės karvių suskirstytos į grupes pagal gydymą skirtingais preparatais. Apskaičiuotas surujojusių ir apsisvaisinusių karvių procentas. Nustatėme, kad susilaikiusius geltonkūnius efektyviau gydyti preparatu *Dalmazin* negu *Estron*.

Raktažodžiai: karvės, kiaušidės, geltonkūniai, folikulai, *Estron*, *Dalmazin*.

Introduction. Acceptable reproductive efficiency requires each cow to calve regularly to maximize economic output of milk production in the face of declining reproductive efficiency over the past 2 decades (Roche *et al.*, 2000). Rapid progress in genetics and management in dairy industry has resulted in decreased milk production per cow. Metabolic demands for more milk negatively impact reproductive function of postpartum cows. In dairy cows, resumption of ovarian activity plays an important role in subsequent fertility (Sakaguchi *et al.*, 2004). Since ultrasound imaging technique has been applied to the study of bovine follicular dynamics, most postpartum follicular development occurs in wave – like manner in normal cycling cattle. Recent studies have revealed, that post partum anovulatory anestrus in dairy cows is not due to a lack of follicular development, but rather the failure of a dominant follicle to ovulate. Ovarian antral follicular development in cattle proceeds through stages of follicular recruitment, selection and dominance. Dominant follicles control development of other follicles through the production of factors that act locally and/or systematically (McGee E. *et al.*, 2000). Development of anovulatory dominant follicles was interrupted by the negative feedback of progesterone from the corpus luteum on the secretion of luteinizing hormone. One of the most common causes of anovulation is the presence of luteal tissue (Wiltbank M. C. *et al.*, 2002). Treatment with

prostaglandin F_{2α} (PGF_{2α}) is likely to be the most effective method to resolve this condition.

The objective of performed study was to characterize postpartum follicular dynamics of corpus luteum persistens using prostaglandins F_{2α} in dairy cows.

Material and methods. Twenty Lithuanian Black and White breed 3-7 year old cows were divided into three groups: two experimental – Group 1 (n=13) and Group 2 (n=4), and control – Group 3 (n=3). Furthermore, the ovarian and follicular measurements were performed using an ultrasound scanner (Scanner 100 LC Vet, the Netherlands).

Ovaries and reproductive tracts were palpated per rectum before initiation of the treatment to confirm presence of a CL and eliminate any cow with morphological abnormalities. The evaluation of ovaries, CL and follicles were made with ultrasound scanner. Cows in Group 1 (n=13) and Group 2 (n=4) with CL persistens were intramuscularly treated with 2 ml of prostaglandin F_{2α} analogues D – (+) – cloprostenolum (0.075 mg, *Dalmazin*®, Fatro S.p.A. Italy) and (0.25 mg, *Estron*, Bioveta, Plc., Czech Republic). Cows in Group 3 (n=3) were served as an untreated control. The average diameter of ovary, CL and follicle was assessed according to the length and width of ovary or follicle (Ginther, 1996 and Sirois, 1988). The arithmetical average of length and width was described as the average measure of ovary and/or follicle. All data was analyzed using SPSS

statistical package (SPSS for Windows 13.0, SPSS Inc., Chicago, IL, USA, 2004) and "Microsoft Excel" spreadsheets. Descriptive statistic was used for the data analysis.

Blood samples for progesterone analysis were taken from all experimental cows. Samples were collected from coccygeal vessels into aseptic silicones disposable vacuum proof tubes ("Venject", Terumo Europe N. V., Belgium) at daily intervals starting at the first day of

experiment until the day of AI. Blood was centrifuged (3000x, 5 min) and serum was stored at -20°C. Peripheral blood progesterone was measured using commercially progesterone diagnostic kit PROG-RIA-CT (BioSource Europe S.A., Belgium) according to Duchens *et al.* (1994) and analyzed in „ГAMMA – 12“ (Russia).

Results. The results of the measurements of ovaries and follicles are shown in Table 1.

Table 1. Size and averages of ovaries, corpus luteum and follicles

	Groups of cows		
	Group I (n=13)	Group II (n=4)	Control group (n=3)
	X±SD		
DKL	31.94±8.19	24.80±8.10	23.50±6.50
DKW	22.63±6.04	17.10±5.23	17.25±3.65
DKM	27.28±6.17	21.00±6.65	19.08±3.52
DKFL	5.79±3.18	6.5±2.94	6.05±3.65
DKFW	4.27±2.82	4.14±1.60	3.95±2.13
DKFM	5.43±1.45	5.61±1.73	4.15±1.35
DKCLL	16.50±4.65	18.10±0.67	12.35±2.16
DKCLW	14.30±5.21	15.50±0.29	8.55±1.87
DKCLM	15.40±4.65	16.85±2.52	10.59±1.24
KKL	27.24±7.05	24.50±4.88	22.65±2.14
KKW	20.28±5.24	19.81±3.63	16.40±3.67
KKM	23.81±5.80	22.14±3.70	18.15±3.48
KKFL	6.51±1.74	7.40±3.30	5.85±1.13
KKFW	5.62±2.53	6.10±2.70	2.95±2.45
KKFM	6.07±2.60	6.82±3.00	4.35±2.15
KKCLL	12.80±3.25	16.44±1.10	14.65±2.35
KKCLW	11.50±4.52	15.90±0.90	7.20±2.61
KKCLM	12.15±3.24	16.20±1.00	10.65±4.35

DKL – length of the right ovary, DKW – width of the right ovary, DKM – average measurements of the right ovary, DKFL – length of the follicle in the right ovary, DKFW – width of the follicle in the right ovary, DKFM – average of the measurements of the follicle in the right ovary, DKCLL – corpus luteum length in the right ovary, DKCLW – corpus luteum width in the right ovary, DKCLM – average of the measurements corpus luteum in the right ovary, KKL – length of the left ovary, KKW – width of the left ovary, KKM – average measurements of the left ovary, KKFL – length of the follicle in the left ovary, KKFW – width of the follicle in the left ovary, KKFM – average of the measurements of the follicle in the left ovary, KKCLL – corpus luteum length in the left ovary, KKCLW – corpus luteum width in the left ovary, KKCLM – average of the measurements corpus luteum in the left ovary.

It was estimated, that on day 1 of experiment average size of cows right ovary in Group 1 was 27.28±6.17 mm, average of follicle size - 5.43±1.45 mm and size of CL persists 15.40±4.65 mm. Sizes of left ovary and follicle were 23.81±5.80 mm and 6.07±2.60 mm, respectively. Corpus luteum persists size in the left ovary was 12.15±3.24 mm. Progesterone level in peripheral blood was 14.75±5.48 nmol/l. In Group 2 average size of cows right ovary was 21.00±6.65 mm, average of follicle size - 5.61±1.73 mm and size of CL persists 16.85±2.52 mm. Average of the left ovary was 22.14±3.70 mm, follicle size - 6.82±3.00 mm, CL persists - 16.20±1.00 mm. Progesterone level in peripheral blood was 15.21±4.9 nmol/l. In Control group average size of right ovary was 19.08±3.52 mm, average of follicle size - 4.15±1.35 mm and CL average size - 10.59±1.24 mm. Sizes of left ovary and follicle were 18.15±3.48 mm and 4.35±2.15 mm, respectively, CL persists - 10.65±4.35 mm.

Progesterone level in peripheral blood was 10.52±5.16 nmol/l.

In Group 1 and 2 average sizes of CL persists and progesterone level were statistically higher compared to controls (Group 3). Progesterone level after administration of Estron reduced from 14.75±0.53 nmol/l on day 1 to 5.04±0.42 nmol/l day 2 (P<0.05). Using prostaglandin F_{2α} analogue D – (+), Dalmazin concentration decrease from 15.21±1.01 nmol/l to 4.73±0.84 nmol/l, respectively (P<0.05).

In Group 1 estrus signs were shown by 12 cows (92.3 %), size of dominant follicle - 12.15±1.15 mm and progesterone level - 1.17±0.56 nmol/l. In Group 2 estrus signs were shown by 3 cows (75.0 %), size of dominant follicle - 9.8±1.1 mm and progesterone level - 2.95±0.96 nmol/l. In Group 3 66.7 % of cows shown estrus signs, size of dominant follicle - 9.2±0.8 mm and progesterone

level - 3.63 ± 1.28 nmol/l. All cows with estrus signs were inseminated.

After administration of Dalmazin (Group 1) follicles growth rate was 2.03 ± 0.16 mm per day. There was significant difference (0.4 ± 0.1 mm per day) between Groups 1 and 2 in follicular growth rate ($P < 0.05$). In all groups was observed two follicular waves, but in Group 1

compared to Group 2 follicular waves were more pronounced (Fig. 1).

Following 45 days after AI, all cows were examined for detection of pregnancy. There was 10 pregnant cows in Group 1, two in Group 2 and one cow in Group 3 (Table 2).

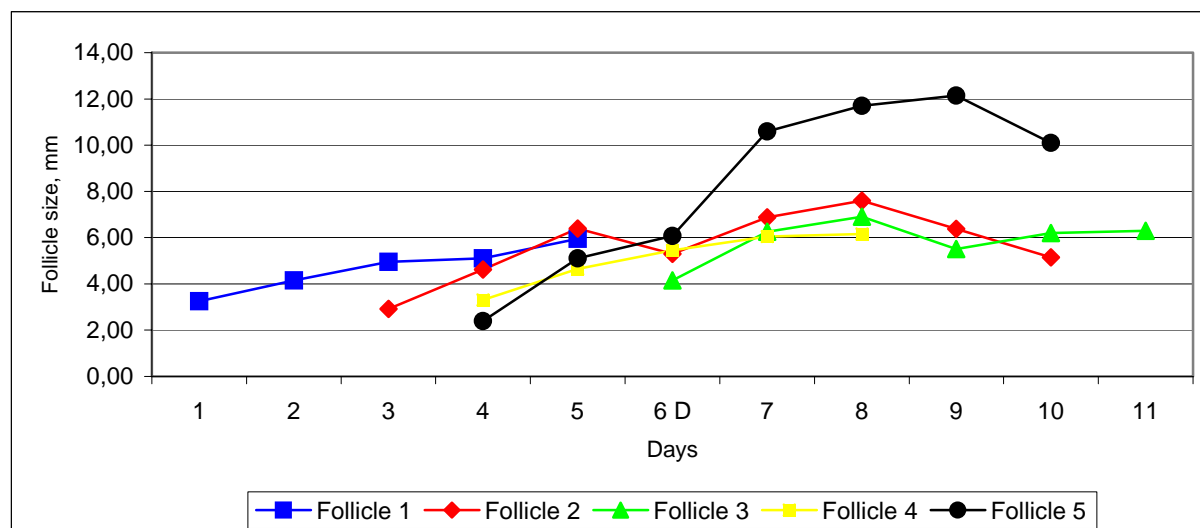


Figure 1. Follicular dynamics in ovaries with corpus luteum persistens and after treatment with *Dalmazin*. Letter "D" show the application of *Dalmazin*. Follicles show in numbers.

Table 2. Estrus signs and pregnancy rates in all groups (%)

	Group I (n=13)	Group II (n=4)	Control group (n=3)
		%	
Estrus signs	92.31	75.00	66.67
Pregnancy rates	83.33	66.67	50.00

Discussion. The results of this experiment demonstrate that lactating cows have tendency to show two follicular waves. This is in agreement with Savio *et al.* (1990), who pointed that cows often show two follicular waves. The prostaglandin $F_{2\alpha}$ analogue D – (+) *Dalmazin* have shown significantly higher follicle growing rate and estrus signs compared to Estron. After administration of *Dalmazin*, CL persistens size and level of progesterone decreased significantly slower compared to group of cows treated with Estron. According results described by Chiesa (2004), prostaglandin $F_{2\alpha}$ analogue D – (+) *Dalmazin* compared to Estron have faster effect because of decresion of sensivity of luteizing hormone receptors. Continously, decreased progesterone level increases follicle stimulating hormone level in blood. After expulsion of FSH, in ovary starts growth of a new follicles. Our results provides support for other observations, because in group of cows treated with *Dalmazin* follicle were on 2.35 ± 0.05 mm larger, estrus signs showed 17.3% more cows, pregnancy rates were on 16.66 % higher compared to cows treated with Estron.

Conclusions.

1. Administration of prostaglandin $F_{2\alpha}$ analogue D – (+) *Dalmazin* have shown higher efficiency compared to Estron for treatment of corpus luteum persistens in dairy cows.
2. Follicular dynamics in ovaries of cows treated with *Dalmazin* were significantly faster.

References

1. Chiesa F. Physiological functions of $PgF_{2\alpha}$ with regards to reproduction and in particular to commencement of post partum recycling in cows. *J. Dairy Sci.*, 2004. Vol. 84; P. 1277 – 1285.
2. Duchens M., Forsberg M., Edqvist L.-E., Gustafsson H., Rodriguez-Martinez H. Effect of induced subabasal progesterone levels around oestrus on plasma concentrations of progesterone, estradiol-17 β and LH in heifers. *Theriogenology*. 1994. No. 42, P. 1159-1169.
3. Ginther O. J., Wiltbank M. C., Fricke P. M., Gibbons J. R., Kot K. Selection of the dominant follicle in cattle. *Biology of Reproduction*. 1996. No. 55, P. 1187-1194.
4. McGee E. A., Hsueh A. J. W. Initial and cyclic recruitment of ovarian follicles. *Endocrine Reviews*, 2000. Vol. 21, No. 2. P. 200-214.
5. Roche J. F., Mackey D. and Diskin M. D. Reproductive management of postpartum cows. *Anim. Reprod. Sci.* 2000. Vol. 22. P. 171 – 180.

6. Sakaguchi M., Sasamoto Y., Suzuki T., Takahashi Y., Yamada Y. Postpartum ovarian follicular dynamics and estrous activity in lactating dairy cows. *J. Dairy Sci.*, 2004. Vol. 87. P. 2114 – 2121.

7. Savio J. D., Boland M. P., Roche J. P. Development of dominant follicles and length of ovarian cycles in post-partum dairy cows. *Journal of Reproduction and Fertility*. 1990. No. 88. P. 581–591.

8. Sirois J., Fortune J. E. Ovarian follicular dynamics during the estrous cycle in heifers monitored by real – time ultrasonography. *Biology of Reproduction*. 1988. No. 39. P. 308-317.

9. Wiltbank M. C., Gumen A., Sartori Physiological classification of anovulatory conditions in cattle. *Theriogenology* 2002. Vol. 57. P. 27-32.