



## Original Scientific Article

**SHORT SYNCH - A REPRODUCTIVE MANAGEMENT TOOL TO INCREASE THE REPRODUCTIVE PERFORMANCE IN DAIRY COWS**

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**ABSTRACT**

Poor estrus detection is the greatest limiting factor for reproductive performance in dairy cows. The study aimed to compare the reproductive performance by assessing the fertility rate (FR) between cows inseminated after detected estrus and those inseminated after synchronized ovulation with the Short Synch protocol. A total of 138 primi- and multiparous dairy cows from 5 dairy farms in North Macedonia were included in the study. At day 65±3 postpartum, an ovarian ultrasound examination was performed for presence of corpus luteum (CL), and body condition scoring (BCS) was made (scale from 1-emaciated to 5-obese). Cows with a CL (n=92) were submitted to two synchronization protocols and grouped accordingly: SPG (n=46) synchronized with a single PGF<sub>2α</sub> injection, and SSG (n=46) synchronized with a Short Synch protocol (PGF<sub>2α</sub>-24h-PGF<sub>2α</sub>-32h-GnRH-16h-TAI). Cows were followed up to the second insemination. Pregnancy diagnosis was done at d30 and d60 after each insemination. Eleven cows (n=11) from the SPG didn't show estrus signs and were excluded from further analysis. Overall, a higher FR (p=0.0008) was observed in the SSG (71.74%, 33/46) compared to the SPG (34.29%, 12/35). Cows in the SSG with BCS>2.75 (76.19%, 16/21) had a higher FR (p=0.0016) than cows in the SPG with BCS>2.75. No differences were observed (p=0.1242) between both groups that had BCS≤2.75 (68.00%, 17/25; 43.75%, 7/16, respectively). Multiparous cows in the SSG had a higher FR (p=0.0021, 71.43%, 25/35) than their counterparts from the SPG (30.43%, 7/23). No significant differences were observed among the primiparous cows. The results indicate that Short Synch protocol might increase the fertility rate in dairy farms.

**Key words:** BCS, cows, fertility rate, Short Synch

**INTRODUCTION**

Poor estrus detection is one of the most important limiting factors for increased reproductive performance in dairy cows. The traditional method of estrus detection is through observation of dairy cows. However, the latter is time-consuming, especially in herds with large numbers of animals. Therefore, this method becomes inappropriate in herds where the number

of cows per worker increases, resulting in a reduced occurrence and poor detection of estrus. Several studies in the last decade show that the percentage of detected animals in estrus is less than 50% (1, 2), which contributes to a prolonged inter-calving period. In addition, several factors (increased milk production, unbalanced feed, stress, and poor welfare) negatively affect the expression of behavioral estrous signs (3). As a solution to this problem, additional tools are used, such as dyeing the tip of the tail or placing pedometers for animal activity monitoring. However, there is still limited guarantee that every cow in estrus will be detected and inseminated on time. Hence, several synchronization protocols have been implemented by using synthetic hormone analogs (4).

In the last 25 years, reproductive management protocols have been developed that allow estrus synchronization, using luteolytic substances such as prostaglandin F<sub>2α</sub> (PGF<sub>2α</sub>) or its synthetic

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analogs (5). The method of estrus synchronization with a single application of  $\text{PGF}_{2\alpha}$  is the most used method on dairy farms in the R.N. Macedonia because it is quite fast and simple. Nevertheless, this management tool does not determine the exact time to perform the artificial insemination, considering that the detection of estrus is still necessary. Therefore, in order to reduce the problems and limitations arising from estrus detection, ovulation synchronization protocols have been developed, allowing insemination in a timely manner without the necessity of estrus detection. More than 12% and 21% of the farmers are using these protocols for the first insemination or resynchronization, respectively, in cows that were not pregnant (4). Although, the detection of estrus is eliminated in these protocols, 10–30% of the cows have asynchronized ovulation (6, 7). There are at least two key factors that negatively affect the effectiveness of these protocols: the inability of the first GnRH injection (G1) to eliminate the current wave and initiate a new wave, and the inability of a single  $\text{PGF}_{2\alpha}$  to cause a complete luteal regression. The first limitation could be eliminated if the protocol is initiated between d5 to d9 of the estrous cycle while the incomplete luteal regression could be reduced if a second application of the  $\text{PGF}_{2\alpha}$  is added 24 hours after the first dose. Therefore, a new modified protocol (Short Synch) has been implemented in this study, that would eliminate the first G1 injection and increase the complete luteal regression by adding a second dose of  $\text{PGF}_{2\alpha}$  24 hours after the first, with a fixed AI without the need for estrus detection.

We hypothesized that cows synchronized with the Short Synch protocol would have a higher fertility rate than cows inseminated at estrus. Therefore, the aim of the study was to compare the fertility rate between cows inseminated with the Short Synch protocol and cows inseminated at detected estrus.

## MATERIAL AND METHODS

The study procedures were compliant with the national Law for the Protection and Welfare of Animals (Off. Gazette of RM, 149/2014), which is harmonized with the Directive of the European Council on the protection of animals used for scientific purposes (2010/63/EU). A total of 138 (n=138) primiparous and multiparous dairy cows from several dairy farms in the Republic of North Macedonia were included in the study. In all farms, the cows were housed on a tied system, were fed twice daily, had unlimited access to water, and

were milked twice a day at a time interval of 12 hours with an average production of 25 liters/day. At day  $65 \pm 3$  after calving, all cows were scored for body condition (BCS, 1-emaciated, 5-obese; 8) and examined by ultrasound (Mindray DP 50, Soma Technology Inc. USA), equipped with a 7.5 MHz linear trans-rectal probe for the presence of CL. Only cows with detectable CL on either ovary (n=92) were included in the study. Cows without detectable CL (n=46) were excluded from further analysis. Cows were randomly allocated into two groups: Short Synch, (SSG group, n=46) and a single  $\text{PGF}_{2\alpha}$  group (SPG, n=46). Cows in the SSG were synchronized with two  $\text{PGF}_{2\alpha}$  injections 24 hours apart, and a single GnRH application 32 hours after the second  $\text{PGF}_{2\alpha}$  injection. They were inseminated in a timely manner, 16 hours after the GnRH injection without estrus detection ( $\text{PGF}_{2\alpha}$ -24h- $\text{PGF}_{2\alpha}$ -32h-GnRH-16h-TAI). In contrast, cows in the SPG group were synchronized by a single injection of  $\text{PGF}_{2\alpha}$  and inseminated when estrus was detected. Eleven cows (n=11) cows from the SPG group did not show signs of estrus and were therefore excluded from further analysis, resulting in 35 cows (n=35) in the SPG group. All cows were followed up to the second insemination. Cows that did not conceive immediately after the first examination were subjected to the same synchronization method and were re-inseminated. The pregnancy diagnosis was done on d30 after each insemination by ultrasound. Confirmation of pregnancy diagnosis was done at d60 after the last insemination. Cows that were diagnosed pregnant at d30 but were not confirmed at d60, were classified as cows that experienced late embryonic death.

The fertility rate and embryonic death was monitored according to the number of inseminations, BCS ( $\text{BCS} > 2.75$  or  $\leq 2.75$ ), and BCS + parity (primiparous or multiparous).

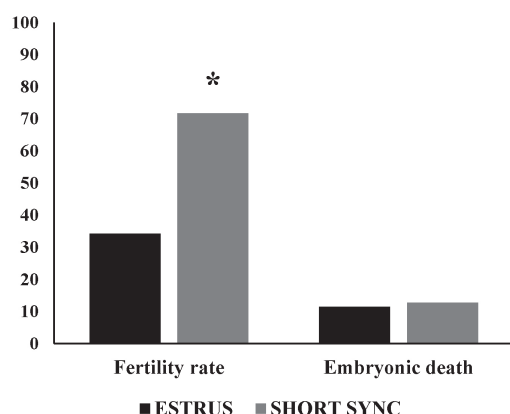
### Statistical analysis

Data were analyzed using the Fisher Exact test (R v 4.2.1). Post hoc power analysis was performed using G\*Power (v 3.1.9.2 Kiel University, Germany), with an effective size of 0.3, an alpha value of 0.05, and a degree of freedom of 1. Results were expressed as number (n) and percentage (%).

## RESULTS

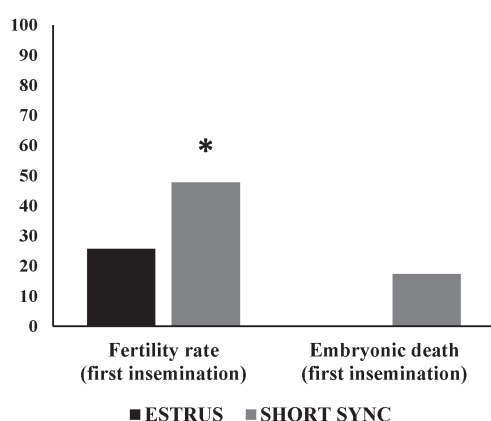
Overall, cows in the SSG had a significantly higher fertility rate (71.74%, 33/46) compared with the cows in the SPG group inseminated after estrus

detection (34.29, 12/35). No significant differences were found between the groups regarding embryonic mortality, although cows in the SSG group had non-significantly higher percentage of embryonic mortality (13.04%, 6/46) than cows in the SPG group (11.43%, 4/35, Fig. 1).



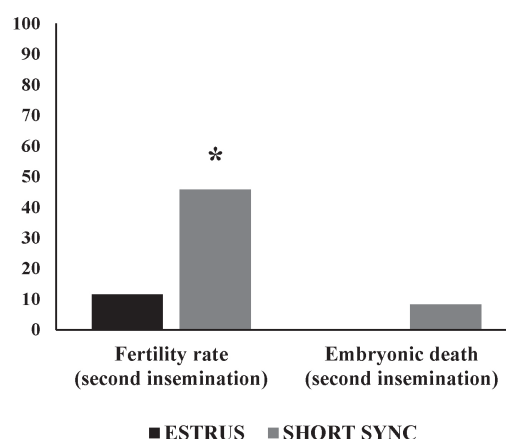
**Figure 1.** Fertility rate and embryonic mortality in both groups. The cows were either submitted to a Short Synch protocol and inseminated in a timely manner or to a single PGF<sub>2α</sub> injection and inseminated after estrus detection

At first insemination, cows in the SSG had a significantly higher fertility rate (47.83%, 22/46) compared to the SPG group (25.71%, 9/35). No embryonic death was recorded in the SPG group, whereas the SSG had 18.18% (4/22, Fig. 2).



**Figure 2.** Fertility rate and embryonic mortality at first insemination in both groups of cows. The cows were either submitted to a Short Synch protocol and inseminated in a timely manner or to a single PGF<sub>2α</sub> injection and inseminated after estrus detection

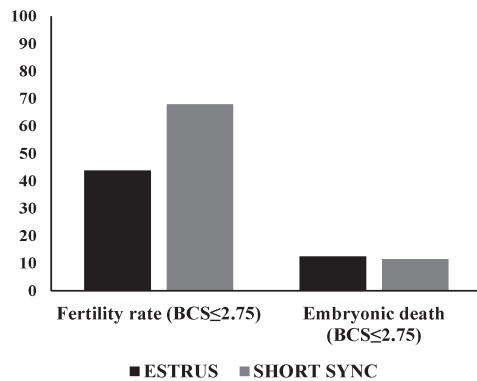
A significantly higher fertility rate was recorded in cows from the SSG group (45.83%, 11/24) compared to the cows from the SPG group (11.54%, 3/26). No significant differences were observed in embryonic mortality between both groups, however, cows in the SPG had a numerically higher percentage (15.38%, 4/26) compared to the cows from the SSG group (8.33%, 2/24, Fig. 3).



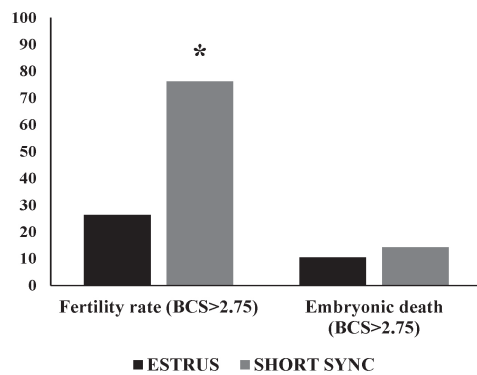
**Figure 3.** Fertility rate and embryonic mortality from second insemination in both groups of cows. The cows were either submitted to a Short Synch protocol and inseminated in a timely manner or to a single PGF<sub>2α</sub> injection and inseminated after estrus detection

Cows in the SSG group which had BCS $\leq$ 2.75 and  $>$ 2.75 had a significantly higher fertility rate (68.00%, 17/25 and 76.19%, 16/21, respectively) compared with the cows from the SPG group (43.75%, 7/16 and 26.32%, 5/19, respectively). SSG group (BSC $<$ 2.75) had non-significantly lower embryonic mortality (11.54%, 3/26) compared to the SPG group (12.50%, 2/16, Fig. 4). In contrast, cows from the SSG group (BSC $>$ 2.75) had non-significantly higher percentage of embryonic mortality (14.29%, 3/21) compared to the SPG group (10.53%, 2/19, Fig. 5).

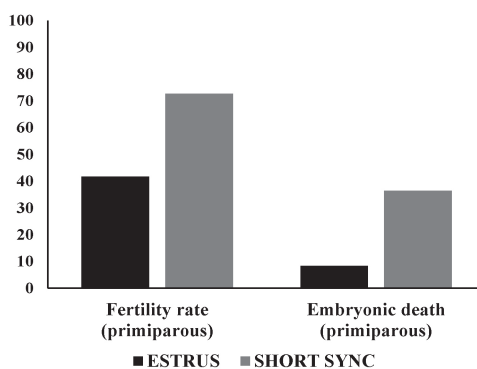
When parity was included in the analysis, primiparous cows from the SSG group had a non-significantly higher fertility rate (72.73%, 8/11) compared to the primiparous cows from the SPG group (41.67%, 5/12). In addition, the embryonic mortality in primiparous cows was non-significantly higher in the SSG group (36.36%, 4/11) compared to primiparous cows from the SPG group (8.33%, 1/12, Fig. 6).



**Figure 4.** Fertility rate and embryonic mortality in cows with body condition scores  $\leq 2.75$ . The cows were either submitted to a Short Synch protocol and inseminated in a timely manner or to a single PGF<sub>2α</sub> injection and inseminated after estrus detection

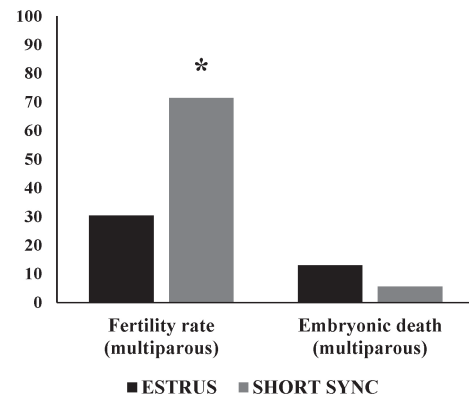


**Figure 5.** Fertility rate and embryonic mortality in cows with body condition scores  $> 2.75$ . The cows were either submitted to a Short Synch protocol and inseminated in a timely manner or to a single PGF<sub>2α</sub> injection and inseminated after estrus detection



**Figure 6.** Fertility rate and embryonic mortality in primiparous cows. The cows were either submitted to a Short Synch protocol and inseminated in a timely manner or to a single PGF<sub>2α</sub> injection and inseminated after estrus detection

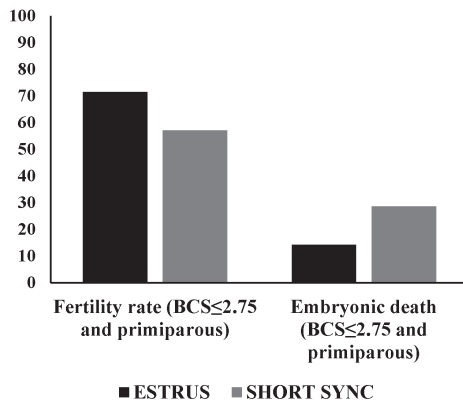
Multiparous cows from the SSG group had significantly higher fertility rate (71.43%, 25/35) than cows from the SPG (30.43%, 7/23). Multiparous cows from the SSG group had non-significantly lower percentage of embryonic mortality (5.56%, 2/36) compared to the cows from the SPG group (13.04%, 3/23, Fig. 7).



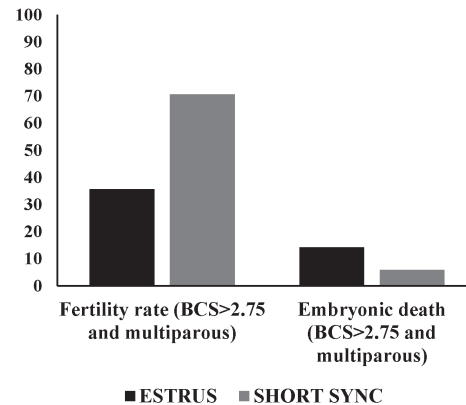
**Figure 7.** Fertility rate and embryonic mortality in multiparous cows. The cows were either submitted to a Short Synch protocol and inseminated in a timely manner or to a single PGF<sub>2α</sub> injection and inseminated after estrus detection

Primiparous cows from the SPG with  $BCS \leq 2.75$  had non-significantly higher fertility rate (71.43%, 5/7) compared to the SSG group (57.14%, 4/7). The embryonic mortality was non-significantly higher in the SSG group (28.57%, 2/7) compared to the SPG group (14.29%, 1/7, Fig. 8). In contrast, multiparous cows with  $BSC \leq 2.75$  from SSG group had a significantly higher fertility rate (72.22%, 13/18) compared to cows from the SPG group (22.22%, 2/9). Cows from the SPG group had a non-significantly higher percentage of embryonic mortality (11.11%, 1/9) compared to cows from the SSG group (5.26%, 1/19, Fig. 9).

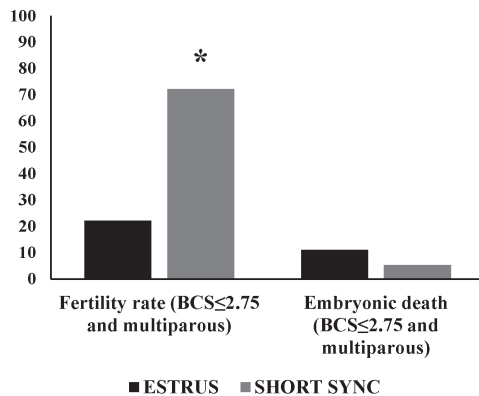
Primiparous cows from the SSG group with  $BSC > 2.75$  were all pregnant (100%, 4/4) whereas none of cows in the SPG group were diagnosed as pregnant (0.00%, 0/5). Consequently, no embryonic mortality was observed in SPG (0.00%), while the SSG group had 50% (2/4, Fig. 10). Multiparous cows from the SSG group having  $BSC > 2.75$  had a non-significantly higher fertility rate (70.59%, 12/17) than the cows from the SPG group (35.71%, 5/14). The SPG group had a non-significantly higher embryonic mortality (14.29%, 2/14) in comparison to the cows from the SSG group (5.88%, 1/17, Fig. 11).



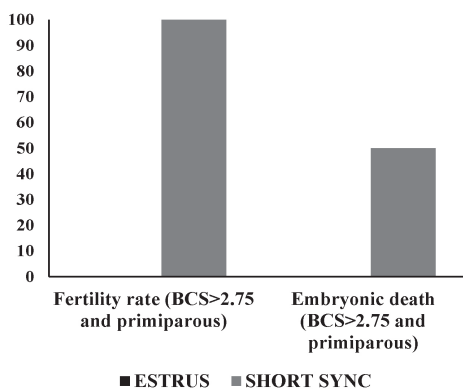
**Figure 8.** Fertility rate and embryonic mortality in primiparous cows with body condition scores  $\leq 2.75$ . The cows were either submitted to a Short Synch protocol and inseminated in a timely manner or to a single  $\text{PGF}_{2\alpha}$  injection and inseminated after estrus detection



**Figure 11.** Fertility rate and embryonic mortality in multiparous cows with body condition scores  $> 2.75$ . The cows were either submitted to a Short Synch protocol and inseminated in a timely manner or to a single  $\text{PGF}_{2\alpha}$  injection and inseminated after estrus detection



**Figure 9.** Fertility rate and embryonic mortality in multiparous cows with body condition scores  $\leq 2.75$ . The cows were either submitted to a Short Synch protocol and inseminated in a timely manner or to a single  $\text{PGF}_{2\alpha}$  injection and inseminated after estrus detection



**Figure 10.** Fertility rate and embryonic mortality in primiparous cows with body condition scores  $> 2.75$ . The cows were either submitted to a Short Synch protocol and inseminated in a timely manner or to a single  $\text{PGF}_{2\alpha}$  injection and inseminated after estrus detection

## DISCUSSION

The aim of the study was to investigate whether cows inseminated in a timely manner by using shortened and modified Ovsynch protocol (Short Synch-SSG) might have a higher fertility rate in comparison with cows inseminated after estrus detection using a single dose of  $\text{PGF}_{2\alpha}$  (SPG). The results have shown that cows inseminated with a Short Synch protocol had a higher fertility rate than cows inseminated after estrus detection. It was assumed that the main reason for the lower fertility rate in the SPG group might be due to incomplete regression of CL-incomplete luteolysis. Atanasov et al. (9) have reported that 25% of cows had an incomplete luteolysis when submitted to an estrus synchronization protocol using a single  $\text{PGF}_{2\alpha}$  application. Incomplete luteal regression leads to a small elevation of progesterone near the insemination period that might block the ovulation and thus reduce fertility (10). The elevated progesterone reduces the magnitude of the GnRH-induced LH surge (4), lowers the motility of the oviducts and uterus affecting the transport of gametes, and increases the endometrium lining leading to compromised fertility. Elevated concentration of progesterone near insemination could be avoided if a second dose of  $\text{PGF}_{2\alpha}$  is applied 24 hours after the first dose, as performed in the current study (SSG group). Similar studies have reported that this approach increases the percentage of luteal regression from 80% to 98% (11, 12), and increases the fertility rate from



6 to 12% (11). These findings were concurring with the current study. Brusveen et al. (11) observed a 6% increase in conception rate at first insemination when a second dose of  $\text{PGF}_{2\alpha}$  was added during the Double OvSynch protocol. Similarly, the current study observed a higher fertility rate in the SSG group cows on the first insemination than the SPG group. Wiltbank et al. (10) reported that the application of a second dose of  $\text{PGF}_{2\alpha}$  induced complete luteolysis in 97% of cows, regardless of P4 concentration at the time of application of the first dose of  $\text{PGF}_{2\alpha}$ . Even a greater increase in pregnancy per AI was observed (12%) when a second dose of  $\text{PGF}_{2\alpha}$  was administered during resynchronization protocols (11).

The current study demonstrated that the cows from SSG group with  $\text{BCS} \leq 2.75$  and  $> 2.75$  had a higher fertility rate than the SPG group. The incomplete luteolysis and higher concentrations of progesterone at the time of artificial insemination could be considered again as an explanation. However, there is no data in the scientific literature to this date that might explain the correlation between BCS and luteolysis. According to a recent study, cows with  $\text{BSC} > 2.75$  showed a higher percentage of luteal regression compared to cows with  $\text{BSC} \leq 2.75$  (9). It is assumed that in leaner cows, the ovulatory follicles are less functional and produce CLs that are less sensitive to the action of  $\text{PGF}_{2\alpha}$  and thus, have an incomplete luteal regression.

Regarding parity, the current study demonstrated significant differences in the fertility rate between multiparous cows from both groups, while no differences were observed in primiparous cows. Multiparous cows from the SSG group had a higher fertility rate compared to cows that were inseminated after estrus detection. Similarly, Wiltbank et al. (10), reported that a higher conception rate was observed in multiparous cows in which two doses of  $\text{PGF}_{2\alpha}$  were administered (45.0%, 45/100) compared to those that underwent the standard OvSynch protocol with a single dose of  $\text{PGF}_{2\alpha}$  (36.6%, 37/101). The latter might be as a result of the higher ovulatory risk in multiparous compared to primiparous cows (13). Multiparous cows had a higher ovulatory response than primiparous cows (38.1 vs 19.6%, respectively), and thus, might have an increased conception rate.

## CONCLUSION

Based on the results of the current study, it can be concluded that the Short Synch protocol could be implemented as a regular reproductive management tool that might increase fertility in dairy farms and

improve the reproductive performance of the cows. Nevertheless, in order to confirm the results of the present study a higher number of cows should be included.

## CONFLICT OF INTERESTS

The authors declare that they have no known conflict of interest in the conduction of the current study.

## ACKNOWLEDGMENTS

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## AUTHORS' CONTRIBUTION

EM, BA and TD planed the study design. BA and TD performed the ultrasonographic examination of the cows. IE and KI performed the proofreading. MN, ST and LjM had run the statistical analysis. DZ, KI and EM performed the hormone injection.

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