



Full Length Article

Estrous Synchronization Efficiency of Buserelin of Different Doses in Combination with Mifepristone and Timed Artificial Insemination in Holstein Cattle

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Abstract

The reproductive performance of lactating dairy cattle remains a major factor affecting herd's profitability; estrous synchronization and timed artificial insemination (TAI) enable producers to maximize the reproductive efficiency of their herd. A total of 88 cows were used to measure the effect of mifepristone 0.4 mg/kg with different doses of Buserelin (10 μ g, 20 μ g and 40 μ g/head) on estrous synchronization efficiency for the same period in cattle. Buserelin was injected at day 0 followed by PG (day=7)-Buserelin + mifepristone (0.4 mg/kg) (day=9) (BPBMH synchronization protocol), results revealed that Buserelin in 20 μ g dose has significantly ($P < 0.05$) increased estrus rate (86.96%) ovulation rate (82.61%) and conception rate (65.22%) among other doses when combined with mifepristone (0.4 mg/kg dose) in cattle. A second experiment was designed to select the best time for FTAI, 95 cows were selected and treated with BPBMH in 0.4 mg/kg mifepristone dose estrus synchronization protocol and timed artificial insemination was performed h 16, 20, 24, 28 and 32 after second Buserelin. The results showed that artificial insemination at 16 h of mifepristone injection was the best insemination time in cattle. © 2019 Friends Science Publishers

Keywords: Buserelin; Mifepristone; Synchronization; FTAI; Cattle

Introduction

China owned 83.34 million dairy cattle and 30.77 million tons of milk output in 2017 (F.A.O., 2018). It represents an important livestock resource which makes China the largest third milk producing country in the world, the annual milk production constitutes more than 5000 L/cow/herd in all 28 provinces with 3.5% fat content (Fu *et al.*, 2013). Reproductive performance of lactating dairy cattle represented one major factor affecting herd's profitability (Azevedo *et al.*, 2014). Reproductive efficiency in high-producing lactating dairy cows decreased due to reduced fertility, low estrus expression, extended calving intervals, variable estrus duration and difficulty in predicting estrus and ovulation time in cattle (Washburn *et al.*, 2002; Lopez *et al.*, 2004; Milo *et al.*, 2006). Therefore, protocols for estrous synchronization and TAI, have been developed, such as Ovsynch, to overcome the restrictions resulting from poor estrus detection especially during the hot season thus improve the success of artificial insemination programs

(Pursley *et al.*, 1995; Souza *et al.*, 2008; Akhtar *et al.*, 2014).

Estrus synchronization protocols that result in highly synchronized estrus and ovulation reduce the time and labor associated with estrus detection (Carvalho *et al.*, 2015). The most commonly used estrus synchronization and FTAI protocol in lactating cattle is the Ovsynch-FTAI program (Campanile *et al.*, 2016; Singh and Balhara, 2016). Studies on estrus synchronization, using either kisspeptin-PGF_{2 α} -kisspeptin protocol or buserelin-PGF_{2 α} -buserelin, Ovsynch protocol, on day 0, 7 and 9, respectively, revealed that the number of follicles and the diameter of dominant follicle, the estrus rate and estrus duration at wave emergence increased after 2nd dose injection of kisspeptin, compared to buserelin (Pottapenjara *et al.*, 2018).

Cows received an i.m. injection of 10 μ g GnRH agonist (Buserelin, Receptal®), followed 7 d later by an i.m. injection of 25 mg PGF 2 α followed by a second i.m. injection of 10 μ g GnRH 48 h later in the Ovsynch (Control) treatment with TAI 16-20 h after the second GnRH, resulted in pregnancy rate of 12.5% (Darras and Alnimer, 2012). Previous studies with Ovsynch synchronization in cattle

reported pregnancy rates of approximately 50% (Hockey *et al.*, 2010). Induced ovulation in postpartum anestrous sahiwal cows and anestrous heifers with Ovsynch protocols resulted in conception rate of 30% and 20% after fixed time AI in sahiwal cows and heifers, respectively (Mohan *et al.*, 2010). In Jordan, pregnancy rates at first AI after Ovsynch application ranged from about 20 to 40% (Alnimer, 2005).

Authors found that Ovsynch treatment resulted in a relatively low pregnancy rate due to not highly synchronized ovulation time (Hassan *et al.*, 2016). Various protocols have been developed to synchronize estrous in large percentage of females at a predetermined time to insemination and increase pregnancy rates (Rajamahendran *et al.*, 2001; Yusuf, 2011). The current direction of estrus synchronization is depend on combining conventional methods of controlling cycle length with the control of follicle development in order to select the ovulatory follicle. The hypothesis is that higher progesterone concentrations inhibit the onset of LH surge resulting in formation of follicular cysts (Khan *et al.*, 2011). Mifepristone as a potent antiprogesterone having a high affinity for the progesterone receptor (Blüthgen *et al.*, 2013; Diantonio *et al.*, 2015). However, there is no information about the efficiency of mifepristone injection to promote ovulation in protocols of ovulation synchronization and FTAI in cattle. We hypothesized that treatment with BPBMH in mifepristone dose of 0.4 mg/kg induces a higher ovulation rate than conventional BPB (Ovsynch) synchronization protocols in cattle.

The objectives of this study were to select the best dose of Buserelin using Ovsynch estrus synchronization protocols combined with mifepristone and to undertake a comprehensive evaluation of the response to BPBMH on synchronization of ovulation and determine appropriate time for FTAI to enhance the reproductive efficiency of cattle.

Materials and Methods

A total of 183 Holstein cows raised in the dairy farm of qiao niu er farm under Hubei qiao niu er Co., Ltd., Hubei province, central China were used for this experiment. The experiment was carried out from November 1st, 2017 to December 30th 2017. The average ambient temperature varied between 4°C and 15°C, with relative humidity ranging between 55% and 65%. Body condition score (BCS) was between 2.5 to 4 points at postpartum stage; at the age of 4 to 8 years old, mean body weight, chest girth, body height, body lengths and abdominal girths were 614.6 ± 118.0 kg, 143.4 ± 14.7 cm, 149.5 ± 5.9 cm, 235.5 ± 8.7 cm, 251.6 ± 14.4 cm, respectively. All the animals were healthy in regular estrus cycle and physical condition and fed with total mixed ration (TMR) consisted of forage (corn silage, peanut vine, rice straw) and concentrate (corn, soybean meal, wheat bran) and were machine-milked twice a day.

Experimental Groups

Experimental site and animal care: All experimental protocols were approved by the Ethical Committee of the Hubei Research Center, Huazhong Agricultural University, China (Approval ID: SCXK (Hubei) 20080005).

Experimental Groups

There are two trials in this experiment. In trial 1, a total of 88 cows were used to optimize the dose of Buserelin in Holstein cattle. These animals were treated with Buserelin (day=0), PG (day=7), and mifepristone 0.4 mg/kg was injected simultaneously with second buserelin (day=9). Three different doses of Buserelin was designed including low dose group (10 µg), medium dose group (20 µg) and high dose group (40 µg). Animal treated with Gonadorelin 100 µg (day=0) PG 0.5 mg (day=7)-Gonadorelin 100 µg + mifepristone (0.4 mg/kg) (day=9) was used as control group. Double AI was performed at 18 h and 28 h insemination after mifepristone injection (Fig. 1).

Trial 2, a total of 95 cows were used to optimize the time of AI in BPBMH estrus synchronization method. All Holstein cows were divided into five groups. Single AI was performed after 16 h, 20 h, 24 h, 28 h, 32 h of mifepristone injection for Holstein cattle.

Follicle Development Detection

Follicle development was evaluated using desktop B-type veterinary ultrasound scanner (WED-9618-v, equipped with LV2-3/6.5 MHz rectal probe, Shenzhen Well.D Medical Electronics Co., Ltd., Guangdong, China) as previously prescribed (Gimenes *et al.*, 2011; Liu *et al.*, 2016). From days 6 (1 day before PGF_{2α} treatment) to 12 (72 h after the second injection of Buserelin). Ultrasonography was performed twice a day at 6 am and 6 pm. Follicle dynamics were analyzed on the basis of follicle diameters. Ovulation was considered to appear at the time of sudden disappearance of dominant follicles (Liu *et al.*, 2016).

Estrus Detection and Pregnancy Diagnosis

Animals examined for signs of estrous twice a day (6 a.m. and 6 p.m.) including vaginal mucus discharge, being mounted, being smelled by other cows or bull (Yindee *et al.*, 2011; Haider *et al.*, 2015). Pregnancy diagnosis was performed by transrectal ultrasonography as described before 40 days after AI (Liu *et al.*, 2016).

Statistical Analysis

Data were statistically analyzed using S.P.S.S. version 17.0 for Windows. The diameter of the ovulatory follicle, the growth speed of dominant follicles and the ovulation rate were represented as mean \pm standard error (SEM).

The chi-squared test were calculated for estrous rate, ovulation rate, conception rate and follicular cyst rate using Graph Pad Prism-6 software package (GraphPad Software Inc.), where results considered as statistically significant at $P < 0.05$ (Liu *et al.*, 2016).

Results

Optimal Dose of Buserelin in BPBMH Methods in Cattle

The results showed that 20 μg Buserelin dose cattle group had the largest non-significant first detection follicle diameter, largest follicle diameter than other groups. Moreover, Buserelin 20 μg dose group showed shorter ($P > 0.05$) estrus duration time, days of ovulation than other groups. Buserelin 20 μg does is the best for estrus synchronization in cattle (Table 1).

The Buserelin 20 μg group total number of estrous rate (73.91%), ovulation rate (82.61%), conception rate (65.22%) were higher than other dose injection, only have significant ($P < 0.05$) difference with high doses of Buserelin 10 μg . Buserelin 20 μg group follicle cysts rate is 3.8%, which is lower than other methods. The results showed Buserelin dose 20 μg was the best dose injection for cattle (Table 2).

In comparison of dynamic change of follicle development among cows synchronized by BPBMH in low (10 μg), medium (20 μg) and high (40 μg) doses of Buserelin, the result showed that Buserelin 20 μg was the best estrus synchronization method for treatment of cattle. Furthermore, the time to attain maximum diameter in BPBMH is relatively short leading to the greatest maximal diameter of follicle than those of others (Fig. 2). Therefore, BPBMH treatment was considered as the best method for Holstein cattle. Considering all the reproduction performance, the medium dose 20 μg of Buserelin combined mifepristone 0.4 mg/kg showed the best synchronization than other methods.

Optimization of Insemination Time in BPBMH Method in Cattle

The results showed that AI time 16 h after mifepristone treatment resulted in estrus rate of 89.47%, ovulation rate of 84.21% and conception rate of 63.16% which were higher than other AI time groups. However, follicle growth rate has no significant difference ($P > 0.05$) between all treatment groups. Moreover, ovulation largest follicle diameter (Fig. 3) showed non-significant difference ($P > 0.05$) between all groups. Considering all factors using BPBMH synchronization protocols in cattle with artificial insemination after 16 h of mifepristone injection is the best artificial insemination time for cattle (Table 3 and Fig. 3)

Discussion

Most dairy producers commonly used Ovsynch-TAI

program for estrus synchronization and TAI in dairy cows (Gümen *et al.*, 2003; Hussein *et al.*, 2004; Bello *et al.*, 2006). However, previous study showed that the ovulation time of the Ovsynch treatment is not highly synchronized, which resulted in a relatively low pregnancy rate (Hassan *et al.*, 2016). Based on the traditional GPG protocol, a new

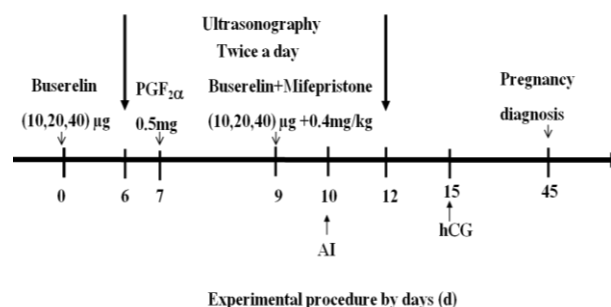


Fig. 1: Schematic diagram of BPBMH estrous synchronization method

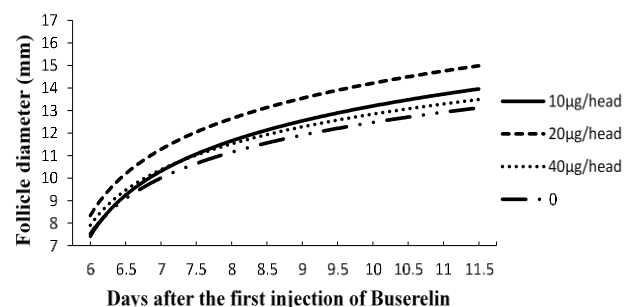


Fig. 2: Dynamic change (diameters, in mean \pm SEM) of follicle development in cows synchronized by BPBMH in low 10 μg , medium 20 μg and high 40 μg doses of Buserelin during the first treatment to the day that a follicle grow in maximal diameter. Values in both X- and Y-coordinates were transformed in logarithm

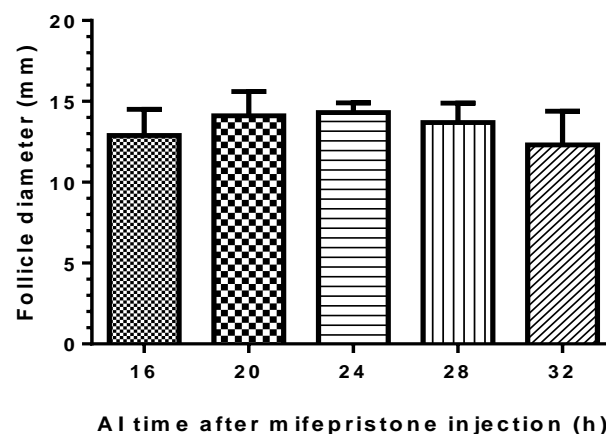


Fig. 3: Maximal diameters (mean \pm SEM) of follicles in cows synchronized by BPBMH in different AI time after mifepristone injection

Table 1: Estrus, ovulation and follicle dynamics of Holstein cows synchronized by BPBMH method in doses of (10, 20 and 40) μg of Buserelin

Buserelin dose ($\mu\text{g}/\text{head}$)	Onset of standing heat after second-Buserelin (h)	estrus duration after onset of standing heat (h)	Time of ovulation after second-Buserelin (h)	Maximum follicle diameter (mm)
10 (n=22)	8.4 ± 0.6^a	16.3 ± 0.5^a	24.7 ± 0.5^a	15.0 ± 3.8^a
20 (n=23)	8.2 ± 0.5^a	16.0 ± 0.4^a	24.5 ± 0.5^a	16.7 ± 4.0^a
40 (n=22)	9.5 ± 0.6^a	15.2 ± 0.3^a	24.6 ± 0.6^a	13.9 ± 2.4^a
0 (n=21)	8.6 ± 0.5^a	15.1 ± 0.4^a	23.8 ± 0.4^a	14.7 ± 2.3^a

Note: The superscripts in different small letters in the same column indicate statistical significance ($P < 0.05$)

Table 2: Estrus, ovulation and pregnancy rates of Holstein cows treated with BPBMH method in Buserelin doses of (10, 20 and 40) μg

Buserelin dose ($\mu\text{g}/\text{head}$)	No of animals treated	No of animals in heat (%)	No of animals ovulated (%)	No of animals pregnant (%)	No of animals with follicular cysts (%)
10	22	16 (72.72 ^b)	15 (68.18 ^a)	10 (45.45 ^{ab})	2 (9.1 ^a)
20	23	20 (86.96 ^b)	19 (82.61 ^a)	15 (65.22 ^a)	1 (4.3 ^a)
40	22	15 (68.18 ^a)	13 (59.09 ^a)	6 (27.27 ^b)	2 (9.1 ^a)
0	21	14 (66.67 ^a)	13 (61.90 ^a)	8 (38.10 ^{ab})	2 (9.5 ^a)

Note: Values in brackets were percentage of treated cows. The superscripts in different small letters in the same column indicate statistical significance ($P < 0.05$)

Table 3: Estrus, ovulation and conception and growth rate of Holstein cows synchronized by BPBMH method in different AI time after mifepristone injection

AI time after mifepristone (h) (n=120)	No of animals treated	No of animals in heat (%)	No of animals ovulated (%)	No of animals pregnant (%)	Growth rate (mm/day)
16	19	17 (89.47 ^a)	16 (84.21 ^a)	12 (63.16 ^a)	1.7182 ^a
20	19	16 (80.00 ^{ab})	15 (75.00 ^{ab})	8 (40.00 ^{ab})	1.6169 ^a
24	21	14 (66.67 ^{ab})	14 (66.67 ^{ab})	7 (33.33 ^{ab})	1.5528 ^a
28	17	11 (64.70 ^{ab})	10 (58.82 ^{ab})	5 (29.41 ^b)	1.6858 ^a
32	18	10 (55.56 ^b)	9 (50.00 ^b)	4 (22.22 ^b)	1.6114 ^a

Note: Values in brackets were percentage of treated cows. The superscripts in different small letters in the same column indicate statistical significance ($P < 0.05$)

synchronization protocol Buserelin(0)-PG(7)-Buserelin(9) +mifepristone (BPBMH) was established in this experiment. The main purpose of this study was to evaluate the effect of mifepristone supplementation in the course of BPBMH protocol in cattle. Many researchers have found that the circulating P4 concentrations are able to control the LH surge (Ireland and Roche, 1982; Roberson *et al.*, 1989), final dominant follicle growth so it is essential for the physical rupture of the follicle and ovulation (Sirois and Fortune, 1990; Savio *et al.*, 1993; Monniaux *et al.*, 2008). Mifepristone can improve ovulation by reducing circulating progesterone level through its antiprogesterone effect and consequently induced LH surge responsible for ovulation (Silvia *et al.*, 2002; López-Gatius *et al.*, 2008; Carvalho *et al.*, 2014; Diantonio *et al.*, 2015; Check *et al.*, 2016).

The results showed that Holstein cows treated with BPB in Buserelin dose of 20 μg induced significantly higher ovulation rate (82.61%) and conception rate (65.22%) in cattle when combined with mifepristone (0.4 mg/kg dose) in comparison with relatively lower ovulation rate of 61.90% and conception rate of 38.10% in control group. These results indicated that combination of BPB with Mifepristone can enhance the ovulation rate induced by Buserelin treatment. These findings are in agreement with results of application of Ovsynch method which resulted in conception rate of 60% in India crossbred cows (Bhattacharyya *et al.*, 2017) and ovulation rate of 75% in Nellore cows (Barros *et al.*, 2000). However it resulted in acceptable pregnancy rate of 45% in cycling cattle

synchronized during breeding season (Mitharwal, 2011). Ovsynch treatment in Pakistan Sahiwal cows resulted in ovulation rate of 50% which seemed less than results of current study (Hassan *et al.*, 2016).

Fixed-time artificial insemination (FTAI) was developed as alternative to improve pregnancy rates resulting from AI (Thomas *et al.*, 2014a, b). The present study demonstrated clearly that ovulation took place 16 h after mifepristone treatment and this is the most appropriate timing of AI in cattle. Likewise, this was observed to be 23 h in cattle by other researchers (Kanitz *et al.*, 2006; Tenhagen *et al.*, 2010). Moreover, combination of mifepristone with the Ovsynch does not affect the timing of ovulation (16 h), hence the best time for FTAI remains around 16 h after mifepristone injection. In the current study results showed that AI time 16 h after mifepristone treatment resulted in higher estrus rate (89.47%), ovulation rate (84.21%) and conception rate (63.16%) than other AI times. Previous study involved cattle submitted to the traditional GnRH/ PGF2 α / GnRH treatment, resulted in pregnancy rate of 28.2% in Ovsynch –TAI synchronization with TAI was performed 16 h after the second injection of GnRH (Campanile *et al.*, 2010).

Conclusion

Considering all of the above factors, it can be concluded that estrous synchronization with BPB 20 μg in combination with mifepristone 0.4 mg/kg is associated with an

acceptable pregnancy rate to FTAI compared with BPB in dairy cattle, treatment with BPBMH protocol tended to be associated with a higher overall pregnancy rate of 65.22%. Results showed that BPBMH synchronization protocol achieved good synchronization of stage of the estrous cycle in cattle and the most appropriate time for TAI was 16 h after mifepristone treatment.

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Disclosure Statement

The authors do not have financial or commercial competing interests.

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