



## RESEARCH ARTICLE

### Effects of Different Doses of PMSG on Reproductive Performance in Chinese Holstein Dairy Cows

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#### ABSTRACT

The current study was designed to evaluate the effect of different doses of pregnant mare serum gonadotropin (PMSG) on reproductive performance synchronized with prostaglandin  $F_{2\alpha}$  ( $PGF_{2\alpha}$ ) in Chinese Holstein dairy cows. The study was conducted on one hundred cyclic dairy cows. Cows were equally divided into five treatment groups. Each group ( $n=20$ ) was administered with an injection of PMSG (Serum Gonadotrophin for Injection, SanSheng Pharmaceutical, China) at dose rate of 2, 2.5, 3, 3.5, or 4 IU/kg BW, followed by an injection of  $PGF_{2\alpha}$  (Cloprostenol Sodium for Injection, SanSheng Pharmaceutical, China) 48 h later. Estrus was detected by a teaser bull three times daily after  $PGF_{2\alpha}$  administration. The cows were artificially inseminated 12 and 20 h after standing heat. On day 30 after artificial insemination, pregnancy rate was confirmed by transrectal ultrasonography while calving rate and multiple calvings were added up after successful parturition. There were no significant difference ( $P>0.05$ ) in estrus response among all treatment groups. Similarly, interval from PMSG administration to estrus was decreased non-significantly ( $P>0.05$ ) with the different doses of PMSG. Pregnancy rate was significantly lower ( $P<0.05$ ) in the highest treatment group (4 IU/kg, 25%) than in low treatment groups (2 and 2.5 IU/kg, 71.4 and 66.7%). Calving rate was non-significant ( $P>0.05$ ) among different regimens. The effects of PMSG dose on multiple calving was found to be statistically significant ( $P<0.05$ ) and administration of 4 IU/kg PMSG resulted in 50% multiple births. The cows failed to get pregnant returned to estrus naturally. The interval from PMSG administration to first natural estrus was significantly higher ( $P<0.05$ ) in high treatment groups ( $>2.5$  IU/kg) than in the lowest treatment group (2 IU/kg), however, later pregnancy rate was non-significant ( $P>0.05$ ) in all groups. In summary, PMSG administration could successfully induce estrus and improve fertility of a dairy herd. Furthermore, low doses of PMSG aid in quick return of non-pregnant cows to subsequent next estrus.

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#### INTRODUCTION

Chinese Holstein dairy cows were developed from cross-breeding between the local yellow cattle and Holstein cows. Since 1979, dairy industry in China has developed with mutual cooperation of government and private investors. The annual milk production in 28

provinces of China is more than 5000 kg/cow/herd with 3.5% fat content (Qiu *et al.*, 1991). These continuous efforts are still in progress with better and properly planned breeding policies. Estrus and ovulation synchronization are the key events in these breeding programs, as they shorten the estrous interval and prevent the time spent on estrus detection, respectively (Pursley *et al.*, 1995).

Pregnant Mare Serum Gonadotrophin (PMSG) is widely used for estrus synchronization programs in small and large ruminants industry due to its cost-effectiveness treatment (Bartolome *et al.*, 2012; Carvalho *et al.*, 2012). PMSG exhibits mainly FSH like activity but also have some LH like activity and its parenteral administration stimulates follicular growth and ovulation in cattle (Dieleman and Bevers, 1987). PMSG is administered; either to grow pool of small follicles or ovulation of large follicles on the ovaries. This was followed by administration of PGF<sub>2α</sub> for the regression of corpus luteum. In cattle, PMSG administration on day 14 postpartum increases the follicular growth and plasma estradiol concentration without affecting the subsequent reproductive performance (Sheldon and Dobson, 2000). In one study, PMSG is used to induce more ovulation rate (Sommer *et al.*, 2007) thus, it increases the chance of multiple ovulation or multiple births (Mehaisen *et al.*, 2005; Talebkhan Garoussi *et al.*, 2012). Therefore, the treatment with PMSG is considered to enhance ovarian follicular growth and fertility at the end of estrus synchronization program in dairy cows (Souza *et al.*, 2009; Rostami *et al.*, 2011).

However, its overall effect on reproductive performance is still debatable. Higher dose of PMSG may lead to excessive follicular development resulting in the failure of ovulation. These un-ovulated follicles secrete abnormally high levels of estradiol, which may have adverse effects on embryo development (Ziecik *et al.*, 2005). It has also been reported that superovulation with PMSG increases the incidence of embryonic mortality and abortion (Kiewisz *et al.*, 2011).

As the different doses of PMSG may vary in response in terms of estrus behavior, fertility and animal productivity in farm animals (Barrett *et al.*, 2004). Therefore, the present study was conducted to evaluate effects of different doses of PMSG on reproductive performance synchronized with PGF<sub>2α</sub> in Chinese Holstein dairy cows.

## MATERIALS AND METHODS

PMSG commercially available as Serum Gonadotrophin for Injection (1000 IU) and PGF<sub>2α</sub> commercially sold as Cloprostenol Sodium Injection (0.2 mg) were purchased from Ningbo Sansheng Pharmaceuticals, China.

This study was conducted on a dairy farm in Hubei province, China. One hundred healthy (Body condition score, 3-3.5), weighing 450-550 kg and cyclic Chinese Holstein dairy cows were selected for this experiment. The cyclicity of animals was based on dairy farm history records. Before the experiment, all the cows were clinically examined to be free from any uterine infection,

peri-parturient diseases, calving difficulties, and other reproductive problems. During the experiment, feeding regimes were maintained according to the recommendations of NRC (2001).

All cows were randomly divided into five groups, each consisting of 20 cows. Synchronization treatment was initiated on day 8-10 of the estrous cycle (day 0=estrus) with a single intramuscular (i.m) injection of PMSG at different dose rates among different regimens groups (2, 2.5, 3, 3.5, 4 IU/kg). This was followed by administration of PGF<sub>2α</sub> 48 h later in all groups. Cows were observed for estrus by a teaser bull thrice a day until reached to heat. The standing heat was confirmed and the cows exhibiting estrus were artificially inseminated twice by the semen of a bull with proven fertility 12 and 20 h after standing estrus. On day 30, all inseminated animals were scanned for pregnancy diagnosis by transrectal ultrasonography (B-mode with 5.0 MHz probe). Calving rate and multiple calvings were recorded after successful parturition of these animals.

All data were presented as means±SEM and were analyzed by one-way ANOVA program of SPSS (version 13.0, SPSS Inc., Chicago, IL). A probability of P< 0.05 was considered statistically significant.

## RESULTS

**Estrus response to PMSG administration:** Regarding estrus response against PMSG administration, no significant effect (P>0.05) was observed among different doses of PMSG. Although, the estrus response was more than 70% in all treatment groups (Table 1). PMSG administration imparted a non-significant (P>0.05) effect on the interval from PMSG administration to estrus. However, the interval was reduced with increase in PMSG administration and the lowest interval recorded was 74 h (3.5 IU/kg) which was significant (P<0.05) to low treatment groups (2 and 2.5 IU/kg) (Table 1).

**Pregnancy rate and multiple calving:** In this experiment, Pregnancy rate was decreased among different regimens with increase in the dosage of PMSG (Table 1). Pregnancy rate was found lowest in the highest treatment group (4 IU/kg) which is significantly lower (P<0.05) than in low treatment groups (2 and 2.5 IU/kg).

The effects of PMSG administration on calving rate, multiple calving are summarized in Table 1. PMSG did not affect (P>0.05) calving rate. Lower doses of PMSG (2 and 2.5 IU/kg) failed to induce multiple calving in these cows; while in other treatment groups, multiple calvings were increased with increase in the dosage of PMSG. In the highest treatment group (4 IU/kg) 50% animals exhibited (n=2) multiple calvings.

**Table 1:** Effects of dose of PMSG on reproductive efficiency of Chinese Holstein dairy cows

PMSG (IU/kg)	n	Interval to estrus (h)	Estrous rate (%)	Pregnancy rate (%)	Calving rate (%)	Multiple calving rates (%)
2	20	98.3±7.6 <sup>a</sup>	14/20 (70.0)	10/14 (71.4) <sup>a</sup>	9/10 (90.0)	0/9 (0.0) <sup>a</sup>
2.5	20	97.3±5.1 <sup>ab</sup>	18/20 (90.0)	12/18 (66.7) <sup>a</sup>	10/12 (83.3)	0/10 (0.0) <sup>a</sup>
3	20	88.0±5.4 <sup>abc</sup>	18/20 (90.0)	8/18 (44.4) <sup>ab</sup>	7/8 (87.5)	1/7 (14.3) <sup>ab</sup>
3.5	20	74.3±6.9 <sup>c</sup>	16/20 (80.0)	6/16 (37.5) <sup>ab</sup>	5/6 (83.3)	2/5 (40.0) <sup>b</sup>
4	20	78.4±8.4 <sup>abc</sup>	16/20 (80.0)	4/16 (25.0) <sup>b</sup>	4/4 (100.0)	2/4 (50.0) <sup>b</sup>

Values within the same column with different superscripts differ (P<0.05).

**Return to estrus and subsequent pregnancy rate:** All the non-pregnant animals in these groups returned to estrus normally and were artificially inseminated. There was a significant linear relationship ( $P < 0.05$ ) between PMSG dose and interval (days) from PMSG administration to first natural estrus. The interval was found shortest (28.6 days) in the lowest treatment group (2 IU/kg), while it was highest (90.2 days) in 4 IU/kg treatment group. However, the pregnancy rates after first natural estruses were similar ( $P > 0.05$ ) between all groups and were ranged from 75 to 83.3% (Table 2).

**Table 2:** Effects of dose of PMSG on interval (days) to first natural estrus and later pregnancy percentage

PMSG (IU/kg)	n	Interval to the second estrus (d)	Pregnancy rate (%)
2	4	28.6±4.1 <sup>a</sup>	3/4 (75.0)
2.5	6	48.3±10.4 <sup>ab</sup>	5/6 (83.3)
3	10	54.4±7.3 <sup>b</sup>	8/10 (80.0)
3.5	10	65.2±8.7 <sup>bc</sup>	8/10 (80.0)
4	12	90.2±10.6 <sup>c</sup>	10/12 (83.3)

Values within the same column with different superscripts differ ( $P < 0.05$ ).

## DISCUSSION

The present study described the effect of different doses of PMSG on different reproductive events in Chinese Holstein cows. Previous reports are inconsistent regarding the effect of various doses of PMSG on estrus response. Some researchers reported that PMSG do not have a significant effect on estrus response at different doses (Sá Filho *et al.*, 2010; Zonturlu *et al.*, 2011), whereas, the others described its negative effects on estrus induction (Zeitoun *et al.*, 1991). In the present study, we found a non-significant effect on estrus response among all treatment groups which varied from 70-90%. Moreover, these results suggested the optimum dose (2.5 and 3 IU/kg) of PMSG for successful estrus induction followed by an injection of PGF<sub>2α</sub> 48 h later in Chinese Holstein cows. Previously, it was reported that PMSG may be included in synchronization protocols to reduce estrus interval and to prompt the estrus in a shorter period of time (Gutiérrez *et al.*, 2009; Kumar *et al.*, 2010). In our study, the interval from PMSG administration to estrus was non-significant. In one previous study (Gonzalez *et al.*, 1994) also failed to find any significance among different regimens of PMSG.

Pregnancy rate was decreased with the increase in dosage of PMSG. The average pregnancy rate in first two treatment groups (2 and 2.5 IU/kg, 69%) was significantly higher than the highest group (4 IU/kg, 25%). Similar results were obtained when high dose of PMSG was used in beef cows (Wang *et al.*, 1988). Lower pregnancy rates in high dose groups may be associated with excessive follicular development, decrease in the percentage of fertilized ova and embryo quality in different species (Paulson *et al.*, 1997; Cornejo-Cortes *et al.*, 2006). Moreover, large doses of PMSG may cause disorders during final follicular and oocyte maturation which is known to proceed according to a chronologically fixed hormonal sequence in normally cyclic cows (Gonzalez *et al.*, 1994) and gilts (Blitek *et al.*, 2010).

The calving rate was similar in five PMSG treatment groups, but multiple births varied among all treatment

groups. There was an increase in the percentage of multiple births as the dose of PMSG increased. These results were consistent to previous reports where results also suggest that multiple calvings occur more frequently at high dosage level in dairy cattle (Andreu-Vázquez *et al.*, 2012) and ewes (Quintero-Elisea *et al.*, 2011). In the current study, it was also stated that injected PMSG doses cause different effects on the interval from PMSG administration to first natural estrus. These results clearly showed that there is a negative effect of large doses of PMSG on subsequent next estrus. There are few studies regarding PMSG administration on return to first estrus, however, there is no study on pregnancy rate after returning to estrus thus, future studies can be designed to explain this phenomena.

**Conclusion:** Synchronization programs with PMSG and PGF<sub>2α</sub> can significantly improve the reproductive efficiency of Chinese Holstein cows. Lower doses of PMSG were suggested to increase the percentage of pregnancy rate in cows with little side effects. In addition to this, larger dose of PMSG can increase the multiple calving rates although; this delays the subsequent next estrus in cows.

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