EFFECT OF COMBINATION OF GnRH AND $PGF_{2\alpha}$ FOR ESTRUS SYNCHRONIZATION ON ONSET OF ESTRUS AND PREGNANCY RATE IN DIFFERENT POSTPARTUM IN SWAMP BUFFALO IN KAMPAR REGENCY

Yendraliza[1], B.P. Zesfin[2], Z. Udin[2], Jaswandi[2] and C. Arman[3]

[1] Faculty of Agriculture and Animal Science, State Islamic University SUSKA Riau, Jl. HR. Soebrantas KM 15 Panam, Pekanbaru 28293 - Indonesia.

[2] Faculty of Animal Husbandry, Andalas University, Kampus Limau Manis Padang 25163 West Sumatera - Indonesia.

[3] Faculty of Animal Science, Mataram University, Jl. Majapahit 62 Mataram, NTB 83125 - Indonesia.

Corresponding E-mail: lizafapet@gmail.com

Received January 10, 2011; Accepted February 25, 2011

ABSTRACT

The purposes of this study were to investigate the effect of administration of GnRH and $PGF_{2\alpha}$ on onset of estrus and conception rate in different postpartum swamp buffalo (*Bubalus bubalis*). Twenty postpartum (pp) buffalo-cows were divided into four groups of five buffaloes as follows: Group A (30 day pp), Group B (45 dpp), Group C (60 dpp), and Group D (75 dpp). The buffaloes in each group were injected intramuscularly with 3 ml GnRH at day 0, followed by intramuscular injection of 2.5 ml $PGF_{2\alpha}$, 10 ml) at day 7 after GnRH injection. Observation of estrus was performed 24 h after injection of $PGF_{2\alpha}$. Artificial insemination was performed 18 h after estrus signs clearly visible. The result of study showed that there were no difference ($p>0.05$) among treatments on the percentages of estrus (100%), onset of estrus (37.4 to 38.4 h), duration of estrus (16.8 to 18.2 h) and conception rate (100%). In conclusion, buffalo-cows in Kampar Regency can be served as early as 30 days post partum using combination of 3 ml GnRH and 2.5 ml of $PGF_{2\alpha}$.

Keywords: swamp buffalo, post partum, estrus, conception rate

INTRODUCTION

The buffalo in Kampar Regency, Riau, Indonesia has an important role due to its endurance and adaptability to the local conditions. The buffalo also serves as capital asset to protect against economic risks such as crop failure, and features in religion and cultural events in the region. In Kampar, buffalo population continuously increased from 2002 to 2007, then slightly decreased in 2008 (21000 buffaloes) thereafter increased again in 2009 (21500 buffaloes).

Reproductive efficiency is the primary factor affecting productivity and is hampered in buffalo-cows by the late attainment of puberty, seasonality of calving, long postpartum anestrus and ubsequent calving interval. Moreover, artificial insemination (AI), which is normal practiced in cattle, is seldom performed in buffalo, because of the weakness of estrus symptoms and the variability of estrus length, which make estrus detection is very difficult.

Uterine involution in buffalo is usually completed in 25-35 days after calving (Hafez *et al*., 2000) and the stimulus of suckling lengthens involution time (Arya and Madan, 2001). Early reestablishment of cyclic ovarian activity after calving is essential because the more estrus cycles of a cow have before 30 days postpartum, the fewer services per conception are required (Metwelly, 2001). Thus, treatments given to initiate normal estrus cycles by the first month postpartum should improve reproductive performance (Zain *et al*., 2001). Silent estrus is the single factor most responsible for poor reproductive efficiency in buffaloes (Prakash, 2002).

Literature investigating the therapeutic efficacy of GnRH when administered less than 40 days after calving have examined various reproductive outcomes but report days to first observed estrus; days to first service; days open (or calving to conception interval); service per conception and first service conception rate has not become common yet (Lean *et al*., 2003).

Prostaglandin induces a premature regression of corpus luteum (luteolysis), as a consequence circulating progesterone concentration decreases that allows a sequence of hormonal and ovarian events which culminate in estrus and ovulation (Gordon *et al*., 1996). Various estrus synchronization protocols, i.e. use of progesterone and progestagens (Hattab *et al*., 2000) and prostaglandin $PGF_{2\alpha}$ and its synthetic analogues (Singh *et al*., 2000; Brito *et al*., 2002) have been utilized to enhance estrus detection, thereby facilitating the use of AI.

Borghese (2005) stated that administration of prostaglandin alone or in combination with GnRH increased the conception rate (CR) up to 56%. The ovsynch protocol (GnRH followed by prostaglandin 7 days later and a second GnRH 2 days later) has been used successfully in buffalo with synchronization of time of ovulation in 70-90% and CR of 33-60% (Baruselli *et al*., 1999; Paul and Prakash, 2005). However, there is no information regarding the use of GnRH and $PGF_{2\alpha}$ as protocol for estrus synchronization and AI in postpartum buffalo-cows in Kampar Regency. Thus, the objective of the present study was to determine the effect of estrus synchronization using combination of GnRH and $PGF_{2\alpha}$ on the onset of estrus and the conception rate in different postpartum of swamp buffaloes raised under tropical conditions in Kampar Regency, Riau, Indonesia.

MATERIALS AND METHODS

The experiment was conducted in Kampar Regency, Riau Province, Indonesia. A total of 20 postpartum buffalo-cows belongs to the farmers was selected by rectal palpation. The age of the buffaloes ranged between 4-8 years. Management of these animals was nearly similar and the animals were housed in collective stall during the night, and they were released extensively during the day for free grazing in the surrounding backyards.

The selected buffaloes were then divided into four groups, each group consisted of five buffaloes. Group A represented 30 days postpartum (dpp) of buffalo-cows, Group B 45 days pp, Group C 60 days pp, and group D 75 days pp. All female buffaloes within each group were injected intramuscularly with 3 ml GnRH (Fertagyl®, Intervet International) on day 0, followed by intramuscular injection of 2.5 ml $PGF_{2\alpha}$ (Dinoprost thromethamine, 10 ml) on day 7 after GnRH injection, followed by observation of estrus 24 hours later. Artificial insemination was conducted 18 hours after sign of estrus was clearly visible. The parameters measured were onset of estrus, duration of estrus, percentage of estrus and conception rate. Buffaloes were rectally palpated to confirm pregnancy 40 days after last AI.

Data were statistically analyzed by analyses of variance (ANOVA) followed by Duncan's Multiple range test. The conception rates obtained in the experimental groups were compared using Chi square test (Steel and Torrie, 1991).

RESULTS AND DISCUSSION

The effects of various treatment regimes on estrus synchronization in buffalo-cows are summarized in Table 1. Results of statistical analyses indicated that there was no significant ($p>0.05$) effects of treatment with GnRH and $PGF_{2\alpha}$ on the onset of estrus, duration of estrus, and conception rate among the four treatment groups.

Table 1 showed that the administration of GnRH at day 0 and followed by injection of 2.5 ml $PGF_{2\alpha}$ at day 7 was able to show spontaneous estrus in all cows between day 2 up to day 5 with a 100 percent estrus. This was characterized by mucus discharge, edema and reddish of vulva, mounting behavior and stand to be mounted.

Onset of estrus among different postpartum groups was achieved around 37 to 38 hours in the buffalo-cows. The difference was not significant ($p>0.05$). This was in agreement with previous study reported in buffaloes (Irrikura *et al*., 2003) that administration of GnRH resulted in spontaneous estrus for the next 6 days in buffalo-cows. This was also supported by the works of Moreira *et al*. (2000) and Twagiramungu *et al*. (1992) who found that the administration of GnRH seems to prolong lifespan of CL and/or partially protects the CL against spontaneous luteolysis. Seven days after GnRH injection, newly recruited follicles and luteal structures sensitive to $PGF_{2\alpha}$ are available to respond to a synchronization treatment with PGF improving the precision of estrous synchronization (Zain *et al*., 2001).

However, our study differed from that of Rao and Venkatramiah (1991) who detected estrus around 88% in Murrah buffalo-cows. El-Wishy (2007) reported in their previous study that GnRH treatment decreased the response to a

Table 1. The Estrus Response and Conception Rate in Buffalo-cows

Treatment	Onset of Estrus (h)	Duration of Estrus (h)	Percentage of Estrus (%)	Conception Rate (%)	P Value
PP30	38.0	16.0	100	100	NS
PP 45	38.2	16.8	100	100	NS
PP 60	38.4	17.0	100	100	NS
PP 75	37.4	18.0	100	100	NS

NS: Not Significant ($p<0.05$)

physiological dose of $PGF_{2\alpha}$ during what was normally, a $PGF_{2\alpha}$ susceptible part of the luteal phase.

Results of the study indicated that all treated buffaloes with different postpartum days responded well having synchronized with GnRH in combination with $PGF_{2\alpha}$ in terms of the onset of estrus. This was expressed by clear signs of estrus. However, our study differed from that of Barile (2005) who reported that the average onset of estrous was 54.6 h in buffaloes. Different onset of estrous of buffalo-cows obtained in the present study and that of Barile (2005) might be due to differences in the protocol used for estrus synchronization, designs of the experiment, breed, and age of the animals.

Rates onset of estrus as well as duration of estrus recorded in the present study (24 to 72 h) are still categorized in the normal range as suggested by Noakes *et al*. (2001) and Baruselli (2001). Duration of estrus tended to slightly increase in the four treatment groups of buffalo cows when days of postpartum increased from 30 to 75 days. Though, no significant ($p>0.05$) difference was observed in this parameter.

The results of estrous duration in our study are supported by Kanai *et al.* (1983), Toelihere (1980) and Shalash (1958) who reported that the average duration of estrus was 19 ± 4.4 h in swamp buffalo in Okinawa, 18 to 19 h in swamp buffalo in Indonesia and 11.9 h in Egyptian buffaloes. According to Baruselli (2001) who reported variable duration of estrus buffaloes was 4 to 64 h. However, Chao *et al*. (2010) reported that the average duration of estrous was 15.13 ± 3.52 h in dairy cows, while Deka *et al*. (2009) recorded that the average duration of estrous was 36.00 ± 3.89 h in Crestar cows. This difference is again might reflect the differences in the type of breed, environment, nutrition and body condition (Nanda *et al*., 2003).

In terms of conception rate, addition of GnRH and $PGF_{2\alpha}$ resulted in a 100 percent conception rate in buffalo-cows even though their days of postpartum were different. All cows in group A, B, C, and D showed a 100% conception rate. This was confirmed during rectal palpation that was conducted two months after the last AI. It was observed as well that during AI, the estrus buffaloes in all group A, B, C, and D, showed discharge mucus. Therefore, semen was deposited more easily in the cervix (position 4) from the all groups.

Conception rate obtained in our study with ovsynch protocol followed by artificial insemination in buffalo-cows is considered high when compared to other similar experiments observed by several workers such as Irikura *et al*. (2003); Rao *et al*. (1991); Mialot *et al*. (1999) and Baruselli *et al*. (1999); who found the conception rates of buffalo were 27.22, 37.5, 36.1 and 42.4%, respectively. The differences in conception rate between the present study with the others, could be due to in present study insemination procedure can be accomplished intrauterine. The differences could also be explained by the fact that buffalo-cows in the study were cycling with the presence of a palpable and active corpora lutea without any abnormalities of the reproductive tract. Besides, our study used different protocol during $PGF_{2\alpha}$ in the luteal phase.

In this study, there was clear improvement in reproductive efficiency in buffalo- cows treated at day 30 postpartum by GnRH followed at day 7 by $PGF_{2\alpha}$. These results are in agreement with El-Wishy (2007), who reported that the sequential administration of GnRH and $PGF_{2\alpha}$ was suggested for management of postpartum reproductive activity in problem herds. De Rensis and Lopez-Gatius (2007) revealed that GnRH can be administered at day 14 postpartum followed by $PGF_{2\alpha}$ 14 days later. They suggested that the logical basis for this treatment regimen was that GnRH treatment would enhance ovulation and subsequent $PGF_{2\alpha}$ treatment would induce luteolysis of CL then ovulation. Hafez (2000) suggested that the administration of $PGF_{2\alpha}$ in early postpartum period would reduce the incidence of subclinical uterine infection and hasten the return to a suitable uterine environment for fertilization and pregnancy. Administration of $PGF_{2\alpha}$ at 7-10 days postpartum was effective in facilitating the uterine involution and resumption of ovarian cyclicity and improving reproductive performance (Noakes *et al*., 2001).

Variations in the results of different studies may be attributed to the response of buffalo that treated by GnRH, that might be influenced by physiologic state at the time of treatment (Baruselli, 2001), reflections of environmental and management factors, reproductive disorder or disease and the interval between calving and treatment.

CONCLUSION

Treatment of buffalo-cows in different postpartum periods with GnRH at day 0, followed by $PGF_{2\alpha}$ 7 days later improved their reproductive efficiency. Thus, under the conditions of the study, it can be concluded that the protocol is effective to resume ovarian activities as early as 30 days postpartum, and appears to be a useful estrus synchronization method as well in improving the reproductive performance of Kampar swamp buffalo-cows.

ACKNOWLEDGMENTS

The authors are very grateful to the Directorate General of Higher Education that funded this experiment through Doctoral Grant Project 2010 Directorate General of Higher Education, Ministry of National Education, Republic of Indonesia.

REFERENCES

Arya, J.S and M. Madan. 2001. Postpartum reproductive cyclicity based on ovarian steroids in suckled and weaned buffaloes. Buffalo J.17;361-369

Baruselli, P.S., E.H. Madureira, V.H. Barnabe, R.C. Barnabe, J.A. Visintin, C.A. Oliveira and R. Amaral. 1999. Estudo da dinamica follicular em bufalas submetidas a sincronizacao da ovulacao para inseminacao artificial em tempo fixo. Arquivos da Faculdade de Veterinaria. UFRGS. 27: 210

Barile, V.L. 2005. Reproductive efficiency in female buffalo. In: Buffalo Production and Research. Ed Antonio Borghese. REU Tech. series 67. FAO-Rome. P.77-107

Borghese, A. 2005. Buffalo Production and Research In: Food and Agriculture Organization of the United Nations. Rome. Italy. Technical Series 67. Page.1

Brito, L.F.C., R. Satrapa, E.P. Marson and J.P. Kastelic. 2002. Efficacy of PGF2a to synchronize estrus in water buffalo cows (*Bubalus bubalis*) is dependent upon plasma progesterone concentration, corpus luteum size and ovarian follicular status before treatment. Anim. Reprod. Sci. 73: 23–35.

Chao, L.M., S. Sato, K. Yoshida, Y. Kawano, T. Kojima and C. Kubota. 2010. Comparison of oestrus intensity between natural oestrus and oestrus induced with Ovsynch based treatments in Japanese Black cows. Repro. Dom. Anim. 45: 168-170

De Rensis, F., F. Lopez-Gatius. 2007. Protocol for synchronization estrus and ovulation in buffalo (*Bubalus bubalis*). A review. Theriogenology. 67: 209-216

Deka, I., J. Goswami, P. Chakraborty, R.K. Biswas, B.K. Sarmah and B.C. Sarmah. 2009. Effect of iliren and norgestomat on synchronization of oestrus in cows. Indian J. Anim. Res. 43 (4): 293-294

El-Wishy, A.B. 2007. The postpartum buffalo II. Acylicity and anestrus. Anim.Reprod. Sci. 97: 216-236

Gordon, P.J, A.R. Peters, S.J. Ward and M.J. Warren. 1996. The use of prostaglandin in combination with a GnRH agonist in controlling the timing of ovulation in dairy cows. Reproduction. 24: 164-168

Hafez, E.S.E. 2000. Physiology of reproduction in farm animals. 8th ed Lea & Febiger, Philadelphia. P.59-93

Hattab, S.A., A.K. Kadoom, R. Palme and E. Bamberg. 2000. Effect of CRESTAR on estrus synchronization and the relationship between fecal and plasma concentrations of progestagens in buffalo cows. Theriogenology. 54:1007–1017

Irikura, C.R., J.C.P. Ferreira, I. Martin, L.U. Cimenes, E. Oba and A.M. Jorge. 2003. Follicular dynamics in buffalo heifers (*Bubalus bubalis*) using the GnRH-PGF2α-

GnRH protocol. Buffalo J. 3: 323-327
Kanai, Y., M. Tokugawa and H. Shimizu. 1987. Hormonal events underlying the prostaglandin induced estrus in Swamp buffaloes. Buffalo J. 1(Suppl.) :23–38.
Lean, I. J, JA Potter, A.R.Rabiee, W.F. Morgan, W.P. Tranter, N. Moss and R.J. Rheinberger, 2003. Comparison of effect of GnRH and prostaglandin in combination and prostaglandin on conception rate and time to conception in dairy cows. Aust. Vet. J., 81: 8.
Metwelly, K.K. 2001. Postpartum anestrus in buffalo cows; cause and treatment. In; Proc sixth.sci Congr. Egypt.Soe Cattle Disease. Assiut University, Egypt. P:259-267
Mialot, J.P., G. Laumonnier, C. Ponsert, H. Fauxpoint, N.E. Barassi, A.A. Ponter and F. Deletang. 1999. Postpartum suboestrus in dairy cows; comparison of treatment with prostaglandin F2α or GnRH + prostaglandin F2α + GnRH. Theriogenology, 52: 901-911
Moreira, F., R.L. de La Sota, T. Diaz and W.W. Thatcher. 2000. Effect of day of estrous cycle at the initiation of a timed artificial insemination protocol on reproductive responses of dairy heifers. J. Anim. Sci. 78: 1568-1576
Nanda, A.S., P.S. Brar and S. Prabhakar. 2003. Enhancing reproductive performance in dairy buffalo; major constrain and achievement in Proceding of the sixth International Symposium on Reproduction In Domestic Ruminants Vol.61, Crieff. Scotland UK, pp: 27-36
Noakes, D.E., T.J. Parkinson and G.C.W. England. 2001. Arthur’s Veterinary Reproduction and Obstetrics. 8th ed. Baillier Tindall, London
Paul, V and B.S. Prakash. 2005. Efficacy of the ovsynch protocol for synchronization og ovulation and fixed time artificial insemination in Murrah buffaloes (*Bubalus bubalis*). Theriogenology. 64: 1049-1060
Perera, B.M.A.O. 2010. Reprodctive cyles of buffalo. Anim. Reprod. Sci. 121: 189-300.
Prakash, B.S. 2002. Influence of environment on animal reproduction. Invited Paper: national workshop on animal climate interaction, held at Izatnagar. Indian J. Anim. pp: 33–47.
Rao, A.V.N and P. Venkatramiah. 1991. Induction and synchronization of estrous and fertility in seasonally an ooestrus buffaloes with GnRH and PGF analog. Anim. Reprod. Sci. 25: 109-113
Singh, J., A.S. Nanda and G.P. Adams. 2000. The reproductive pattern and efficiency of female buffaloes. Anim. Reprod. Sci. 60:593–604.
Steel, R.G.D. and J.H. Torrie. 1991. Principles and Procedures of Statistics. McGraw-Hill Book Co. Inc. New York.
Toelihere, M.R. 1980. Biological aspects of reproduction and artificial insemination of the swamp buffalo, 1n:Buffalo Production for Small Farms, Food and Fertilizer Technology Center. Taipei. pp: 120-135
Zain, A.E., A. KH. Abdel-Razek and M.M. Anwar. 2001. Effeect of combined using of GnRH and PGF2α on ooestrus synchronization and pregnancy rate in buffalo-cow. Assiut Vet.Med.J. 45: 89.