ENHANCING PROSTAGLANDIN-BASED ESTRUS SYNCHRONIZATION PROTOCOL FOR ARTIFICIAL INSEMINATION IN WATER BUFFALOES

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ABSTRACT

Estrus synchronization (ES) in tandem with artificial insemination (AI) is a breeding technique which expands the use of male germplasm for genetic propagation. However, in buffalo reproduction in general, the difficulty in estrus detection does not permit the accurate timing of AI leading to low reproductive efficiency. The present study was conducted to evaluate pregnancy rates following four ES protocols aimed at improving reproductive efficiencies of water buffaloes. Post-partum water buffaloes (n=262) with corpus luteum, with body condition score of ≥3, and with ovary size of ≥2 cm were used in this study. The animals were randomly allocated into four treatment groups: T1, control - ES with single injection of prostaglandin (PGF2α, 25 mg i.m.) and inseminated after 3 days; T2 - the same as T1 except that the administration of gonadotropin releasing hormone (GnRH; 100 μg i.m.) was done a day before AI; T3 - same as T2 but with the administration of GnRH on the day of AI; and T4 - same as T3 but with the administration of human chorionic gonadotropin (hCG; 1500 IU i.m.) at AI. Determination and confirmation of pregnancy through rectal palpation was done at day 60 post AI. Results of the present study revealed a significant difference (P<0.05) between the control and the treatment groups; however, no significant difference was observed among the three groups of ovulatory hormones. These findings suggest that the administration of ovulatory hormones improved pregnancy rate in water buffaloes and the use of GnRH or hCG at the time of AI is advantageous and cost-effective approach to conduct AI activities in the field.

Keywords: *Bubalus bubalis*, buffaloes, estrus synchronization, gonadotropin, prostaglandin, water buffaloes

INTRODUCTION

The water buffalo (*Bubalus bubalis*) is a major component of the Philippine agriculture as an important source of milk, meat, hide and draft power. There are about 2.89 million buffaloes in the Philippines, 99% of which is with small-hold farmers and 0.1% is at commercial level. The Philippine Carabao Center (PCC) takes the

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lead in conserving, propagating, and promoting water buffaloes aimed at increasing income and improving the general well-being of the Filipino farming communities. However, the productivity and profitability from buffalo raising is affected by its reproductive efficiency because of various factors such as poor estrus expression, breeding seasonality, long anestrous period and calving interval and estrus/ovulation asynchrony. In addressing these reproductive concerns and in keeping with the mandate, PCC has developed the Genetic Improvement Program with the use of Assisted Reproductive Technology (ART) as a key breeding strategy.

Artificial insemination (AI) is a major tool for expanding the usage of superior germplasm of paternal origin, and currently is the widely used breeding technique in buffaloes in the Philippines. It is considered the banner program for genetic improvement program of buffaloes with national scope and being an important component of the socio-economic program of PCC. Along with AI technology is the conduct of estrus synchronization for greater applicability of AI technology in the country. Generally, with this synchronization strategy, the female animals are artificially inseminated based on the manifestation of estrous signs. However, buffaloes are generally known to exhibit "silent heat" phenomenon, causing difficulties in estrus detection by human mainly due to poor expression of estrus by the animal with less precise timing of AI which eventually results in low conception rate and calf drop.

The Estrus Synchronization and Artificial Insemination technologies are indispensable reproductive tandem for buffalo propagation in the Philippines over the last three decades. It has been implemented targeting a large number of female buffaloes to be inseminated at a pre-determined time. Under this technique, female animals are pooled together, examined for reproductive status, and non-pregnant animals are induced to come into synchronous estrus and inseminated at the same time. This technique is suitable under Philippine setting because of the limited numbers of trained technicians and the scattered demographic location of the animals handled by individual farmers. Linkages with partners in the implementation of the AI program include agricultural officers of the Local and Regional Government Units, and the village-based AI technicians. It is worth mentioning though that the reproductive condition of the animal is exacerbated by the logistical concern/requirement of the program. Over the last 10 years, the number of animals that were estrus synchronized and artificially inseminated tremendously increased; however, the overall efficiency remains far from the desired target. Given the current set up, scope and span of the Artificial Insemination Program in the country, there is a serious need to develop a reproductive management strategy most suitable to the current breeding system or AI program. Specifically, enhancing the prostaglandin-based estrus synchronization protocol is the most purposeful area to focus on considering its close functional association with AI efficiency. Moreover, in the light of climate change, there could be limitations in the current estrus synchronization program, thus it is the need of the hour to come up with a climate change-responsive estrus synchronization program.

To synchronize the estrous cycle, ovarian activity is manipulated so that the time of ovulation can be predicted. This is achieved by controlling the luteal phase of the cycle through the administration of prostaglandins or progesterone analogues or controlling the follicular development and ovulation using different combinations of prostaglandins, progesterone, GnRH, hCG, eCG and estradiol (De Rensis and Lopez-Gatius, 2007). Cumulative works on the use of PGF2α and its synthetic analogues in buffaloes have been reported (Chantaraprateep, 1987; Kamonpatana *et al*., 1987; Pant *et al*., 1992; Brito *et al*., 2002). Prostaglandin causes lysis of the corpus luteum (CL) during the responsive phase and a consequent decrease in the levels of progesterone leading to the development of follicles of the next wave (Galina and Orihuela, 2007). The use of gonadotropin releasing hormone (GnRH) and prostaglandin (PGF2α) has proven to be very successful in synchronizing estrus in cattle and buffaloes (Lamb *et al*., 2004; Amaya-Montoya *et al*., 2007). In addition, various estrus synchronization protocols using progesterone and progestagens (Saini *et al*., 1986; Singh *et al*., 1988; Luthra *et al*., 1994; Subramaniam *et al*., 1994) have been utilized to enhance estrus manifestation and detection, thereby facilitating the use of AI for genetic improvement program.

The Philippines through its Research and Development efforts to improve AI efficiency in water buffaloes have been strengthened over the years. In a related research work, the follicular response and occurrence of ovulation in estrus synchronized buffaloes was investigated by transrectal ultrasonography. A widely variable ovulation time among the estrus synchronized animals after prostaglandin injection was observed with some animals failed to ovulate at all. There is a great need to shorten the range of ovulation at the end of the estrus for a more precise timing of AI, a need to ensure follicular growth and maturation, and a need to increase rate of ovulation following estrus synchronization treatment. Thus, enhancing the current prostaglandin-based estrus synchronization protocol with ovulatory hormones is the premise of the present study. Specifically, the efficacies of different estrus synchronization protocols were compared in an attempt to improve conception rate following AI in water buffaloes.

MATERIALS AND METHODS

Animal selection and determination of body condition score

Water buffaloes of at least 40 days postpartum, with approximately ≥2 cm size of ovaries, with corpus luteum (CL) and with a body condition score (BCS) of not less than 3 were selected and used in the present study. The ovarian sizes were measured and detection of CL was done using an ultrasound scanner (HS-1600, Honda Electronics Co., Ltd. Japan). Evaluation of BCS was done according to the method described by Alapati *et al*. (2010). Briefly, a BCS of 1 stands for emaciated animals; a BCS of 2 indicates a dorsal spine that is pointed to the touch, with the hips, pins tail head and ribs being prominent; a BCS of 3 represents those buffaloes whose ribs are usually visible with little fat cover and dorsal spine are barely visible; a BCS of 4 is for animals that are smooth and well covered, but with no marked fat deposits; and BCS of 5 is for heavy deposits of fat clearly visible on the tail head and brisket, with the dorsal spines, ribs, hooks and pins fully covered and unable to be felt even with firm pressure. The tonicity of the uterus is evaluated following the system used at PCC which is soft/flaccid for tone 1; slightly hard for tone 2; and very hard for tone 3. The selected animals were randomly assigned into four (4) treatment groups namely: Treatment 1 (Control); At day 0, the animals were injected with prostaglandin (PGF2α, Lutalyse, 25 mg, Pharmacia and Upjohn Co., MI, USA) and first artificial insemination (AI) was performed at least 72 h after PGF2α injection with a follow-up AI after 8 h. In Treatment 2, the animals were injected with gonadotropin releasing hormone (GnRH, Cystorelin, 100 µg, Merial Ltd., GA, USA) a day before AI; Treatment 3, GnRH was given at the time of AI; and Treatment 4, human chorionic gonadotropin (hCG, Chorulon, 1500 IU; Intervet Inc. Summit, NJ 07001, USA) was administered at the time of AI. All treatments were replicated 4 times.

Artificial insemination and pregnancy diagnosis

All the animals were artificially inseminated 72 h after PGF2α injection and repeated 8 h later with frozen-thawed buffalo semen derived from bulls with proven fertility. Artificial insemination was done by two (2) selected AI technicians of the Center. Selection of AI technician was based on their performances for the last 4 years. Presence of mucus discharge and tonicity of the uterine horn were determined at the time of artificial insemination. Detection of pregnancy was done at 60 days post AI through rectal palpation.

Statistical analysis

The data were analyzed and compared by analysis of variance (ANOVA) followed by Tukeys-Kramer's HSD, as *post hoc* test. Data were presented as means±SD. All analysis was performed using JMP Statistical software (version 11.1.1 SAS Institute Inc., Cary, NC, USA). The minimum considered level of significance was set at $P<0.05$.

RESULTS AND DISCUSSION

Artificial insemination efficiency is affected by various intrinsic and extrinsic factors; however, of major concern is the timing of semen introduction into the female reproductive tract to fertilize the matured oocytes released from the ovary. The basis of conducting AI to female animal is the presence of behavioral estrus, either naturally or by estrus synchronization.

The results of the present study revealed that the four protocols generally induced estrus synchronization among the treated animals showing signs of estrus (mainly mucus discharge and with uterine tone of 2 to 3), ranging between 97 to 100% at 72 to 80 h after the injection of prostaglandin (Table 1). For the first protocol the percentage of animal that manifested estrus signs was 97.37%, followed by 98.46 for the second protocol, and 100% each for the third and fourth protocols and there were no significant differences ($P>0.05$) among the four treatments. Expression of estrus after prostaglandin injection and before fixed-time AI has been reported to change the uterine environment, increase accessory sperm numbers, fertilization rates, and overall embryo survival (Richardson *et al*., 2016). The result of the present study indicates that the parameters used in the selection of animals for hormonal treatment: presence of functional CL, a body condition score of not less than 3, and size of the ovaries of not lower than 2 cm are effective approach to achieve better response and outcome for estrus synchronization protocols. In some cases, the animals could have ≤5 days old CL which is insensitive to PGF2α treatment thus failed to respond to the action of prostaglandin.

The present study underscores body condition of the animals as critically important factor influencing AI outcome. It was shown that the body condition at the beginning of the breeding period and at estrus affected estrus manifestation but did not affect conception rate (Crudeli *et al*., 1999). However, post-partum buffaloes having a BCS of 2.5 to 3.5 are more likely to get pregnant than thin (≤2.0) or over-conditioned (>3.5) buffaloes (Rahman *et al*., 2012). Thus, management of BCS has a tremendous impact on estrus expression and eventually conception rate.

Essentially, the pregnancy rate of artificially inseminated water buffaloes has been improved following treatment with ovulatory hormones in comparison with the non-treated group (Table 1). Significant differences (P<0.05) exist between Treatment 1 (Control) and Treatments 2, 3 and 4, which indicate that administration of GnRH and hCG around the time of AI results in higher rates of pregnancy. This finding is in agreement with the previous reports by other researchers (Berber *et al*., 2002; Pursley *et al*., 1995) on the superiority of AI performed when GnRH is injected before insemination. The difference observed between the control or prostaglandin alone and GnRH treated protocols could be attributed to the action of GnRH at the level of the anterior pituitary gland to stimulate the release of LH to cause ovulation of the dominant follicle that eventually yielded a good corpus luteum. On the other hand, for Treatment 3, GnRH given at the time of AI may help in the survival of fetus and may prevent the lysis of CL which is required for embryonic development. For Treatment 4, in which exogenous hCG replaced GnRH at the time of AI, it could be possible that hCG could have acted directly on the dominant follicle in the ovary that eventually caused ovulation and CL formation for the maintenance of pregnancy. The present result is however is not in agreement with previous study wherein the pregnancy achieved with progesterone alone is highly comparable with GnRH supplemented protocols (Gupta *et al*., 2008). Moreover, the pregnancy rates achieved for prostaglandin (64.3%) and GnRH given before and after AI (64% and 63%, respectively) were higher compared with the present results. This noted discrepancy could be attributed to the different management of the animals especially after insemination and the environmental condition or season at the time of the study. Moreover, De Rensis and Lopez-Gatius, (2007) pointed out that the animal's follicular status at the beginning of treatment could account for the differences of results obtained following synchronization protocols.

In our earlier experiment (unpublished), ovulation of dairy animals subjected to estrus synchronization following a single injection of prostaglandin was monitored by ultrasound. A wide variation in the ovulations time was noted, taking several days to ovulate, with some animals not ovulating at all after the manifestation of estrus. This phenomenon could be the possible reason for the low pregnancy rate with PGF2α alone, which can induce estrus but not necessarily the subsequent ovulation, thus the need for use of exogenous ovulatory hormones. In the present study, the higher pregnancy rate of animals that were given GnRH or hCG a day before or at the time of AI could be attributed to their LH-like action leading to tighter ovulation after the occurrence of estrus. Furthermore, the gonadotrophins could help in the final development of the follicles and maturation of the oocytes before ovulation occurs. To ensure ovulation, GnRH was given at the time of insemination (De Rensis and Lopez-Gatius, 2007). Similar pregnancy rates were achieved in both animals given GnRH and those managed under the classic Ovsynch protocol (48 vs. 50%).

The present study demonstrated that administration of ovulatory hormones around estrus period enhanced AI efficiency in water buffaloes as it also underscores other important parameters at the initiation of the treatment contributing to the overall success rate achieved in the present study. Specifically, this work emphasizes the effectiveness and recommends the estrus synchronization protocol: prostaglandin with GnRH or hCG at the time of AI, to be adopted in a wide scale AI program to minimize logistics, labor, and cost of the conduct of AI. Essentially, any improvement in buffalo reproductive efficiency holds huge influence on animal productivity and potential impact on the socio-economic aspect of the genetic improvement program in water buffaloes.

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Table 1. Percent pregnancy of artificially inseminated water buffaloes treated with different estrus synchronization protocol.

Treatments	No. of animals treated	No. of animals observed in estrus (%)	No. of animals pregnant	Percent pregnancy
Treatment 1	76	74 (97.37)	16	21.052 ± 2.62^{b}
Treatment 2	65	64 (98.46)	24	36.923 ± 1.34^{a}
Treatment 3	59	59 (100.00)	21	35.593 ± 1.74^{a}
Treatment 4	62	62 (100.00)	24	38.709 ± 1.68^{a}

^{a,b} Values are means±SD of 4 replicates. Within columns, values with different superscripts are significantly different(P<0.05).

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