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Improvement of First-Service Conception Rate in Dairy Cows after the Injection of Pregnant Mare Serum Gonadotropin Six Days Postpartum

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Abstract

BACKGROUND: Early resumption of ovarian activity after parturition could positively influence the fertility of dairy cows. Ovulation of the first postpartum follicular wave and formation of corpus luteum that secretes progesterone can substantially benefit the resumption of ovarian cyclicity in cows. In this regard, the presence of a dominant follicle in the ovary before day 10 postpartum could enhance ovulation rate in the first postpartum follicular wave. Pregnant mare serum gonadotropin (PMSG) is one of the hormones with the potential to improve the growth of follicles.

OBJECTIVES: Accordingly, the present study was conducted to evaluate the effect of PMSG injection six days after parturition on reproductive parameters in dairy cows.

METHODS: The subjects were randomly assigned to the two groups of control (N=74) and treatment (N=79). Cows in the control group received no treatment, while animals in the treatment group received 500 IU PMSG on day six postpartum. In both experimental groups, cows were subjected to Presynch-Ovsynch protocol 30-35 days postpartum. The subjects that experienced estrus during the Presynch-Ovsynch protocol were inseminated. On the other hand, the cows that were not in estrus during this period were subjected to fixed-time insemination at the end of the Presynch-Ovsynch protocol. Pregnancy was diagnosed 45 days after artificial insemination using the rectal examination.

RESULTS: In the control group, 66.22% of cows were inseminated based on estrus detection and 33.78% of cows were inseminated at fixed times. In the intervention group, 68.35% of cows were inseminated based on estrus detection and 31.65% of cows were inseminated at fixed times. The first-service conception rate (FSCR) was 27.03% and 48.1% in the control and treatment groups, respectively (P=0.01).

CONCLUSIONS: The present study showed that the injection of 500 IU PMSG six days after parturition could enhance FSCR in dairy cows.

KEYWORDS: Dairy cows, Estrus synchronization, PMSG, Postpartum, Presycnh-Ovsynch

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Introduction

The genetic selection of dairy cows to increase milk production has reduced the fertility of these animals over the past two decades (Berry *et al.*, 2016; Rearte *et al.*,

2018). Although few studies have investigated the dynamics of reproductive performance in Iranian dairy herds, the reports originating from cattle breeding centers indicate that high producing cows experience inferior fertility with longer days open and higher services per conception, compared to low producing cows (Ansari *et al.*, 2010). One of the reasons for such decrease in the fertility of cows is the elevation in anestrous cows over recent decades (Lucy *et al.*, 2001; Berry *et al.*, 2016) because early resumption of ovarian activity could culminate in a higher level of reproductive performance (Galvão *et al.*, 2010; López-Helguera *et al.*, 2016). Moreover, postpartum anestrus could cause a delay in days to first service after parturition, particularly in herds in which artificial insemination is performed based on estrus detection (Walsh *et al.*, 2007).

Indeed, postpartum anovulatory anestrus in dairy cows is not due to the absence of a follicular wave in ovaries because a new follicular wave emerges about 5-7 days after parturition in cows (Beam and Butler, 1998) and may reach to the large size by day 10 postpartum (Savio *et al.*, 1990). However, the newly emerged follicular wave has not the same fate in all cows and some cows fail to ovulate, predisposing them to anestrus (Roche *et al.*, 1998). Therefore, interventions that enhance the growth of the first postpartum follicular wave could potentially augment the proportion of ovulating cows leading to improved fertility. In this context, pregnant mare serum gonadotropin (PMSG), also called equine chorionic gonadotropin, has both follicle-stimulating hormone- and luteinizing hormone-like activities and has been substantiated to stimulate follicular growth and ovulation in cattle (Rostami *et al.*, 2011; Canadas *et al.*, 2019). The injection of PMSG on day six postpartum has been previously reported to improve the reproductive performance of dairy cows inseminated based on the detection of behavioral estrus (Vojgani *et al.*, 2013).

One of the other negative consequences of substantial increment in milk production of cows over recent decades has been the weakened intensity and shortened period of estrus expression, which led to a 60% reduction in estrus detection rate in dairy herds (Berry *et al.*, 2016; Nowicki *et al.*, 2017). To circumvent this issue, various pre-synchronization and synchronization protocols were developed, which coordinate follicular growth and corpus luteum lysis to perform artificial insemination at the appropriate time without estrus detection (Wiltbank and Pursley, 2014; Nowicki *et al.*, 2017), and in turn, elevate the reproductive performance of dairy herds (Bisinotto *et al.*, 2014; Carvalho *et al.*, 2015; Dirandeh *et al.*, 2018).

Ovarian cyclicity of cows at the beginning of synchronization protocols could affect the efficiency of protocols with cyclic cows responding better to synchronization protocols than non-cyclic cows (Galvão KN and Santos, 2010; Yilmazbas-Mecitoglu *et al.*, 2012; Borchardt *et al.*, 2020). Consequently, the present study aimed to test the hypothesis that PMSG injection six days postpartum could improve the reproductive performance of dairy cows subjected to the Presynch-Ovsynch protocol.

Materials and Methods

Study Setting

The present study was conducted during September 2016-June 2017 at a commercial Holstein dairy farm located in Parsabad Moghan, Ardabil province, Iran (Latitude:39 39N; Longitude: 4748E; Altitude: 1200 m).

Animals, Diet, and Management

Healthy Holstein cows (N=153) without puerperium disorders, such as retained placenta and dystocia, or digestive and/or reproductive disorders, including clinical endometritis,

clinical mastitis, lameness, and displaced abomasum were included in the study. Cows received a balanced ratio in accordance with the National Research Council recommendation for dairy cows (NRC, 2001).

The first and second postpartum examinations of cows were performed on days 15-20 and 30-35 postpartum, respectively. The voluntary waiting period (VWP) was 40 days and cows that had estrus following the termination of VWP were inseminated 12 h after the detection of standing estrus. Estrus detection was performed three times a day by visual observation for at least 30 min each time. All artificial inseminations were conducted by the same technician and pregnancy diagnosis was performed 45 days after artificial insemination by transrectal palpation.

Experimental Design

On day 5 postpartum, cows were examined in terms of body condition score (BCS) and were blocked based on BCS and were randomly assigned to the control and treat-ment groups. Cows in the test group (N=79) received 500 IU of PMSG (GONASER®, HIPRA, Spain) on day six postpartum. The Cows in the control group (N=74) received no treatment on day six postpartum. In both experimental groups, cows were subjected to Presynch-Ovsynch protocol starting 30-35 days postpartum. The Presynch-Ovsynch protocol was initiated by two injections of PGF2α (GESTAVET PROST®, HIPRA, Spain) with intervals of 14 days. Twelve days after the second PGF2α, cows received an injection of GnRH (Vet-aroline, Aburaihan Co., Tehran, Iran) followed by a dose of PGF2α seven days later, which was followed by a second injection of GnRH 56 days later. The cows that were in estrus during the Presynch-Ovsynch protocol were inseminated 12 h after the detection of estrus (Youssefi *et al.*, 2013). However, the cows that were not in estrus during this period were eventually inseminated at fixed times 16 h after the second GnRH injection (Figure 1).

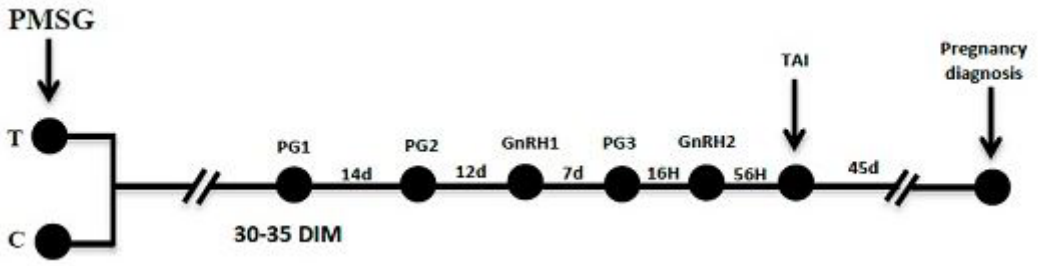

Figure 1. Experimental design**:** T: Treatment group; C: Control group; DIM: Days in milk; AI: Artificial insemination: TAI: Timed artificial insemination. Cows in treatment group received 500 IU of PMSG on day 6 postpartum, but cows in the control group received no treatment on day 6 postpartum. In both experimental groups, cows subjected to Presynch-Ovsynch protocol which was as follows: Presynch (PGF2α -14d- PGF2α) and Ovsynch (GnRH -7d- PGF2α -56h- GnRH -16h- TAI) with an interval of 12 d.

Statistical Analysis

Data were analyzed by logistic regression using the GENMOD procedure, including function link logit in the model. Logistic regression analysis produced an adjusted odds ratio as the strength of the difference between groups. All analyses were conducted utilizing SAS (Statistical Analysis Systems Institute) version 9.4 (Cary, NC, SAS Institute; 2013). *P*-value $\leq$ 0.05 was considered statistically significant.

Results

Conception rate, days in milk (DIM), milk production, and BCS of cows based on the group and parity of cows are presented in Table 1. The proportion of cows that experienced estrus after the initiation of the

Presynch-Ovsynch protocol and were inseminated based on detected estrus did not differ between the control (49/74: 66.22%) and treatment (49/79: 68.36%) groups (P>0.05) (Table 1).

Table 1. Conception rates in the treatment and control groups based on estrus AI or fixed time AI

Control (N=74)	CR (%)	DIM (days)	MP (kg)	BCS
Based-on-estrus AI(N=49)				
Primiparous	54.54(11/6)	34.82	32.08	3.00
Multiparous	28.94(38/11)	35.79	34.68	3.13
Timed AI(N=25)				
Primiparous	0 (0/3)	33.33	28.33	2.92
Multiparous	13.63 (3/22)	34.73	38.35	3.08
Treatment (N=79)				
Based-on-estrus AI(N=54)				
Primiparous	42.1 (8/19)	34.84	26.79	3.01
Multiparous	60 (21/35)	34.37	36.77	3.14
Timed AI(N=25)				
Primiparous	42.85 (3/7)	33.00	30.29	3.11
Multiparous	33.3 (6/18)	33.83	36.17	3.13

AI: Artificial insemination; CR: Conception rate; DIM: Days in milk; MP: Milk production; BCS: Body condition score

Conception rate in animals treated with PMSG on day six postpartum (38/79: 48.1%) was greater than untreated cows (20/74: 27.03; P=0.01) (Table 2). Moreover, conception rate was higher in subjects inseminated based on detected estrus (46/103: 44.66%), compared to cows subjected to fixed-time insemination at the end of Presynch-Ovsynch protocol (12/50: 24%; P=0.011) (Table 2). However, conception rate was not influenced by station, BCS, parity, milk production, and the DIM on which the Presynch-Ovsynch protocol was started (P>0.05) (Table 2).

Table 2. Conception rates in the treatment and control groups

Effect	Class	CR (%)	AOR	95% CI	P-value
Treatment	1	48.10 (38/79)	2.610	1.260-5.405	0.010
	2	27.03 (20/74)	—	—	—
AI	Based on observed estrus	44.66 (46/103)	2.855	1.268-6.431	0.011
	Timed	24.00 (12/50)	—	—	—
Station	A	38.46 (30/78)	1.255	0.607-2.596	0.539
	B	37.33 (28/75)	—	—	—

Effect	Class	CR (%)	AOR	95% CI	P-value
BCS	—	—	0.344	0.047-2.509	0.293
Parity	Primiparous	42.50 (17/40)	0.715	0.297-1.724	0.455
	Multiparous	36.28 (41/114)	—	—	—
Milk production	—	—	0.974	0.931-1.019	0.259
DIM	—	—	1.059	0.966-1.161	0.222

CI: Confidence interval; AOR: Adjusted odds ratio, DIM: Days in milk; AI: Artificial insemination: TAI: Timed artificial insemination.

Discussion

The present study was conducted to assess whether the injection of PMSG on day six postpartum could enhance the reproductive performance of dairy cows. It was revealed that PMSG injection six days after calving improved the first-service conception rate (FSCR) even though it did not affect the estrus expression of cows after the commencement of the Presynch-Ovsynch protocol. Consistent with our finding, Vojgani *et al.* (2013) found a positive impact of PMSG injection six days postpartum on the fertility of cows that were not assigned to ovulation synchronization protocols after parturition.

An earlier study indicated that the injection of PMSG on day six postpartum stimulated follicular growth, augmented the proportion of cows that ovulated by day 20 postpartum, and advanced resumption ovarian activity after parturition (Rostami *et al.*, 2011). Early postpartum ovulation would prevent long-term progesterone deprivation in cows, and in turn, help to re-coordinate the hypothalamic-pituitary-ovary axis, which is essential for regular ovarian activity in cows (Plant, 2015). Regulation of the hypothalamic-pituitary-ovary axis leads to frequent estrous cycles before the time of insemination (Kawashima *et al.*, 2006). The concentration of circulating estradiol peaks during the estrus phase resulting in hastened uterine involution due to reduced size, a marked increase in uterine tone and contractility, and benefits for the uterine defense mechanisms. Consequently, a higher number of estrous cycles facilitates the uterine clearance and improves fertility (Kawashima *et al.*, 2006; Mueller *et al.*, 2006; Vojgani *et al.*, 2013; Sugiura *et al.*, 2018). In corroboration of this notion, Canadas *et al.* (2019) found that the injection of PMSG during the early postpartum period reduced vaginal discharge score and improved uterine involution in cows. Furthermore, early resumption of ovarian activity has been reported to be associated with higher reproductive performance in cows (Galvão *et al.*, 2010; López-Helguera *et al.*, 2016). Therefore, the beneficial impact of PMSG injection six days after calving in the present study could be due to the positive influence of this hormone on the early resumption of ovarian activity.

Herein, it is worth noting that the day of PMSG injection relative to parturition day is of importance for enhancing reproductive performance in cows using this type of hormone therapy. Injection of PMSG on day six

postpartum in the present investigation, as well as the study conducted by Vojgani *et al.* (2013) and on day eight postpartum in the study performed by Canadas *et al.* (2019) advanced resumption of ovarian activity, improved uterine health status, and enhanced reproductive performance in cows. However, injection of this hormone on days 9-15 postpartum (Freick *et al.*, 2017) or on days 11-17 postpartum (Patron-Collantes *et al.*, 2017) failed to positively affect the fertility of cows. As a result, it could be inferred that the injection of PMSG for improving the fertility of cattle could be suggested during a limited period after parturition (e.g., 6-8 days postpartum).

Regardless of the effect of PMSG injection on FSCR, conception rate was observed to be greater in animals that were inseminated based on detected estrus than cows that were fixed-time inseminated at the end of Presynch-Ovsynch protocol. This result is in agreement with the findings of previous studies indicating higher fertility following insemination based on the observation of spontaneous estrus than insemination based on estrus synchronization protocols (Dalton *et al.*, 2005; Gugssa *et al.*, 2016; Machado *et al.*, 2017). One of the major reasons for this phenomenon is that not all cows respond to the first GnRH of ovsynch protocol, which leads to failure of the protocol to synchronize ovulation in cows. Therefore, some animals would be in estrus at the time of fixed-time insemination (Wiltbank and Pursley, 2014; Nowicki *et al.*, 2017; Khalil, 2019).

Conclusion

The present study showed that PMSG injection six days after parturition improved FSCR. However, it did not affect the cows that expressed behavioral estrus before the first service postpartum. Furthermore, it was observed that the conception rate was higher in cows that were inseminated following spontaneous estrus than in cows that were fixed-time inseminated.

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Conflict of Interest

None.

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بهبود باروری اولین تلقیح پس از زایش در گاوهای شیری متعاقب تزریق PMSG ۶ روز پس از زایمان

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زمینه مطالعه: بازیابی زودهنگام فعالیت تخمدانی پس از زایش می‌تواند بر باروری گاوهای شیری تأثیر مثبت داشته باشد. تخمک‌گذاری اولین موج فولیکولی پس از زایش و تشکیل جسم زردی که پروژسترون ترشح کند می‌تواند کمک چشمگیری بر بازیابی فعالیت تخمدانی در گاوها داشته باشد و در این رابطه، حضور یک فولیکول غالب روی تخمدان پیش از روز ۱۰ پس از زایمان می‌تواند نرخ تخمک‌گذاری در اولین موج فولیکولی پس از زایش را ارتقا دهد. گونادوتروپین سرم مادیان آبستن (PMSG) یکی از هورمون‌هایی است که می‌تواند رشد فولیکول‌های تخمدانی را بهبود دهد.

هدف: بنابراین، مطالعه حاضر به‌منظور ارزیابی اثر تزریق PMSG ۶ روز پس از زایمان بر پارامترهای تولیدمثلی در گاوهای شیری به انجام رسید.

روش کار: گاوها به‌صورت اتفاقی در دو گروه شامل شاهد (تعداد = ۷۴) و تیمار (تعداد = ۷۹) قرار گرفتند. گاوهای گروه شاهد هیچ تیماری دریافت نکردند، اما گاوهای گروه تیمار در روز ۶ پس از زایمان ۵۰۰ واحد بین‌المللی PMSG دریافت کردند. در هر دو گروه آزمایشی، گاوها از روز ۳۰-۳۵ پس از زایش تحت پروتکل presynch-ovsynch قرار داده شدند. گاوهایی که از آنها طی انجام پروتکل presynch-ovsynch فحلی مشاهده شد بر اساس فحلی تلقیح شدند و گاوهایی که از آنها طی این دوره فحلی مشاهده نگردید در انتهای پروتکل presynch-ovsynch در زمان ثابت تلقیح شدند. تشخیص آبستنی ۴۵ روز پس از تلقیح مصنوعی با استفاده از معاینه رکتال انجام گرفت

نتایج: در گروه شاهد، ۶۶/۲۱٪ گاوها بر اساس فحل یابی و ۳۳.۷۸٪ گاوها در زمان ثابت تلقیح شدند و درگروه تیمار، ۶۸/۳۵ ٪ گاوها بر اساس فحل‌یابی و ۳۱/۶۵ ٪ گاوها در زمان ثابت تلقیح شدند. نرخ باروری در تلقیح اول در گروه‌های شاهد و تیمار به ترتیب ۲۷/۰۳٪ و ۴۸/۱۰٪ بود (P=۰/۰۱).

نتیجه‌گیری نهایی: مطالعهٔ حاضر نشان داد که تزریق ۵۰۰ واحد بین‌المللی PMSG، ۶ روز پس از زایمان می‌تواند نرخ باروری در اولین تلقیح پس از زایش را بهبود دهد.

واژه‌های کلیدی: باروری، پرسینک اووسینک، گاو شیری، همزمان‌سازی، PMSG

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