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Full Length Article

Mifepristone Synchronization of Estrus and Timed Insemination for Swamp and River Crossbred Buffalo Heifers

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Abstract

The objectives of this study were to optimize the best dose of mifepristone injections in combination with PmPG synchronization protocols and the use of fixed–time artificial insemination (FTAI) to enhance the reproductive efficiency and the use of superior genetics of Chinese crossbred buffalo heifers. A total of 155 crossbred Buffalo heifers were used in this experiment, first 76 heifers were synchronized with PmPGMH in different doses (0.3, 0.4 and 0.5 mg/kg) of mifepristone. PmPGMH in mifepristone dose of 0.3 mg/kg showed the highest significant ($P < 0.05$) estrus rate (84.2%), ovulation rate (89.5%) and conception rate (52.6%) among other methods. Furthermore, 79 crossbred buffaloes were subjected to single FTAI using PmPGMH at h 16, 20, 24, 28 and 32 of mifepristone dose (0.3 mg/kg) treatment to select the best AI time, the results showed that TAI at h 24 was the best insemination time.

Key words: PMSG; Mifepristone; TAI; Buffalo heifers

Introduction

Buffalo is the most common domestic farm animal which can tolerate high temperature and humidity of tropical and subtropical countries so have been used for long time in Asia (Lu *et al.*, 2015). It plays an important role in livestock production and the economy in china. However, the productivity of such animal is significantly diminished by poor reproductive efficiency represented in late puberty, seasonal breeding, low expression of estrus signs and difficult detection of ovulation time, higher fetal and embryonic mortality (Singh *et al.*, 2000; Baruselli *et al.*, 2001; Giuseppe *et al.*, 2005). Silent estrus was one of the biggest problems in buffaloes which increased calving interval due to the failure of the estrous detection (Rajesh *et al.*, 2017). That may be explained due to lower concentrations of hormones in buffalo than in cattle (Lokeshwar, 1990; Jainudeen and Hafez, 2016). Estrus detection greatly influenced the efficiency of artificial insemination programs in buffalo (Akhtar *et al.*, 2014), so different protocols have been developed to synchronize follicular waves and ovulation in predetermined time to support FTAI, these protocols depend mainly on the combination ofP4, E2, eCG (Rensis and López–Gatius, 2007; Carvalho *et al.*, 2013, 2016, 2017; Monteiro *et al.*, 2018), PMSG, GnRH and PGF2 (Turk *et al.*, 2008; Kumar *et al.*, 2010).

PMSG treatment showed great efficiency in estrous synchronization and improved incidence of multiple ovulation in sheep and goat during the breeding season (Greyling and Niekerk, 1991; Mehaisen *et al.*, 2005; Ustuner *et al.*, 2007; Garoussi *et al.*, 2012). Moreover, it enhanced dairy cow ovarian follicular growth and fertility (Souza *et al.*, 2009; Rostami *et al.*, 2011). PMSG combination with CIDR resulted in estrus rate of 59.1% and conception rate of 66.6% and consequently improved fertility in true anoestrus buffaloes. However, PRID+PMSG treatment showed better ovarian follicular growth with average pregnancy rates of 70% (Presicce *et al.*, 2005; Kumar *et al.*, 2010; Caesar *et al.*, 2011).

Wide variation in estrus duration and low estrus expression resulted in difficult prediction of the time of ovulation so the use of AI for genetic improvement in buffaloes is limited (Baruselli, 1994; Baruselli, 2001). The use of these hormonal protocols can control follicular development and ovulation at a predetermined time to induce estrus in large percentage of females to allow TAI without estrus detection (Carvalho *et al.*, 2016; Monteiro *et al.*, 2018). This enable the beneficial use of AI for genetic improvement in buffalo industry (Berber, 2002).

Many recent studies has been performed on synchronization programs in buffaloes, however the average

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herd fertility remained low when compared to dairy cows (Arshad *et al.*, 2017). Moreover, FTAI estrus synchronization protocols gave un satisfactory results in heifers (Dahlen *et al.*, 2003; Lamb *et al.*, 2006). Lower pregnancy rates resulted from FTAI in heifers are mainly due to an inability to synchronize follicular waves with the same success that occurred in cows (Lamb *et al.*, 2006). Improving the reproductive performance of buffalo heifers became an important necessity in order to supply herd with suitable replacements, so attention needs to be focused on better understanding of the mechanisms controlling ovarian follicular growth and development for improving super ovulation responses and conception rates, and reducing embryonic mortality.

In order to optimize existing synchronization protocols in buffalo heifers, it is mandatory to improve the degree of induced synchronization between ovulation of dominant follicle and the time of AI, and to understand the fate of dominant follicle after the end of the synchronization protocol. Many synchronization protocols used a combination of exogenous hormones to regulate the estrous cycle (Muth–Spurlock *et al.*, 2016), However, there is no information about the efficiency of mifepristone injection to promote ovulation in protocols of ovulation synchronization and TAI in buffalo. Mifepristone is a progesterone receptor (PR) antagonist, which works as antiprogestogen by blocking the effects of progesterone to induce ovulation (Carvalho *et al.*, 2014). Thus, the current study aimed to evaluate the efficiency of combining mifepristone in different doses with PmPG synchronization protocols and to undertake a comprehensive evaluation of the response of Chinese crossbred buffalo heifers to this new estrus synchronization protocol. We hypothesized that PmPG treatment with mifepristone will induce a higher ovulation rate than PmPG synchronization protocols, thus increase herd fertility and reduce embryonic losses.

Materials and Methods

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 Animals

A total of 155 crossbred buffalos (Mediterranean × Murrah × Nili Ravi × Jianghan) were raised in a Buffalo Farm in Jinniu, Hubei province, China. The average ambient temperature varied from5 to 28 buffalo Farm in humidity ranging from 55% and 73% in breeding season (September to March) of year 2018. The body condition scores (BCS) of buffaloes varied from points 2.5 to 3.5 at sexually mature stages, with mean body weight, chest girth, body height, body lengths and abdominal girths averaged 433.0 ± 72.1 kg, 186.9 ± 12.9 cm, 136.1 ± 5.8 cm, 128.9 ± 5.8 cm and 120.6 ± 5.5 cm, respectively. All the animals were healthy in regular estrus cycle and physical condition, fed with total mixed ration (TMR) consisted of forage (corn silage, rice straw, peanut vine) and concentrate (corn, wheat bran, soybean meal), and were machine–milked twice a day.

Experimental Groups

There were 2 trials in this study. Trial 1 was performed to optimize the dose of mifepristone in combination of PmPG with hCG in buffalo heifers. A total of 76 buffalo heifers were divided into 4 groups and were synchronized by PmPGMH protocol in 4 doses (0, 0.3, 0.4 and 0.5 mg/kg body weight) of mifepristone respectively. Mifepristone was injected at same time as the injection of GnRH (day 4). Artificial insemination was performed at the afternoon of day 5, which is 24 h after mifepristone injection. An injection of hCG (2000 IU) was given on day 5 after insemination.

Trial 2 was performed to optimize the time of AI in PmPGMH method. A total of 79 crossbred buffalo heifers were treated by PmPGMH protocols in 0.3mg/kg dose of mifepristone. Animals weredivided into 5 groups and given a single AI at h 16 (group 1), 20 (group 2), 24 (group 3), 28 (group 4), and 32 (group 5), respectively after mifepristone injection. An injection of hCG (2000 IU) was given on day 5 after insemination (Fig. 1).

Evaluation of Follicle Development and Ovulation

Follicle development was evaluated by ultrasonography on day 1st (1 day before $PGF_{2\alpha}$ treatment) to 7th day (72 h after the injection of GnRH) by use of 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). Ultrasonography was performed twice a day at 6 am and 6 pm. Follicle dynamics were evaluated on the basis of follicles diameters. The time of sudden disappearance of dominant follicles were considered as ovulation to appear (Gimenes *et al.*, 2011; Liu *et al.*, 2016).

Estrus Detection and Pregnancy Diagnosis

Estrus was observed twice a day (6 a.m. and 6 p.m.) including the presence of vaginal mucus discharge, animal being mounted by others and the presence of > 9 mm follicle. Pregnancy diagnosis was performed by transrectal ultrasonography (Desktop B–type veterinary ultrasound scanner) day 40 after AI (Yindee *et al.*, 2011; Haider *et al.*, 2015; Liu *et al.*, 2016).

Statistical Analysis

Data were analyzed using statistical software package (S.P.S.S. version 17.0 for Windows). The ovulation rate, the growth speed of dominant follicles and the diameter of the ovulatory follicle were expressed as mean ± standard error (SEM). Data for estrous rate, ovulation rate, follicular cyst rate and conception rate were calculated by the chi–squared test using Graph Pad Prism–6 software package (GraphPad Software Inc.), where $P < 0.05$ was considered as statistically significant. Data about follicle diameters were transformed firstly in logarithm (Fig. 3).

Results

Optimal dose of Mifepristone in PmPGMH Methods

Table 1 results showed that buffalo heifers treated by PmPGMH protocol in low dose (0.3 mg/kg) of mifepristone showed shorter ($P > 0.05$) estrus duration time, days of ovulation and initial follicle diameter (Table 1) and the biggest maximum follicle diameters than other groups (Fig. 2).

Table 2 results showed that higher estrous rate (84.2%), ovulation rate (89.5%), conception rate (47.4%), and the lowest silent estrus rate (0.5%) and follicular cysts rate (0.5%), although the follicle diameters at maximal size were not significantly different among all groups (Fig. 2). In comparison of dynamic change of follicle development among buffalo heifers synchronized by PmPGMH in low (0.3 mg/kg), medium (0.4 mg/kg) and high (0.5 mg/kg) doses of mifepristone, we found that the follicle diameter at maximal size in dose of 0.3 mg/kg was greatest among those in other doses. A comprehensive analysis on estrous rate, ovulation rate, conception rate and dynamic of follicle development showed that the dose of 0.3 mg/kg of mifepristone was the best in PmPGMH method for synchronization and TAI (Fig. 3).

Optimization of Insemination time in PmPGMH Method

Table 3 results showed that, after 24 h mifepristone injection artificial insemination time estrus rate (88.89%), ovulation rate (83.33%) and conception rate (50.00%) were higher ($P < 0.05$) than all other groups (Table 3), However follicle growth rate and Ovulation largest follicle diameter (Fig. 4) were not significantly different in all five groups. Considering the economic and management, we suggested that the best AI time in buffalo heifers is 24 h after mifepristone injection.

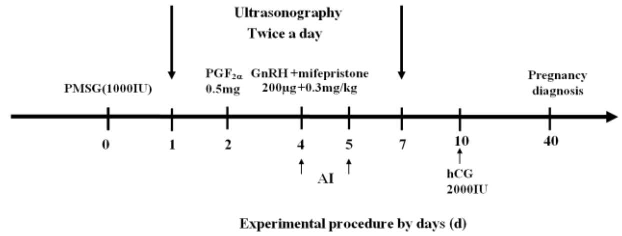

Fig. 1: Schematic diagram of PmPGMH method

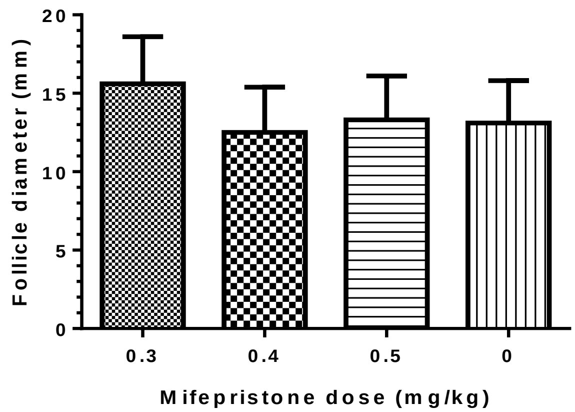

Fig. 2: Follicle diameters (mean ± SEM) at maximal size of buffalo heifers synchronized by PmPGMH in different doses of mifepristone

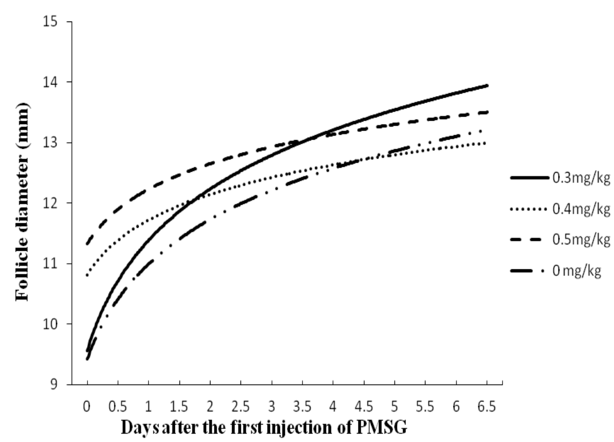

Fig. 3: Dynamic change (diameters, in mean ± SEM) of follicle development in buffaloes synchronized by PmPGMH in low (0.3 mg/kg), medium (0.4 mg/kg) and high (0.5 mg/kg) doses of mifepristone during the first treatment to the day that a follicle grown in maximal diameter. Values in both X- and Y-coordinates were transformed in logarithm

Discussion

Many strategies have been performed to improve estrus and ovulation synchronization and TAI programs for buffalo. However, it failed to attain the same success in heifers (Dahlen *et al.*, 2003; Lamb *et al.*, 2006). The present study developed a new synchronization protocol combined with mifepristone based on the conventional PmPG protocol named PmPGMH–TAI. The effects of different mifepristone doses, as well as the AI time on different reproductive events were also described in Chinese crossbred buffalo heifers in this new protocol.

Table 1: Onset of standing heat, Estrus duration, onset of ovulation and follicle diameter of buffalo heifers synchronized by PmPGMH method in doses of 0, 0.3, 0.4 and 0.5 (mg/kg) of mifepristone

Mifepristone dose (mg/kg)	Onset of standing estrus after second GnRH (h)	Estrus duration after onset of standing estrus (h)	Time of ovulation after second GnRH (h)	Maximum follicle diameter (mm)
0.3 (n=19)	8.3 ± 0.9^{a}	15.2 ± 0.4^{a}	24.2 ± 1.2^{a}	11.4 ± 1.4^{a}
0.4 (n=19)	8.1 ± 0.5^{a}	15.3 ± 0.5^{a}	23.5 ± 1.5^{a}	10.7 ± 1.8^{a}
0.5 (n=20)	9.0 ± 0.7^{a}	15.4 ± 0.8^{a}	24.3 ± 0.8^{a}	11.4 ± 1.5^{a}
0 (n=18)	8.0 ± 0.6^{a}	16.2 ± 0.6^{a}	24.2 ± 1.3^{a}	11.1 ± 1.6^{a}

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

Table 2: Estrus, ovulation and conception of buffalo heifers synchronized by PmPGMH method in doses of 0, 0.3, 0.4 and 0.5 (mg/kg) of mifepristone

Mifepristone dose (mg/kg)	No of animals treated	No of animals in silent estrus (%)	No of animals in heat (%)	No of animals ovulated (%)	No of animals pregnant (%)	No of animals with follicle cysts(%)
0.3	19	1 (5.3^{b})	16 (84.2^{a})	17 (89.5^{a})	9 (47.4^{b})	1 (5.26^{a})
0.4	19	4 (21.1^{ab})	14 (73.7^{ab})	13 (68.4^{ab})	6 (31.6^{ab})	2 (10.5^{a})
0.5	20	6 (30.0^{a})	11 (55.0^{b})	11 (55.0^{b})	4 (20.0^{a})	2 (10.0^{a})
0	18	3 (16.6^{ab})	12 (66.7^{ab})	11 (72.2^{b})	6 (33.3^{ab})	2(11.1^{a})

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

Table 3: Estrus, ovulation and conception and follicle dynamics of buffalo heiferssynchronized by PmPGMH method in different AI time after mifepristone injection

AI time after mifepristone (h) (n=79)	No of animals treated	No of animals in heat (%)	No of animals ovulated (%)	No of animals pregnant (%)
16	15	9 (60.00^{ab})	8 (53.33^{ab})	3 (20.00^{ab})
20	14	11 (78.57^{ab})	10 (71.42^{ab})	5 (35.71^{ab})
24	18	16(88.89^{a})	15 (83.33^{a})	9 (50.00^{a})
28	17	11 (64.71^{ab})	11 (64.71^{ab})	4 (23.53^{ab})
32	15	7 (46.67^{b})	6 (40.00^{b})	2 (13.33^{b})

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

Previous findings suggested that PMSG combination with Crestar implants resulted in higher estrus induction of more than 80% and conception rates of 45– 66.67 % in true anestrous cattle and buffaloes (Nayak *et al.*, 2009; Kumar *et al.*, 2010; Jerome *et al.*, 2016). However, other findings recorded that pregnancy rate was reduced with the increase in dosage of PMSG in dairy cows, it was significantly lower (25%) in the 4 IU/kg dose group than that of 2 and 2.5 IU/kg dose groups (71.4 and 66.7%, respectively). Moreover, higher dose of PMSG resulted in failure of ovulation due to excessive follicular development (Fu *et al.*, 2013).

High progesterone level in follicular fluid resulted in preventing the onset of LH surge, a primary hormone responsible for ovulation, by negative feedback upon the hypothalamus or pituitary thus leading to formation of follicular cysts with the incidence of 2.8% in buffalo heifers to 4.2% in buffalo cows (Luktuke and Arora, 1972; Khan *et al.*, 2011). This follicles abnormally produced high levels of estradiol, which may interfere with embryo development (Barrett *et al.*, 2004; Kiewisz *et al.*, 2011). Mifepristone, as antiprogestogen, can block the effect of progesterone and induce ovulation (Parra *et al.*, 2000; Carvalho *et al.*, 2014; Diantonio *et al.*, 2015; Check *et al.*, 2016). Moreover, A desirable effect on ovulation synchronization was reported after GnRH Injection at 20 and 24 h after progesterone removal, with LH surge created 2 h after its injection in season or out of the reproductive season (Reyna *et al.*, 2010) The results in present study showed that buffaloes treated with Mifepristone (0.3 mg/kg body weight, at the same time with GnRH injection) combined with PmPG method reduced follicular cyst rate (5.0%, 1/19), compared with PmPG treatment that induced relatively higher follicular cyst rate (11.1%, 2/18), thus improved estrous rate (84.2%, 16/19), ovulation rate (89.5%, 17/19) and pregnancy rate (47.4%, 9/19) in buffalo heifers.

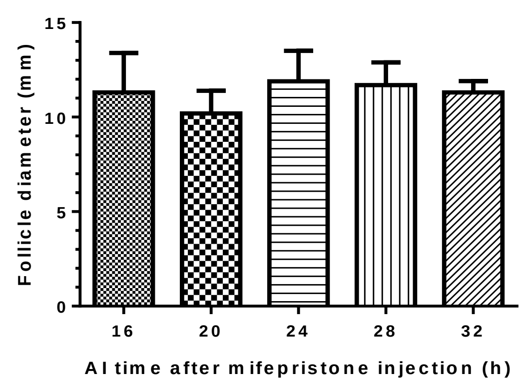

Fig. 4: Follicle diameters (mean ± SEM) at maximal size of buffalo heifers synchronized by PmPGMH in different AI times after mifepristone injection

Silent estrus represented the main obstacle threat in productivity and reproductive efficiency in water buffaloes because of prolonged calving intervals and great loss in milk production (Awasthi *et al.*, 1998; Madam, 1998; Prakash, 2002). It was reported that 30% of buffaloes ovulated without obvious estrous signs after PGF2 treatment (Awasthi *et al.*, 2007). $PGF_{2\alpha}$ treatment in previous studies showed reduction in the concentration of progesterone in plasma in cyclic Murrah–cross buffaloes (Brito *et al.*, 2002). So declined silent estrous rate in present study can be explained due to the antiprogestogenic mechanism of mifepristone to reduce p4 concentration in plasma after mifepristone treatment. The present study showed that buffaloes treated with mifepristone (0.3 mg/kg body weight) combined with PmPG method decreased silent estrus rate (5.3%) in comparison with PmPG treatment (16.6%).

FTAI have been used to improve pregnancy rates by delaying AI of anestrous females 20 to 24 h after the predicted time (Thomas *et al.*, 2014). Previous studies in cows synchronized with PMSG in combination with PGF2α and artificially inseminated 12 and 20 h after standing heat resulted in pregnancy rate of 66.7% (Fu *et al.*, 2013). Present study revealed that PmPGMH synchronization method with TAI at 24 h after mifepristone injection resulted in the best estrus rate (88.89%), ovulation rate (83.33%) and conception rate (50%) in buffalo heifers.

Considering all the reproduction performance, it can be concluded that PmPGMH protocols showed the best synchronization achievements of stage of the estrous cycle 84.2%, induced greater ovulation rate 89.5% and improved pregnancy rate (47.4%) compared with PmPG method (33.3%) after TAI in swamp and river crossbred buffalo heifers undergoing commercial milking.

Conclusion

A new synchronization and fixed–time artificial insemination method named PmPGMH have been developed in crossbred buffalo heifers (Fig. 1). Heifers synchronized with PmPGMH in mifepristone dose of 0.3 mg/kg resulted in better synchronization than other methods. Moreover, AI at 24 h of mifepristone injection is the best artificial insemination time for heifers. The PmPGMH estrus synchronization method is as the following: (1) Crossbred buffalo heifers were subjected to intramuscular injection (I/M) of 100OIU PMSG on day 0, followed by an injection of PGF2α (0.5 mg） on day 2; (2) Simultaneous injection of second GnRH (400 μg) and one injection of mifepristone (0.4 mg/kg) on day 4; (3) Artificial insemination was performed in the afternoon of day 5,which is 24 h after mifepristone injection; (4) I/M injection of hCG (2000 IU) in the afternoon of day 10; (5) pregnancy diagnoses was performed on day 40 after AI.

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