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Follicular dynamics following induced luteolysis and transvaginal ultrasound-guided aspiration of the largest follicle in dairy cows

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ABSTRACT

In practice, many veterinarians inseminate cows 'blindly' 72 hours following a single injection of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). In this paper we describe the results of a study in which we examined follicular growth dynamics following $PGF_{2\alpha}$ induced luteolysis, aiming at a better insight into the chances for pregnancy when cows are inseminated in a timed manner. A total of 62 dairy cows (CL>25 mm and largest follicle >12 mm) were enrolled in the study and divided over three treatment groups. On day 0 (start of the experiment) all animals received an intramuscular injection of 500 µg cloprostenol, while 42 cows were subjected to ultrasound-guided trans-vaginal follicle aspiration of the largest follicle and were further subdivided into ablation ≤8.5 mm (n = 31) and ablation >8.5 mm (n = 11), according to the size (<8.5 vs. 8.5 to 12 mm, respectively) of the second largest follicle present at the moment of aspiration. The remaining cows (n = 20) were allocated as controls, representing cows that did not undergo follicle aspiration. The duration of follicular growth (DG), follicular growth rates (GR) as well as luteolysis (LL) were estimated in all cows, based on repeated ultrasound examinations and blood analyses of progesterone levels. Duration of growth (days from $PGF_{2\alpha}$ to follicular size ≥15 mm) significantly differed between the cows in ablation ≤8.5 mm and control (5.12 ± 0.23 vs. 3.31 ± 0.21 days) as well as between ablation >8.5 mm and control cows (4.34 ± 0.28 vs. 3.31 ± 0.21 days, $P<0.05$), whereas no differences were observed between the cows in ablation ≤8.5 mm and ablation >8.5 mm ($P>0.05$). On the basis of the results of the present study, we conclude that cows bearing a CL and treated with one single luteolytic dose of prostaglandins exhibit large variations in the duration of final follicular maturation as a result of a variety in development status of the follicles present at the time of $PGF_{2\alpha}$ application.

Key words: follicular growth, prostaglandin $F_{2\alpha}$, luteolysis, cows

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Introduction

Hormonal interventions are used to increase the probability of estrous detection and insemination, and to subsequently increase the number of pregnant cows in dairy herds (LUCY et al., 2004). In cows, ovarian follicular growth occurs in a regular wave-like pattern. Each wave is comprised of successive phases referred to as recruitment, selection, deviation, dominance, and atresia (MOORE and THATCHER, 2006). A wave of follicular growth is characterized by the synchronous emergence and development of a group of follicles (recruitment). One of these follicles becomes dominant (selection, deviation) and achieves the greatest diameter (dominance), suppressing the growth of the subordinate smaller follicles (atresia, NOSEIR, 2003).

Luteolytic substances, such as prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) or its synthetic analogues, are used widely in dairy cow practice. for example for estrus-synchronization protocols. An intramuscular injection of $PGF_{2\alpha}$ to cows with a functionally mature corpus luteum (CL) leads to luteolysis in 85 % to 95 % of cases followed by the initiation of a new follicular phase (MacMILLAN et al., 1983; STEVENSON and PHATAK, 2010), but the interval from treatment to ovulation differs between treated individuals. The latter is affected either by the size of the dominant follicle (KASTELIC et al., 1990) or by the stage of the follicular wave at the time of $PGF_{2\alpha}$ treatment (TWAGIRAMUNGU et al., 1992). Cows with mature follicles at the time of luteolysis show estrus sooner than cows with immature follicles (ROCHE et al., 1998). Variations in the time of the cycle when the prostaglandin injection is administered, in combination with variations in follicular growth, will cause a great variety in the onset of estrus following one single $PGF_{2\alpha}$ injection, so that veterinarians and farmers are encouraged to inseminate the cows only after estrus is detected. Indeed, insemination following visually detected estrus, instead of "blind" insemination at 72 hours after $PGF_{2\alpha,}$ has been reported to significantly affect conception rates (NOAKES et al., 2001). When timed AI after a single $PGF_{2\alpha}$ injection was applied in lactating dairy cows, pregnancy rates were substantially lower in comparison with those following AI after a detected estrus (ARCHBALD et al., 1992). FOLMAN et al. (1990) reported a conception rate of 39.4 % in cows inseminated at observed estrus, whereas AJITKUMAR et al. (1995) found conception rates of 24.0 and 37.5 % in cows inseminated 72 and 96 hours, respectively, after a single prostaglandin administration. Therefore, it was concluded that fixed-time insemination after a single injection of $PGF_{2\alpha}$ seldom yields acceptable results (DE JARNETTE, 2004: Estrus synchronization: a reproductive management tool, e-book http://www.selectsires.com/resources/fertilitydocs/estrus_syn_reproman.pdf).

Ultrasound-guided transvaginal follicle aspiration (UTFA) has been approved as a standard low invasive procedure for retrieval of bovine oocytes (SENEDA et al., 2001), for evaluating the intra-follicular environment of the oocyte (LEROY et al., 2005), as well as a tool for artificial control of follicular dynamics using ablation procedures (BERGFELT et

al., 1994). It has been shown that ablation of the dominant follicle by UTFA at a random stage of the estrous cycle, induces a new follicular wave and therefore will synchronize follicular development (BERGFELT et al., 1994). When all follicles >5 mm were aspirated, an additional 4 d was required for new follicular recruitment to proceed to ovulation (BERGFELT et al., 1994), without any effect on the number of follicular waves, the size of the ovulatory follicles, and the subsequent progesterone concentration (STUBBINGS and WALTON, 1994). However, this method is not suitable for routine practice because it is time-consuming and requires expensive equipment**.**

In the present study, we examined follicular growth dynamics following $PGF_{2\alpha}$-induced luteolysis, aiming for a scientific explanation of the lowered pregnancy results when cows are inseminated in a timed manner. In order to create variable but precisely defined scenarios of follicular growth in some cows, the largest follicle was removed by UTFA.

Materials and methods

Animals and experimental design. A total of 62 [primiparous (n = 20) and multiparous (n = 42)] Holstein-Friesian dairy cows from two dairy farms in the Republic of Macedonia were included in the study. On Farm 1, cows were housed in a free stall barn, whereas cows on Farm 2 were housed in a tie-stall barn on deep straw bedding. Cows fulfilling the following criteria were included in the study: having regular cyclicity (regular cycles of approximately 21 days), and on day 0 (start of the experiment) the presence of a mature CL (>25 mm) and a follicle ≥12 mm. In order to create treatments with a precisely defined status of follicular development, 42 cows were submitted to ultrasound-guided transvaginal follicle aspiration for ablation of the largest follicle (LF) on day 0. According to the size of the second largest follicle (<8.5 mm versus 8.5 to 12 mm) present at the moment of aspiration, the cows were subdivided into ablation ≤8.5 mm, (n = 31) and ablation >8.5 mm, (n = 11), respectively, whereas the remaining cows were allocated to a control group (n = 20) representing the cows that did not undergo any follicle aspiration. In total, each treatment represented a specific developmental stage of the dominant follicle at the time of the injection of a luteolytic dose of prostaglandins: no dominant follicle (ablation ≤8.5 mm), the presence of a dominant follicle from 8.5 to 12 mm (ablation >8.5 mm) and the presence of a dominant follicle ≥12 mm (control). On the same day (day 0) all cows received 500 μg of cloprostenol i.m for induction of luteolysis. Ovarian follicular development was monitored daily by transrectal ultrasonography, starting at day 0 until the dominant follicle reached the size of ≈ 15 mm. Blood collection for progesterone determination and ultrasonographic measurement of the CL were performed daily in all cows until the end of the experiment (7 days after $PGF_{2\alpha}$ injection).

Ultrasonography examination. Ultrasonography examination of the ovaries was performed using a B-mode scanner Aloka SSD 500, (Tokyo, Japan), equipped with a 7.5 MHz linear-array transducer for intrarectal use and convex-array transducer for intravaginal use. Before insertion of the lubricated transducer, the rectum was emptied, and the ovaries were first manually located before introducing the transducer. The diameters of the follicles, as well as of the CL, were measured by means of electronic calipers located on the ultrasound device after freezing the pictures on the screen. Duration of growth, growth rates, as well as luteolysis were estimated in all cows using the following definitions:

Duration of growth (days) - number of days from the first visualization of the follicle (day of $PGF_{2\alpha}$ injection) until the follicle reached 15 mm.

Daily growth rate (cm/day) - the difference between the first visualization of the follicle and 15 mm divided by the number of days necessary to attain 15 mm (modified by KOJIMA et al., 2003).

Luteolysis - (structural and functional regression of the CL), decreasing of the CL diameter concomitantly with a decline in P4 concentration below 0.5 ng/mL (modified from MARTINS et al., 2011).

Aspiration of the largest follicle. Ovarian follicle ablation was done using ultrasound-guided transvaginal follicle aspiration, as described by BERGFELT et al. (1994). Briefly, caudal epidural anesthesia was induced using 3 to 6 mL of 2 % lidocaine (Rotexmedicca, Trittau - Germany). Next, the perineal area was disinfected using 10 % povidone-iodine (Betadine, Alkaloid, R. Macedonia). The convex-array transducer was fitted with a 65 cm long plastic handle equipped with a needle guide to facilitate placement of the transducer and guidance of the aspiration needle to the vaginal wall and the ovaries. While the transducer was positioned in the vaginal fornix, the free hand was used to transrectally position the ovary, against the vaginal wall. The follicle was stabilized over the transducer face. A 17-gauge, single lumen needle (55 cm long) was placed in the needle guide and advanced through the vaginal wall into the follicular antrum. Ultrasonic hyper-echogenicity of the needle tip as well as a guided line on the screen of the ultrasound device allowed determination of its location and direction during follicle puncture. Follicle ablation was visualized by collapse of the antral follicle following evacuation of the follicular contents. Follicular fluid was manually aspirated by applying a vacuum using a 10 mL plastic syringe.

Blood progesterone analysis. Blood samples were daily collected, starting from Day 0 until the end of the experiment, in all cows from the coccygeal vein into glass tubes and centrifuged (3000 rpm ×g 10 minute) within 3 hours after collection. The samples were stored at -20^0 C until determination of progesterone by enzyme-immune assay (EIA). The determination was made at the laboratory of endocrinology at the Faculty of Veterinary Medicine - Skopje (Macedonia), using a commercially available kit (HUMAN,

Progesterone ELISA Test - Germany). The intra- and inter-assay coefficients of variation were <10 %. The lower detection limit was 0.04 ng/mL.

Data analysis. All statistical analyses were performed using the SAS version software 9.2 (Statistical Analyses System). Descriptive statistics were created using PROC MEANS and PROC FREQ of SAS (Institute, Inc., Cary, NC, USA, 2010). The distribution of all variables was checked to approximate the normal Gaussian distribution. All analyses were performed using the PROC GLIMMIX procedure from SAS version 9.2 for Windows (Institute, Inc., Cary, NC, USA, 2010). Analysis of the duration of growth was performed using the normal distribution function whereas growth rate, luteolysis and P4 were analyzed with the lognormal distribution function from SAS. The statistical models contained fixed effects for all treatments, parity, and the interaction between group and parity. Least square means and contrasts were computed using the LSMEANS statement. Data are reported as least square means with standard errors (LSM ± SE), except for the growth rate. Lognormal distributions were back-transformed and reported with the 95 % confidence intervals. Significance and tendency were declared at $P<0.05$ and $0.05<P<0.1$, respectively.

Results

Originally, 62 cows were included in the study based on the predefined inclusion criteria. The average size of the CL on Day 0, (25.61 ± 0.74 mm, 26.35 ± 2.08 mm and 26.49 ± 0.98 mm, $P>0.05$) as well as the mean progesterone (P4) concentrations (3.89 ± 0.55 ng/mL vs. 3.78 ± 0.44 ng/mL and 4.54 ± 0.56 ng/mL, $P>0.05$), did not differ between ablation ≤8.5 mm, ablation >8.5 mm and control groups, respectively. In contrast, among the 62 cows originally included, 15 cows [24.19 %, (ablation ≤8.5 mm, n = 8, ablation >8.5 mm, n = 4, control, n = 3)] had progesterone <1 ng/mL at the time of prostaglandin treatment and were therefore excluded from further analysis. After administration of $PGF_{2\alpha}$, 5 cows from ablation ≤8.5 mm and 5 cows from the control failed to complete luteolysis. Progesterone concentrations (Fig. 1) on day 0 in cows which failed to fully regress their CL (7.24 ± 3.71 ng/mL) were greater in comparison with cows in which luteolysis was successfully completed (4.38 ± 0.34 ng/mL, $P<0.05$). Cows in which luteolysis failed were excluded for further analysis, leading to a total number of 37 cows remaining for further analyses. No differences were observed in luteolysis and in the decline in P4 concentration among treatments.

Resumption of the follicular growth (defined as continuous development of the follicles, five days after the beginning of the experiment) failed in 8 (ablation ≤8.5 mm) treated cows and they were hence deleted from further analysis of duration of growth and growth rate, leading to a total of 29 cows. Duration of growth (Fig. 2) was different ($P<0.05$) between the ablation ≤8.5 mm and the control groups, (5.12 ± 0.23 days vs.

3.31 ± 0.21 days), respectively, as well as between the ablation >8.5 mm and the control groups (4.34 ± 0.28 days vs. 3.31 ± 0.21 days,). Furthermore, cows in ablation ≤8.5 mm had a numerically longer duration of growth than cows in ablation >8.5 mm (5.12 ± 0.23 days vs. 4.34 ± 0.28 days, P>0.05). Second parity cows showed a numerically shorter duration of growth (4.03 ± 0.24 days) when compared with first (4.63 ± 0.22 days) and greater parity cows (4.52 ± 0.20 days).

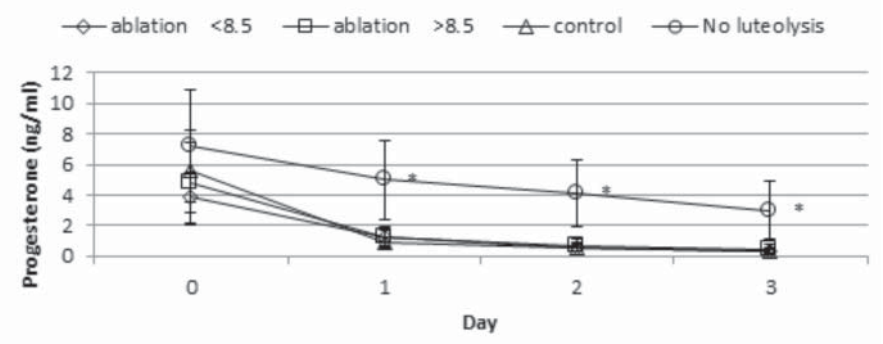

Fig. 1. Decline of the serum P4 concentration after $PGF_{2\alpha}$ injection in cows with or without complete luteolysis. Ablation ≤8.5 mm (no dominant follicle, n = 18), ablation >8.5 mm (presence of a dominant follicle from 8.5 to 12 mm, n = 7), presence of a dominant follicle ≥12 mm (control n = 12) and no luteolysis (n = 10).

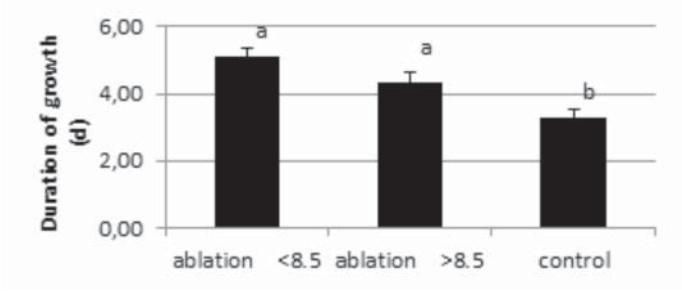

Fig. 2. Duration of growth between treatments. Ablation ≤8.5 mm (n = 10), ablation >8.5 mm (n = 7) and control (n = 12) cows. Superscripts show the difference among treatments (a,b).

No significant differences in growth rate (Fig. 3) were detected between treatments (P>0.05). Cows in the ablation ≤8.5 mm group had a numerically greater growth rate (0.23 ± 0.09 cm/day) than the cows of ablation >8.5 mm (0.17 ± 0.07 cm/day) and control groups (0.15 ± 0.05 cm/day).

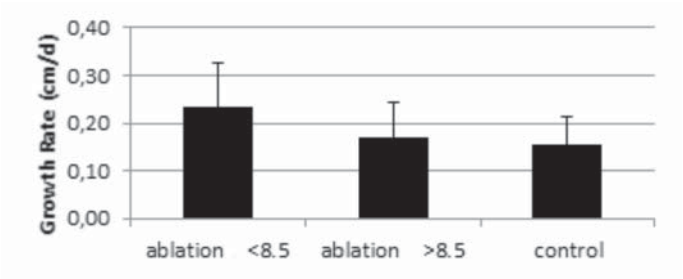

Fig. 3. Growth rate of the dominant follicle among treatments

First-parity cows (Fig. 4) had numerically greater growth rates (0.22 ± 0.08 cm/day) in comparison to the second and greater parity cows (0.17 ± 0.07 cm/day and 0.15 ± 0.06 cm/day) respectively, but no differences were detected (P>0.05), either between the former or latter two parity groups (P>0.05).

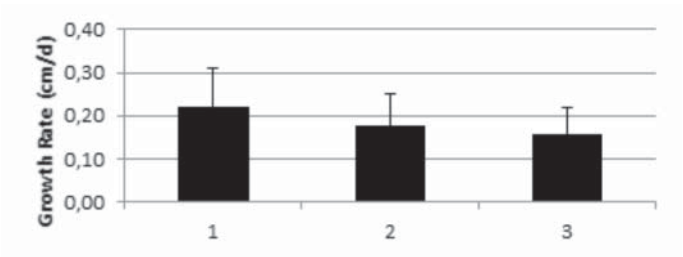

Fig. 4. Influence of parity on the growth rate of the dominant follicle among parity groups. (1 = first parity, 2 = second parity, 3 = greater parity).

Cows that failed to recommence follicular growth, surprisingly, had a slower decline of the progesterone during 24 hours after treatment in comparison with cows which resumed regular follicular growth (mean 1.61 ng/mL vs. 3.24 ng/mL, P<0.05).

Discussion

The main intention of the present study was to discourage veterinary practitioners from routinely performing "blind" inseminations in cows 72 hours following one single injection of $PGF_{2\alpha}$. The starting point was that the length of the interval from $PGF_{2\alpha}$ treatment to estrus depends upon the developmental stage of the dominant follicle present at the time of treatment (MARTINEZ et al., 2009). It has been well demonstrated that a luteolytic dose of $PGF_{2\alpha}$ will cause regression of the CL starting from day 5-7 after estrus (MOMONT and SEGUIN, 1984). Indeed, in the present study most of the cows undergo luteal regression; however, our results have shown that a single $PGF_{2\alpha}$ treatment might

cause partial luteolysis, especially in the cows that produce higher amounts of P4 in comparison with the cows that produce less P4. The latter is in contrast with the results reported by MARTINS et al. (2011), in which higher P4 concentrations were associated with higher probability of luteolysis. Nevertheless, partial luteolysis has been shown to result in subluteal peripheral P4 concentrations (2-3 ng/mL) impairing further follicular growth (KINDER et al.,1996) and finally blocking the ovulation (SAVIO et al., 1993), leading to unsynchronized estrus and presumably subsequent reduced fertility if those cows are inseminated blindly 72 hours after a single $PGF_{2\alpha}$ injection.

Some studies (DOVENSKI et al., 1999; VERONESI et al., 2002) demonstrated a good correlation between ultrasonography determination of the diameter of the CL and peripheral P4 concentrations. In contrast, the results of the present study have shown that 24.19 % of the cows had P4 concentrations lower than 1 ng/mL at the time of prostaglandin treatment, even if a CL larger than 25 mm in diameter was detected by ultrasonography. The latter suggests that spontaneous regression had already occurred, despite the fact that the CL still showed a relatively large diameter at ultrasonography examination. SIQUEIRA et al. (2009) observed similar results, and reported that the decline in plasma progesterone concentration usually precedes luteal tissue regression. It was assumed that the primary decline in systemic progesterone is probably not due to the regression of steroidogenic cells, but it is a result of the decreased blood flow to the CL, which significantly impairs the steroidogenic capacity of luteal cells. After cloprostenol-induced luteal regression, there were no significant differences in the rate of luteolysis between treatments in the present study. Significant differences were observed in the decline of progesterone levels at 24 h between cows which resumed and those that did not recommence regular follicular growth. It can be assumed that plasma estradiol concentrations may have a significant impact on the luteolysis rate (ARAUJO et al., 2009). In this regard, cows with normal follicular growth possibly had higher plasma estradiol concentrations in comparison with cows in which regular follicular growth did not occur. COLAZO et al. (2002) observed similar results, linking the effect of the diameter of the ovulatory follicle at cloprostenol treatment and the decline in plasma P4 concentrations 24 h after treatment. The decline in plasma P4 concentration was significantly affected by the diameter of the ovulatory follicle at the time of treatment. When the eventual ovulatory follicle was small (5-8 mm) at the time of the cloprostenol treatment, there was a greater ($P<0.05$) decline in mean plasma P4 concentration than when the ovulatory follicle was large (13-16 mm) at the time of treatment (5.8 ng/mL vs 3.9 ng/mL over 24 h). The authors assume that larger follicles were already less estrogen active than small follicles at the time of cloprostenol treatment, and the latter may have been collectively producing higher estradiol concentrations, resulting in a greater decline in progesterone 24 h later.

The results of the present study furthermore show that cows treated with $PGF_{2\alpha}$ when no dominant follicle is present need a longer interval to attain 15 mm in comparison with cows that already have follicles larger than >8.5 mm at the time of treatment. These findings are similar with the results reported by KASTELIC et al. (1990). Heifers treated on Day 5 after ovulation had a shorter interval from $PGF_{2\alpha}$ treatment to ovulation (3.0 days) than those treated on Day 12, (4.5 days). The reasons for this were the different development phases of the dominant follicle at the time of treatment. Treatment on Day 5 was followed by ovulation of the dominant follicle of wave 1 (near its maximum diameter at the time of treatment), whereas treatment on Day 12 was followed by ovulation of the dominant follicle of wave 2, which required more time to attain ovulatory size. From a practical point of view, the situation when no dominant follicle is present at the time of injection of $PGF_{2\alpha}$ is possible when cows are at 1 to 4 days after ovulation. In this regard, there will be no estrus synchronization (refractoriness of CL), so an additional $PGF_{2\alpha}$ injection, 11 or 14 days apart is required. In practice, protocols based on a double prostaglandin injection with 11 to 14 days apart are popular. Usually, the regimen based on a 14-day interval between the two injections shows an improved response over the 11 day protocol, because two injections given 14 days apart ensure that most animals are in the late luteal stage (cycle day 11 to 14) when they receive the second $PGF_{2\alpha}$ dose (MURUGAVEL et al., 2008). After day 4 of the cycle, follicular deviation takes place, which is characterized by a divergence in growth rates and the distinction between the dominant and subordinate follicles (GINTHER et al., 2003). Removing the largest follicle after deviation triggers the second largest follicle (>8.5 mm) present to achieve dominance and become the pre-ovulatory follicle, leading to a shorter interval from treatment to largest diameter, as shown in the ablation >8.5 mm group in the present study. This result corresponds to the reports of VASCONCELOS et al. (1999) and DESCÔTEAUX et al. (2010), in which it was demonstrated that follicles >8 mm generally have a sufficient amount of LH receptors to resume follicular growth to dominance and the capability to ovulate once they reach full development. From a practical approach, by administrating one single $PGF_{2\alpha}$ injection early (day 5 - 9) or late (day 14 - 16) in the estrous cycle, when there is a follicle >8.5 mm present, the time needed to detect estrus is shorter (i.e., estrus will occur earlier and with less variation), whereas administration on day 10-12 of the estrous cycle will lead to an interval of 3 to 7 days before estrus will occur (LUCY et al., 2004). When $PGF_{2\alpha}$ was injected on Day 5-8, the mean interval to estrus was 49.5 ± 6 h (STEVENSON et al., 1984), whereas prostaglandin administration at mid-cycle (day 10) or later in the luteal phase (day 14 to 16), resulted in a mean time to estrus of 86.9 ± 5.9 h (SIQUEIRA et al., 2009) and 60.6 ± 8 h (STEVENSON et al., 1984) respectively.

Since parity may have an effect on follicular growth, we also tested its effect in the present study. Our results show that second parity cows experienced no significantly shorter duration of growth in comparison to primi- and multiparous cows, which is not

in accordance with the results reported by ROSENBERG et al. (1990), where older cows entered estrus sooner than younger ones ($P<0.05$)**.** In the present study, the follicular growth rate did not differ between treatments. Cows that started a new follicular wave (ablation ≤8.5 mm) experienced a numerically faster growth rate in comparison to the cows where the second largest follicle took over or grew to be the greatest follicle (ablation >8.5 mm and control). ARAUJO et al. (2009) reported that follicular growth rates following ablation of the dominant follicle tend to depend on the stage of the estrous cycle ($P = 0.07$), which is in accordance with the results presented here. Furthermore, our results were different from the results reported by ASSEY et al. (1993), who observed a mean growth rate of 0.9 ± 0.3 mm/day of the ovulatory follicle starting from the moment of a cloprostenol injection until ovulation. Currently, there is a lack of data in literature to clarify all ambivalent results reported in the different studies, which necessitates further investigation.

Conclusion

The present study yields some very essential information for cattle practitioners. First of all, we showed that 24.19 % of the cows which were thought to have an active CL based on ultrasonography examination, had peripheral progesterone levels <1 ng/mL and hence had already started luteal regression. Secondly, we showed that in some cows having high progesterone levels, no complete luteolysis occurred. Both phenomena do occur in real life and will lead to a great asynchrony of ovulation and hence disappointing results, if the cows are inseminated in a timed manner. However, most cows did experience luteal regression and subsequently resumed regular follicular growth, which initiated a new follicular phase of the estrous cycle. The period of the follicular phase was affected by the diameter of the follicles present at the time of injection, whilst parity did not have any influence on the duration of growth, the growth rate or on luteolysis. Therefore, from a practical point of view, in terms of treatment - estrus intervals, in general, it can be stated that the main factor affecting the length of the treatment - estrus interval is the development status of the follicles present at the time of $PGF_{2\alpha}$ application, accompanied by the success of CL regression. Since $PGF_{2\alpha}$ has no impact upon follicular development and, therefore, no control over follicle wave emergence, cows possessing a CL at the time of $PGF_{2\alpha}$ injection will come into estrus with little precision in terms of time, which may result in a lack of synchronization between ovulation and insemination if the animals are inseminated without detection of heat. In this regard, performing “blind” insemination at 72 h after a single $PGF_{2\alpha}$ injection will result in disappointing fertility results, while satisfactory conception rates can only be achieved if artificial insemination is performed after the detection of (standing) heat.

References

AJITKUMAR, G., E. MADHAVAN, C. P. N. IYER (1995): Management of estrous cycle in crossbred cows using prostaglandin. J. Vet. Anim. Sci. 26, 117-120.

ARAUJO, R. R., O. J. GINTHER, J. C. FERREIRA, M. M. PALHAO, M. A. BEG, M. C. WILTBANK (2009): Role of follicular estradiol-17beta in timing of luteolysis in heifers. Biol. Reprod. 81, 426-437.

ARCHBALD, L. F, T. TRAN, R. MASSEY, E. KLAPSTEIN (1992): Conception rates in dairy cows after timed insemination and simultaneous treatment with gonadotrophin releasing hormone and/or prostaglandin F_2 alpha. Theriogenology 37, 723-31.

ASSEY, R. J., B. PURWANTARA, T. GREVE, P. HYTTEL, M. H. SCHMIDT (1993): Corpus luteum size and plasma progesterone levels in cattle after cloprostenol-induced luteolysis. Theriogenology 39, 1321-1330.

BERGFELT, D. R., K. C. LIGHTFOOT, G. P. ADAMS (1994): Ovarian synchronization following ultrasound-guided transvaginal follicle ablation in heifers. Theriogenology 42, 895-907.

COLAZO, M. G., M. F. MARTÍNEZ, J. P. KASTELIC, R. J. MAPLETOFT (2002): Effects of dose and route of administration of cloprostenol on luteolysis, estrus and ovulation in beef heifers. Anim. Reprod. Sci. 72, 47-62.

DESCÔTEAUX, L., G. GNEMMI, J. COLLOTON (2010): Practical Atlas of Ruminant and Camelid Reproductive Ultrasonography. A John Wiley & Sons, Inc., Publ. p.54-56.

DOVENSKI, T., LJ. KOCOSKI, P. TROJACANEC, V. PETKOV, K. POPOVSKI, G. MICKOVSKI, F. POPOVSKA-PERCINIC, LJ. MICKOV (1999): Comparison of ultrasonic morphology of bovine corpora lutea and plasma progesterone concentration during estrous cycle and early pregnancy. Mac. J. Reprod. 5, 47-57.

FOLMAN, Y., M. KAIM, Z. HERZ, M. ROSENBERG (1990): Comparison of methods for the synchronization of estrous cycles in dairy cows. 2. Effects of progesterone and parity on Conception. J. Dairy Sci. 73, 2817-2825.

GINTHER, O. J., M. A. BEG, F. X. DONADEU, D. R. BERGFELT (2003): Mechanism of follicle deviation in monoovular farm species. Anim. Reprod. Sci. 78, 239-257.

KASTELIC, J. P., L. KNOPF, O. J. GINTER (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.

KINDER, J. E., F. N. KOJIMA, E. G. BERGFELD, M. E. WEHRMAN, K. E. FIKE (1996): Progestin and estrogen regulation of pulsatile LH release and development of persistent ovarian follicles in cattle. J. Anim. Sci. 74, 1424-1440.

KOJIMA, F. N., E. G. M. BERGELT, M. E. WEHRMAN, A. S. CUPP, K. E. FIKE, D. V. M. AGUAYO, T. S. TORRES, M. G. WINDER, D. T. CLOPTON, A. J. ROBERTS, J. E. KINDER (2003): Frequency of luteinizing hormone pulses in cattle influences duration of persistence of dominant ovarian follicles, follicular fluid concentration of steroids and activity of insulin-like growth factor binding proteins. Anim. Reprod. Sci. 77, 187-211.

LEROY, J. L. M. R., T. VANHOLDER, B. MATEUSEN, A. CHRISTOPHE, G. OPSOMER, A. DE KRUIF, G. GENICOT, A. VAN SOOM (2005): Non-esterified fatty acids in follicular fluid of

dairy cows and their effect on developmental capacity of bovine oocytes *in vitro*. Reproduction 130, 485-495.

LUCY, M. C., S. McDOUGALL, D. P. NATION (2004): The use of hormonal treatments to improve the reproductive performance of lactating dairy cows in feedlot or pasture-based management systems. Anim. Reprod. Sci. 82-83, 495-512.

MacMILLAN, K. L. (1983): Prostaglandin responses in dairy herd breeding program. N. Z. Vet. J. 31, 110-113.

MARTÍNEZ, F. M., A. G. BÓ, R. J. MAPLETOFT (2009): Synchronization of follicular wave emergence and ovulation for reproductive biotechnologies. Proceedings of 1st Simpósio internacional de reprodução animal aplicada, Londrina, Brazil 26-55.

MARTINS, J. P. N., R. K. POLICELLI, L. M. NEUDER, W. RAPHAEL, J. R. PURSLEY (2011): Effects of cloprostenol sodium at final prostaglandin $F_{2\alpha}$ of Ovsynch on complete luteolysis and pregnancy per artificial insemination in lactating dairy cows. J. Dairy Sci. 94, 2815-2824.

MOMONT, H. W., B. E. SEGUIN (1984): Treatment of unobserved estrus in lactating dairy cows with prostaglandin $F_{2\alpha}$ products. Compend. Cont. Edu. Pract. Vet. Univ. Minnesota, St. Paul., 28.

MOORE, K., W. W. THATCHER (2006): Major advances associated with reproduction in dairy cattle. J. Dairy Sci. 89, 1254-1266.

MURUGAVEL, K., J. L. YÁNIZ, P. SANTOLARIA, M. LÓPEZ-BÉJAR, F. LÓPEZ-GATIUS (2008): Prostaglandin based estrus synchronization in postpartum dairy cows: An Update. Int. J. App. Res. Vet. Med. 11, 31-36.

NOAKES, D. E., J. T. PARKINSON, C. W. G. ENGLAND (2001): Arthur's Veterinary Reproduction and Obstetrics. W. B. Saunders, 56, book.

NOSEIR, W. M. B. (2003): Ovarian follicular activity and hormonal profile during estrous cycle in cows: the development of 2 versus 3 waves. Reprod. Biol. Endocrinol. 50, 1-6.

ROCHE, J. F., M. MIHN, M. G. DISKIN, J. IRELAND (1998): A review of regulation of follicle growth in cattle. J. Anim. Sci. 76, 16-29.

ROSENBERG, M., M. KAIM, Z. HERZ, Y. FOLMAN (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.

SAVIO, J. D., W. W. THATCHER, L. BADINGA, R. L. DE LA SOTA, D. WOLFENSON (1993): Regulation of dominant follicle turnover during the estrous cycle in cows J. Reprod. Fert. 97, 197-203.

SENEDA, M. M., C. R. ESPER, J. M. GARCIA, J. A. OLIVEIRA, R. VANTINI (2001): Relationship between follicle size and ultrasound-guided trans-vaginal recovery. Anim. Reprod. Sci. 67, 37-43.

SIQUEIRA, L. G. B, C. A. A. TORRES, L. S. AMORIM, E. D. SOUZA, L. S. A. CAMARGO, C. A. C. FERNANDES, J. H. M. VIANA (2009): Interrelationships among morphology, echotexture, and function of the bovine corpus luteum during the estrous cycle. Anim. Reprod. Sci. 115, 18-28.

STEVENSON, J. S., A. P. PHATAK (2010): Rates of luteolysis and pregnancy in dairy cows after treatment with cloprostenol or dinoprost. Theriogenology 73, 1127-1138.

STEVENSON, J. S., M. K. SCHMIDT, E. P. CALL (1984): Stage of estrous cycle, time of insemination, and seasonal effects on estrus and fertility of Holstein heifers after prostaglandin $F_{2\alpha}$. J. Dairy Sci. 67, 1798-1805.

STUBBINGS, R. B., J. S. WALTON (1994): Effect of ultrasonically-guided follicle aspiration on estrous cycle and follicular dynamics in Holstein cows. Theriogenology 43, 705-712.

TWAGIRAMUNGU, H., L. A. GUILBAULT, J. PROULX, P. VILLENEUVE, J. J. DUFOUR (1992): Influence of an agonist of gonadotropin-releasing hormone (buserelin) on estrus synchronization and fertility in beef cows. J. Anim. Sci. 70, 1904-1910.

VASCONCELOS, J. L., R. W. SILCOX, G. J. ROSA, J. R. PURSLEY, M. C. WILTBANK (1999): 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 52, 1067 -1078.

VERONESI, M. C, G. GABAI, M. BATTOCCHIO, A. MOLLO, F. SOLDANO, G. BONO, F. CAIROLI (2002): Ultrasonographics appearance of tissue is a better indicator of CL function than CL diameter measurement in dairy cows. Theriogenology 58, 61-68.

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ATANASOV, B., M. HOSTENS, I. CELESKA, K. ILIESKA, G. OPSOMER, T. DOVENSKI: Dinamika rasta folikula nakon izazvane luteolize i ultrazvukom nadzirane transvaginalne aspiracije najvećeg folikula u mliječnih krava. Vet. arhiv 85, 247-260, 2015.

SAŽETAK

U praksi, mnogi veterinari osjemenjuju krave "na slijepo", 72 sata nakon jedne injekcije prostaglandina $F_{2\alpha}$ ($PGF_{2\alpha}$). U radu su opisani rezultati istraživanja dinamike rasta folikula nakon luteolize izazvane prostaglandinom $PGF_{2\alpha}$ s ciljem dobivanja boljeg uvida u mogućnost za gravidnost kod pravovremeno osjemenjenih krava. Ukupno su 62 mliječne krave (žuto tijelo >25 mm i najveći folikul >12 mm) bile uključene u istraživanje i podijeljene u tri skupine. Nultog dana (početak pokusa) svim je životinjama intramuskularno bilo primijenjeno 500 µg kloprostenola. Istovremeno je kod 42 krave uz pomoć ultrazvuka provedena transvaginalna aspiracija najvećeg folikula. Krave su dalje bile razvrstane na osnovi veličine (<8,5 i 8,5 do 12 mm) drugog po veličini folikula u trenutku aspiracije na skupinu s veličinom odstranjenog dijela ≤8,5 mm (n = 31) i veličinom odstranjenog dijela >8,5 mm (n = 11). Ostale krave (n = 20) smatrane su kontrolnom skupinom koja nije bila izložena aspiraciji folikula. Kod svih krava je, uz pomoć ponovljene ultrazvučne pretrage i mjerenjem razine progesterona u krvi, utvrđeno trajanje rasta folikula zatim stopa rasta folikula te luteoliza. Trajanje rasta folikula (dani od $PGF_{2\alpha}$ do veličine folikula ≥15 mm) signifikantno se razlikovalo između krava s veličinom odstranjenog dijela ≤8,5 mm i krava kontrolne skupine (5,12 ± 0,23 prema 3,31 ± 0,21 dana) kao i između krava s odstranjenim dijelom >8,5 mm i krava kontrolne skupine (4,34 ± 0,28 prema 3,31 ± 0,21 dana, P<0,05). Između krava s odstranjenim dijelom ≤ 8,5 mm i >8,5 mm nisu utvrđene signifikantne razlike (P>0,05). Na temelju postignutih rezultata može se zaključiti da krave sa žutim tijelom, koje su dobile jednu luteolitičku

dozu prostaglandina, pokazuju velike varijacije u trajanju konačnog sazrijevanja folikula. Navedeno je rezultat različitog razvojnog stupnja folikula koji su prisutni u vrijeme primjene $PGF_{2\alpha}$.

Ključne riječi: rast folikula, prostaglandin $F_{2\alpha}$, luteoliza, krave