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Progesterone concentration in milk and blood serum and reproductive efficiency of cows after Ovsynch treatment

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Abstract: An experiment was conducted to investigate the effects of hormonal synchronization of ovulation on progesterone concentrations in milk and blood serum and on reproductive performance. Sixty Holstein–Friesian cows averaging 8000 L over 305 days of lactation were divided into 2 groups of 30 animals. One-factor ANOVA and a t-test of progesterone concentrations in venous serum and milk revealed that the lowest concentrations of progesterone in milk (Ovsynch 5.8 ± 1.0 ng/mL; control 4.5 ± 0.6 ng/mL) and blood serum (Ovsynch 1.3 ± 0.9 ng/mL; control 1.4 ± 0.5 ng/mL) in both groups were found on the day of estrus or artificial insemination (AI) (both $P < 0.05$). Increases in the concentrations of progesterone in milk (Ovsynch 17.6 ± 4.3 ng/mL; control 10.5 ± 1.9 ng/mL) and blood serum (Ovsynch 3.6 ± 1.1 ng/mL; control 4.0 ± 1.0 ng/mL) were observed on day 7 after AI (both $P < 0.05$). Concentrations of progesterone in milk and blood serum in nonpregnant cows were reduced on day 21 after AI. Assessment of reproductive performance revealed that the application of treatment shortened the duration of the service period (Ovsynch 76 days; control 83 days) and the calving interval (Ovsynch 376 days; control 382 days).

Key words: Ovsynch, synchronization of estrus, dairy cows, reproductive parameters

1. Introduction

High producing dairy cows used in modern dairy herds produce between 8000 and over 10,000 L of milk per cow annually. However, the energy demands for high milk production and the various stressors to which the animals are exposed during intensive production conditions are directly related to a decline in the reproductive efficiency of the cows. It is recognized that reproductive disorders are indirect indicators of the adverse conditions in which the animals live (1,2).

In Serbia, as in other countries, there is a significant problem of reduced fertility in high-dairy Holstein–Friesian cow herds. The reduced fertility is manifested as a prolonged interval from calving to the first estrus, which results in an increased frequency of repeat breeding and, consequently, an increase in the required inseminations for successful conception. All this results in a significant extension of the service period and the calving interval duration, thus reducing the efficiency of milk and calf production (3–5). Using appropriate biotechnological methods to control reproductive function, it is possible to increase the level of reproductive efficiency of dairy cows (5). One of the most common biotechnological methods for control of ovarian activity and establishment of an estrous cycle postpartum is the use of natural or synthetic hormonal preparations. The success of their applications, measured by the degree of estrous activity and the values of conception rates after insemination in induced estrus, depends on numerous factors such as: the interval from the moment of partus in which the treatment is made, the status of the functional activity of the ovaries, reproductive and general health conditions of the cows, level of milk production in the previous and current lactations, season and year, microenvironmental climate parameters, technology of accommodation and milking, and exposure to a variety of other stressors (5,6). The most commonly used hormonal therapies on dairy farms are the Ovsynch protocols for synchronization of estrus and ovulation, and these are based on the use of gonadotropin-releasing hormone (GnRH) in combination with prostaglandins. The first results (7) of the application of the so-called Prostaglandin–Gonadotropin–Prostaglandin program

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were presented. The essence of the protocol is that the initial dose of GnRH causes luteinization or dominant follicle atresia. At that moment a new wave of follicular growth, follicle recruitment, and the concurrent creation of a corpus luteum begin, thus further enhancing follicle function. Seven to 8 days later a single prostaglandin dose is injected, which causes luteolysis when the dominant follicle is estrogen active, and rapidly finalizes the growth of the preovulatory follicle. A second injection of GnRH is injected 48 h after the prostaglandin injection in order to ensure ovulation; insemination should then be done during the next 16 to 20 h. While the Ovsynch treatment is common in many areas of the world, it has only recently been implemented on Serbian dairy farms. Consequently, the objective of this research was to evaluate the effects of Ovsynch treatment on the reproductive performance of dairy cows raised under Serbian production conditions.

2. Materials and methods

The research was carried out on a farm of Holstein–Friesian dairy cows with a tied-stall system. Sixty cows were chosen and divided into two groups of 30 animals each (group A was Ovsynch treated and group B was the control group). The cattle were equal in age, parity, and level of milk production as measured in the previous lactation period. Another selection condition of cows was that they had to be in optimal physical condition, with a healthy and functional mammary gland and no signs of functional disorders of the locomotor apparatus. In the selected animals there was no history of postpartum disorders of the reproductive system (retentio secundarium, endometritis, or mastitis), and partus was at least 40 and at most 90 days. The animals from group A were acyclic (anestric) with dysfunctional ovaries or had follicular cysts until the moment of treatment. The cows from this group were treated with the Ovsynch method (7) on average on day 61 after calving, according to the regime described in Table 1.

The cows in group B were not treated with hormone preparations. They were inseminated on average on day 57 after calving, after spontaneous estrus, which was recorded visually and by rectal examination. Artificial insemination (AI) of the cows in both groups was carried out by veterinary technicians and in accordance with regular procedures at the farm. Cows were monitored several times a day in two shifts (in the morning and afternoon). In cows that showed external signs of estrus a rectal examination was performed; those with apparent estral mucus and a follicle in one of the ovaries were inseminated. The health and reproductive status of the cows (including all reproductive parameters and body condition score) were monitored and recorded by veterinary specialists in cow reproduction. The concentration of progesterone in the milk and blood serum in group A was monitored during the experiment. Blood samples were taken from the jugular vein on days 0 (the day of the first GnRH injection), 7, 9, and 10 (day 10 is when the AI was done), and on days 7, 14, 21, and 35 post-AI. In group B, milk and blood samples were taken on the day of AI (day 10), and on days 7, 14, 21, and 35 post-AI. Analyses of progesterone concentrations were performed using commercial kits for analysis of milk (OvuCheck Milk test, Biovet Inc., Canada) and blood (progesterone ELISA kits, Immunolab GmbH, Germany). Data analysis was performed using standard methods of descriptive statistics; one-factor ANOVA and t-test (STATISTICA 8.0 Software, StatSoft, Inc.) were used to determine the significance of differences in the average values.

3. Results

The concentrations of progesterone (ng/mL) measured in the milk and blood of cows treated with the Ovsynch method and those in the control group are presented in Tables 2 and 3. Results revealed that the milk progesterone concentrations in samples collected on the day of AI and on the 7th day post-AI were significantly higher ($P < 0.01$) in the cows treated with the Ovsynch method than in the control cows, but did not differ ($P > 0.05$) on the other

Table 1. Chart of Ovsynch protocol by days of treatment.

Days	Treatment	Preparation and dose	Result of the treatment
0	I/M injection of GnRH	Receptal (Intervet (UK) Ltd.), 2 mL	Ovulation of follicle and the beginning of follicle growth.
7	I/M injection of PGF2α	Dinolytic (Dinolytic, Pfizer), 5 mL	Regression of the corpus luteum.
9	I/M injection of GnRH	Receptal (Intervet (UK) Ltd.), 2 mL	Ovulation of follicle, about 26 to 32 h after the second injection of GnRH.
10	Term of AI 12 to 16 h after the second injection of GnRH (without revealing signs of estrus, so-called fixed insemination)		High value of successful conception.

Table 2. Concentration of progesterone in milk of cows treated with the Ovsynch method and of cows in the control group.

Day	Pregnancy	Mean ± SD progesterone in milk (ng/mL)		Statistical significance (P)
		Ovsynch (A)	Control (B)	
0	+	9.6 ± 1.1	-	-
	–	6.2 ± 1.9	-	-
7	+	17.6 ± 4.3	-	-
	–	12.1 ± 3.2	-	-
9	+	5.6 ± 0.9	-	-
	–	6.1 ± 0.6	-	-
10 (day of AI)	+	5.8 ± 1.8	4.5 ± 0.6	**P < 0.01**
	–	5.8 ± 1.0	5.2 ± 1.5	**P < 0.01**
7 days post-AI	+	13.0 ± 1.2	10.5 ± 1.9	**P < 0.01**
	–	13.9 ± 8.8	8.6 ± 1.3	**P < 0.01**
14 days post-AI	+	18.0 ± 2.6	19.0 ± 3.8	P > 0.05
	–	19.6 ± 7.1	15.3 ± 2.4	P > 0.05
21 days post-AI	+	17.4 ± 1.4	19.4 ± 4.2	P > 0.05
	–	13.5 ± 7.2	9.6 ± 5.1	P > 0.05
35 days post-AI	+	15.9 ± 2.2	20.0 ± 1.7	P > 0.05
	–	21.2 ± 4.5	17.2 ± 1.7	P > 0.05

Pregnancy: + means pregnant; – means nonpregnant.
P: probability; highly significant differences at the level of $P < 0.01$ are given in bold; $P > 0.05$ was considered not significant.

days post-AI (Table 2). Conversely, the mean progesterone concentrations in the blood were significantly higher ($P < 0.01$) on days 14 and 21 post-AI in the Ovsynch-treated cows as compared with those in the control cows, but did not differ on the other days of comparison (Table 3).

4. Discussion

The reason for the higher concentrations of progesterone in milk as compared with those in blood is the liposolubility of the steroid hormone and its transition from blood to milk (8), as well as its de novo synthesis in the mammary gland (9). The results obtained in the present study are consistent with reports of lower progesterone concentrations in blood during the early luteal phase (2 to 3 days after estrus) than during the middle of the luteal phase (10,11). Moreover, the concentration of progesterone in blood was higher in the middle of the luteal phase as compared with that in the early and late luteal phase (12). Comparing both groups in terms of progesterone concentrations in milk showed that there is a significant difference only during the first 7 days after insemination ($P < 0.01$) between cows in the control and Ovsynch groups. From day 21 until day 35 post-AI, the concentration of progesterone in the milk of cows with interrupted pregnancy increased in both groups. The additional increase of progesterone values found on the 35th day post-AI (i.e. 14 days after repeat breeding) indicates the establishment of a new luteal phase of the estrous cycle that was not clinically manifested. The level of progesterone in milk reliably indicates prenatal mortality rather than an irregular cycle (13). On the other hand, our results show that the mean concentrations of progesterone in milk and blood serum of pregnant Ovsynch-treated cows increased until day 21 post-AI, while concentrations were slightly lower by day 35 post-AI. The mean values of progesterone concentrations from day 12 and until day 17 post-AI were higher in pregnant cows following insemination than in nonpregnant cows over the same period, with considerable variation among individual animals of both groups (14). In the period after day 14 of AI, the mean values of progesterone in the Ovsynch group were lower than those in the control group. The early luteal phase (5–12 days of estrus) is

Table 3. Concentration of progesterone in blood of cows treated with the Ovsynch method and of cows in the control group.

Day	Pregnancy	Mean ± SD progesterone in blood (ng/mL)		Statistical significance (P)
		Ovsynch (A)	Control (B)	
0	+	2.7± 1.5	-	-
	–	1.8± 1.0	-	-
7	+	5.3 ± 1.4	-	-
	–	4.9 ± 0.7	-	-
9	+	1.2 ± 0.3	-	-
	–	0.7 ± 0.4	-	-
10 (day of AI)	+	2.1 ± 1.3	1.5 ± 0.2	P > 0.05
	–	1.3 ± 0.9	1.4 ± 0.9	P > 0.05
7 days post-AI	+	3.6 ± 1.1	4.0 ± 1.0	P > 0.05
	–	2.7 ± 0.4	2.1 ± 0.5	P > 0.05
14 days post-AI	+	8.4 ± 1.1	4.0 ± 0.4	**P < 0.01**
	–	4.1 ± 0.7	3.2 ± 0.7	**P < 0.01**
21 days post-AI	+	13.6 ± 2.8	4.9 ± 1.0	**P < 0.01**
	–	4.5 ± 0.7	3.0 ± 3.50	**P < 0.01**
35 days post-AI	+	7.0 ± 1.1	5.1 ± 2.0	P > 0.05
	–	1.7 ± 0.3	2.7 ± 1.37	P > 0.05

Pregnancy: + means pregnant; – means nonpregnant.
P: probability; highly significant differences at P < 0.01 are given in bold; P > 0.05 was considered not significant.

the optimal period for starting Ovsynch treatment (15). Moreover, lower values of progesterone concentrations in milk and blood were found by day 17 post-AI in cows with failed conception as compared with those values in pregnant cows (13). A strong negative correlation between progesterone concentrations in milk and embryo survival was previously reported (16). It was recommended (17) that the optimal values of progesterone concentrations in milk for embryo survival on days 5, 6, and 7 were 7.4, 13.2, and 16.8 ng/mL, respectively. Milk progesterone concentrations in pregnant and nonpregnant cows in the present study on day 7 post-AI in the Ovsynch group were 13.0 and 13.9 ng/mL, respectively, and in the control cows they were 10.5 and 8.6 ng/mL, respectively (Table 2).

A comparison of the reproductive efficiency recorded on the farm revealed differences in calving intervals between the two groups of cows (Ovsynch : control = 375.7 : 382 days); yet, these were shorter in duration than the calving intervals recorded for cows reared under production conditions in the tested farm (443.8 days). In order to shorten the interval between calving, numerous authors have recommended that inseminations of cows begin in the period from 45 and 50 days after calving as this provides opportunity for reinsemination and implementation of intensive programs for estrus detection to support successful insemination (18–21). Considering the values obtained for the insemination index in the experimental group and the other cows reared on the farm, it can be noted that they are above the optimal values reported by many authors (1.5 and 1.7). Early secretion of prostaglandin F2α (PGF2α) and early regression of the corpus luteal were reported to be responsible for low levels of conception (22–24). In the present experiment, the lowest insemination index was recorded in the control group and the cows from the Ovsynch group (2.1 and 2.0, respectively). The insemination index of the other animals (not included in our study) on the tested farm was 3.0.

The values obtained for the duration of the service period in the groups of experimental cows are in agreement with those reported by the majority of authors, who state that the optimal service period of cows is less than 90 days and on average would range from 85 to 110 days. The service period of cows treated with the Ovsynch method (76 days) was shorter than the service period of those in the control group (83 days). Additionally, the values obtained for the duration of the service period in the cows of our study groups were lower than those in the production cows on the tested farm (179 days). The obtained values concerning the reproductive parameters of untreated cows are in agreement with data obtained in previous investigations on Serbian farms (3). It was confirmed (25) that for insemination after calving, the third estrus postpartum (between 40 and 50 days) is most commonly used, and the latest period is between 75 to 90 days; that way cows can give birth every year.

These facts clearly show that the application of the studied hormonal treatments can greatly increase the reproductive efficiency of cows, and thus increase milk production and the number of calves per cow annually. These results may lead to a better understanding of the physiology of establishing postpartum estrous cycles and the definition of effective control technologies and improvements in the reproduction of cows in high milk yield herds.

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