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INDUCTION AND SYNCHRONIZATION OF ESTRUS IN DAIRY COWS USING A SINGLE INJECTION OF PGF2α AND GnRH

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The aim of this study was to examine the effects of treatment with a single injection of GnRH and PGF2α on estrous response, fertility and service period. A total of 120 lactating Simmental cows were divided into four groups of 30 cows each: group PGF2α 40 was treated on the 40th day post partum with a single injection of 2 mL prostaglandin analogue (Estrumate), group PGF2α 50 was treated in the same way on the 50th day, group GnRH was treated on the 40th day post partum with a single injection of 2 mL GnRH analogue (Receptal), and the fourth group (control) was not hormonally treated. Fertility of cows was not significantly different ($p>0.05$). The difference in the estrous response and service-period between the control group and experimental groups was statistically significant ($p<0.01$).

Key words: cows, fertility, GnRH, induction, PGF2α,

INTRODUCTION

There is a growing trend towards decreased reproductive efficiency in dairy cattle, especially high-yielding dairy cows. Intensive selection for milk production has had a negative effect on the reproductive performance, mainly due to clinical problems in the postpartum period, poor expression of external estrous signs, and defective oocytes and embryos (Nakada, 2006; Dobson *et al*., 2007). Also, a negative energy balance in early postpartum (Diskin *et al*., 2003), organizational failure to detect estrus in a timely manner (Mayne *et al*., 2002; Groehn and Rajala-Schultz, 2000), and an inadequate insemination technique (García-Ispierto *et al*., 2007) can lead to unsatisfactory reproductive performance on dairy farms.

In order to improve the reproductive efficiency of dairy cows, a number of different hormonal protocols are used to systematically affect their physiological and reproductive processes. Of greatest importance in commercial production is the induction of synchronized estrus in the postpartum period, because it helps to establish a synchronized ovarian function. For this purpose, one can use luteolitics, primarily prostaglandin F2α (PGF2α) or its analogues in combination

with gonadotropin-releasing hormone (GnRH) or its analogues according to a specified schedule of application of each hormone. In this way, luteal regression is induced in a targeted manner by means of prostaglandins (Lauderdale *et al*., 1974) and ovulation of the dominant follicle is induced by using GnRH (Britt *et al*., 1974).

The dairy sector in Bosnia and Herzegovina is largely dominated by milk producers who raise dual purpose Simmental cattle. Although the production is mainly focused on milk production, the profits earned from the sale of calves significantly affect the total cost of production. In this sense, the service period or intercalving interval is not only a reproductive, but also a very important production parameter that directly affects the economic results of dairy farms. Although improved reproductive performance is known to simultaneously improve the entire production, milk producers are relatively unlikely to use conventional hormonal treatments to induce and synchronize estrus, mainly due to insufficient budgets. Taking into account the above facts, our research was based on the hypothesis that a treatment with only one injection of PGF2α or GnRH analogues in the final stage of puerperium will result in better reproductive performance, especially estrous response. At the same time, induction and synchronization will be less labor intensive and more financially acceptable for the average milk producer, compared to conventional hormonal protocols.

The aim of this study was to examine the effect of different methods of estrous induction and synchronization on estrous response, pregnancy rate and duration of service period in lactating Simmental cows.

MATERIALS AND METHODS

The research was conducted on a dairy farm in the vicinity of Bihac in Bosnia and Herzegovina. A total of 120 Simmental cows aged 3 to 6 years, with average annual milk production of 7 000 kg, were used in this research. All cows were kept in the free stall system with seasonal access to pasture in the period from May to September.

The cows were divided into four equal groups of 30 animals each: in the PGF2α 40 group were cows treated with a single injection of 2 mL i.m. PGF2α analogue (cloprostenol sodium, Estrumate, Schering-Plough) on the 40th day postpartum, in the PGF2α 50 group were cows treated in the same way on the 50th day postpartum, in the GnRG group were cows treated with 2 mL i.m. of GnRH analogue (buserelin acetate, Receptal, Intervet) on the 40th day postpartum, and in the control group were cows that were not hormonally treated. All cows were kept under the same nutrition, nursing and health care conditions.

A complete gynecological examination was performed before hormonal treatment in order to determine the involutional processes of the uterus and ovarian functional activity. All treated cows were in the luteal phase of the estrous cycle. Estrous detection was carried out by observation for external signs of estrus.

Insemination was performed by usual bimanual method after expression of external signs of estrus. Insemination of cows in the control group was performed

at the first spontaneous estrus which appeared after the 40th day postpartum. All cows were inseminated with semen from the same bull. Diagnosis of pregnancy was performed by rectal palpation 11–12 weeks after insemination.

Statistical analysis of the obtained data was performed using standard methods of descriptive analysis. Chi-square test was used to compare the value of estrous response and pregnancy rate, and Student's t-test to compare the values of the interval treatment – estrous response and service period between the groups (Petrie and Watson, 2006). The significance of the difference was based on the possibility $p<0.05$, unless specified otherwise.

RESULTS

The distribution of estrous response by days after hormonal treatment and the average duration of the interval from treatment to estrus response are shown in Table 1.

Table 1. Distribution of estrous response and average duration of the interval from treatment to estrus

Parameters	Groups							
Days of estrous response	$PGF_{2\alpha}$ 40		$PGF_{2\alpha}$ 50		GnRH		Control	
	n	%	n	%	n	%	n	%
1-3	10	33.34	12	40.00	11	36.67	–	–
4-6	12	40.00	101	33.34	8	26.67	5	16.66
7-9	1	3.33	11	3.33	7	23.33	4	13.33
≥ 10	1	3.34	3	10.00	2	6.67	10	33.33
Total	24	80.00	26	86.67	28	93.34	19	63.34
Interval treatment – estrous response (mean±stand. error)	3.92 ± 0.49^{b}		4.69 ± 0.68^{b}		5.14 ± 0.76^{b}		11.26 ± 1.19^{a}	

^{ab}Means without a common superscript within row are significantly different ($p<0.01$)

In the PGF2α 40 group 80.00% (24/30) of the treated cows reacted. The cows were most likely to exhibit estrus between the 4th and the 6th day after treatment, when 40.00% (12/30) of the cows reacted, while 6.67% (2/30) reacted after 7 days or more. Estrous response in the PGF2α 50 group was registered in 86.67% of treated cows (26/30). In the first three days after treatment 40.00% (12/30) of the cows entered into estrus, while 3.33% (1/30) reacted between the 7th and the 9th day, and 10.00% (3/30) after 10 days. Treatment with GnRH resulted in estrous response in 93.34% (28/30) of the treated cows. The largest number of cows 36.67% (11/30) reacted in the first three days, while only 6.67% (2/30) of the cows reacted after the 10th day. In the control group a total of 63.34% (19/30) of the cows reacted. The greatest estrous response occurred after the 10th day, when

33.33% of cows (10/30) reacted, while not a single cow reacted in the first three days. The durations of treatment – estrous response interval were 3.92±0.49, 4.69±0.68, 5.14±0.76 and 11.26±1.19, respectively. A statistically high significant difference was found between experimental and control groups ($p<0.01$). The pregnancy rate and insemination index are shown in Table 2.

Table 2. Pregnancy rate and insemination index

Groups	Parameters						
	Number of inseminated cows		Pregnancy rate at first insemination		Total pregnancy rate		Insemination index
	n	%	n	%	n	%	
$PGF_{2\alpha}$ 40	23	95.83	16	69.56	18	78.26	1.12
$PGF_{2\alpha}$ 50	24	92.30	18	75.00	20	83.33	1.11
GnRH	26	92.86	17	65.38	19	73.08	1.12
Control	16	84.21	7	43.75	13	81.25	1.86

[a, b]Means without a common superscript within column are significantly different ($p<0.05$)

The pregnancy rate of cows in PGF2α 40 at first insemination was 69.56% (16/23), and the total pregnancy rate was 78.26% (18/23). In the PGF2α 50 group the pregnancy rate at the first insemination was 75.00% (18/24), and the total pregnancy rate was 83.33% (20/24). Cows in the GnRH group achieved a pregnancy rate of 65.38% (17/26) at first insemination and a total pregnancy rate of 73.08% (19/26). In the control group the pregnancy rate achieved at the first insemination was 43.75% (7/16) and total pregnancy rate was 81.25% (13/16). No statistically significant ($p>0.05$) difference was observed in pregnancy rates at first insemination and total pregnancy rates between the groups. Insemination indices by groups were 1.12, 1.11, 1.12 and 1.86, respectively. The duration of service period (in days) is shown in Table 3.

Table 3. Duration of service period

Groups	Mean ± standard error	Minimum	Maximum	CV %
$PGF_{2\alpha}$ 40	52.54 ± 0.66 [A a]	45	62	6.17
$PGF_{2\alpha}$ 50	54.69 ± 0.73 [A b]	50	64	6.84
GnRH	60.46 ± 1.03 [B]	52	69	9.04
Control	68.32 ± 1.46 [C]	57	78	9.34

[A, B]Means without a common superscript within column are significantly different ($p<0.01$)
[a, b]Means without a common superscript within column are significantly different ($p<0.05$)

The service period of cows in the PGF2α 40 group varied from 45 to 62 days, with an average value of 52.54±0.66 days; in the PGF2α 50 group from 50 to 64

days, with an average value of 54.69±0.73 days; in the GnRH group from 52 to 69 days, with an average value of 60.46±03.01 days, and in the control group from 57 to 78 days, with an average value of 68.32±1.46 days. The duration of the service period of cows in all experimental groups (40 PGF2α, PGF2α 50, GnRH) was statistically different from cows in the control group ($p<0.01$). A statistically significant difference ($p<0.05$) was found between groups PGF2α 40 and PGF2α 50, while a statistically highly significant difference ($p<0.01$) was observed between PGF2α 40 and GnRH, PGF2α 50 and GnRH.

DISCUSSION

The possibility that cows receive a single application of prostaglandins is dependent on the confirmation of the existence of *corpus luteum*. If the application of PGF2α was performed during diestrus, luteolysis and estrus could be expected to occur 2–7 days after application. Treating cows in this way, Seguin (1980) reported that 34% of the cows entered into estrus on the third day and 32% on the fourth day. However, 2% of the cows came into estrus on the first day, 8% on the second day, 17% on the fifth day, 3% on the sixth day, and 4% on the seventh day. In our study, the estrous response in the PGF2α 40 group was 80.00% (24/30) and in group PGF2α 50 86.67% (26/30), where 73.34% (22/30) of cows in both groups entered into estrus within the first 6 days. These results were expected because PGF2α regresses the *corpus luteum* and breaks the negative feedback of progesterone, and brings the cows into a new estrous cycle. It is not surprising that some of the cows treated with PGF2α failed to respond, given the difficulties in distinguishing ovarian structures by rectal palpation in the period from the 5th to the 7th day relative to the period of extremely well-developed *corpus luteum*. Our results of estrous response are better than those of Amer (2008), who reported that 68.3% of Holstein cows exhibited estrus after the first treatment with prostaglandin and 71.7% after the second treatment. Positive experiences with the aim of inducing luteolysis after PGF2α application are reported by Elmarimi *et al*. (1983), who significantly shortened the treatment–estrous response interval in Holstein and Jersey cows. In our study, treatment with GnRH resulted with estrous response of 93.34% (28/30). These results are in agreement with those of Benmrad and Stevenson (1986), who, by applying either PGF2α or GnRH only, increased the estrous response and shortened the treatment–estrus interval in Holstein cows with normal and abnormal puerperium.

In reproduction management it is very important to establish a reliable system for timely detection of estrus, because otherwise extension of the service period may occur (Opsomer *et al*., 1996). The analysis of reproductive records of a large number of dairy farms showed that the percentage of detected estrus ranged from 48.3% (Kinsel and Etherington, 1998) to 71.0% (Mayne *et al*., 2002). On the other hand, according to Rhodes *et al*. (2003), between 11 and 38% of cows in the first 50-60 days postpartum were anestrous, and the reasons for this state, as cited by researchers, include negative energy balance and the emergence of peripartum diseases. The estrous response of hormonally treated

cows in our study was high. The reason for this lies in the fact that the genetic potential of cows is not fully utilized, and that cows are not forced to maximum milk production. Hormonal treatment was performed at the optimal time, physiological conditions for the establishment of synchronized estrus were present. The cows were healthy, of low parity structure, and in breeding condition. Free stall system with seasonal access to pasture, combined with a standardized diet, further contributed to the results of estrous response of analyzed cows.

Based on the analysis of reproductive records of a large number of dairy farms, conception at first insemination ranged from 37.1% (Mayne *et al*., 2002) to 40.7% (Galon *et al*., 2010), which is certainly an unsatisfactory result. The pregnancy rate in our study was above 70%, which is considered satisfactory, but there was no statistically significant difference between control and experimental groups. The conception rate according to Amer (2008) after the first injection of prostaglandin was 56.3% and 50.0% after the second. Fallah and Ajami (2010) after two treatments with PGF2α achieved a pregnancy rate of 81.48%. Répási *et al*. (2005) reported a significantly higher pregnancy rate in cows that were treated twice with PGF2α. Benmrad and Stevenson (1986) reported that treatment only with PGF2α or GnRH improves the fertility of dairy cows, especially those with puerperal problems, while Stevenson and Call (1988) concluded that the treatments in early postpartum did not improve reproductive performance. In our study the treatment was carried out in the final stage of puerperium, which had a favorable effect on fertility results. Application of PGF2α can improve conception to a greater degree, but its application at an exactly defined time limits its use. However, it is not possible to give a clear recommendation for the use of PGF2α for routine control of ovulation, because only cows with a mature *corpus luteum* will respond to a single application of PGF2α. Looking at the average value of the insemination index of cows in the experimental groups, we can conclude that it was better than that of Cilek and Tekin (2005), who reported a value of 1.76 after the expression of spontaneous estrus. The results obtained in experimental groups are do to the fact that the reproductive service on the farm is well organized, and following detection of estrus artificial insemination is carried out promptly, using semen of known origin and good quality.

In adequate conditions of accommodation, nutrition and care, Simmental breed cows achieve optimal service period of 60-90 days. This includes the time required for complete involution of genital organs. According to the findings of Pantelic *et al*. (2008), the average duration of the service period was 115.19 days, while a duration of 153.82 days was reported by Petrovic (2007). Slightly shorter duration of 93.87 days was reported by Cilek and Tekin (2005) and 94 days by Prandi *et al*. (1994). Generally, the results obtained in our study were significantly better. Also, in all experimental groups the service period was significantly shorter ($p<0.01$) compared to the control group, which was one of the objectives of the study.

Estrous response, insemination index and duration of service period in our study were satisfactory. Examined hormonal treatments can be easily applied on small-scale farms. Hormone treatment causes a high estrous response, which facilitates the detection of estrus and timely insemination of cows. Also, we

achieved increased calving over a short period on an annual basis and a desired lactation period of 305 days. All the aforementioned improve reproductive efficiency and directly affect production efficiency.

CONCLUSION

Based on the obtained results, it can be concluded that the hormonal treatment used in this study achieved a satisfactory degree of estrous response and shortened the duration of the service period. The obtained results justify the application of this hormonal treatment in practical production, which together with the optimization of other production factors would improve reproductive performance on dairy farms and thus increase the efficiency of milk production.

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PRIMJENA JEDNOKRATNE INJEKCIJE $PGF_{2\alpha}$ I GnRH U SVRHU INDUKCIJE I SINHRONIZACIJE ESTRUSA KOD MLIJEČNIH KRAVA

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SADRŽAJ

Cilj ovog rada bio je da se ispita uticaj tretmana sa $PGF_{2\alpha}$ i GnRH na estrusno reagovanje, fertilitet i trajanje servis-perioda krava simentalske rase u laktaciji. Ukupno 120 krava bilo je podijeljeno u četiri grupe po 30 krava: prva grupa ($PGF_{2\alpha}$ 40) je tretirana 40 dana post partum sa jednom injekcijom 2 ml analoga prostaglandina (Estrumate), dok je druga grupa ($PGF_{2\alpha}$ 50) tretirana na isti način 50 dana, treća grupa (GnRH) tretirana je 40 dana post partum sa jednom injekcijom 2 ml GnRH analoga (Receptal), a četvrta grupa nije hormonalno tretirana. Fertilitet krava nije se statistički značajno razlikovao između grupa ($p>0.05$), dok je kod estrusnog reagovanja krava i dužine trajanja servis-perioda između oglednih grupa i kontrolne grupe ustanovljena statistički značajna razlika ($p<0.01$).