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RESPONSES OF LOCAL BUFFALOES TO ESTRUS SYNCHRONIZATION AND FIXED TIMED ARTIFICIAL INSEMINATION IN SUBTROPICAL BHUTAN

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ABSTRACT: The study evaluated four different hormonal synchronization and FTAI protocols on estus response and conception rate of local buffaloes under Samtse district. In total, 38 potential buffaloes were selected and divided into four groups. The animals of either primiparous or multiparous were randomly selected irrespective of parity, age, breed, milk yield and body weight due to small female population base. The protocols were evaluated in two different seasons; seasonal anestrus (March to April) and breeding season (September), 2018. The data were descriptively analyzed to deduce estrus and conception responses from synchronization and FTAI. The findings indicated that there was a marked difference in the conception rate using four different protocols. The conception rate of 30% in buffalo with the application of P3 protocol during the breeding season was found to be higher when compared to other protocols. It can be inferred that application of P3 protocol during breeding season in buffaloes is found to be promising. However, owing to disproportion of sample size bewteen treatment groups, the findings from current study may not be recommended for wider appliaction in the field. For future research, it is suggested to use appropriate sample size with standard estrus synchronization and FTAI protocols in other buffalo rearing areas for better outcomes. The use of germplasm from superior dairy buffalo breeds like Nili Ravi and Murrah is also recommended to revive and improve the productivity of local or non-descript buffaloes in the country.

Keywords: Buffaloes; estrus synchronization; fixed time AI; Nili Ravi.

1.INTRODUCTION

Buffalo (*Bubalus bubalis*) plays an important role in the world for the production of animal protein. Buffalo has a significant role in many developing countries as it provides milk, meat, hide and draught power. The world population of buffalo is estimated at 199 million (FAOSTAT 2012), of which more than 96% are found in Asia. In recent decades, buffalo farming has expanded widely in the Mediterranean and Latin America. Central/ Northern Europe also introduced several herds of buffaloes. The global buffalo population is estimated to be increasing by 10% annually, reflecting the growing interest worldwide (Cruz 2007). Dairy buffaloes have been used for milk production in India, Pakistan, other South Asian countries, the Middle East and Italy. Dairy characteristics are also being induced in the local population of Indo-Chinese Region and South America through crossbreeding with genetic materials of Pakistani Nili Ravi and Indian Murrah buffaloes. In China, the milk yield of buffaloes increased from 700 to 2,000 kg/year through crossbreeding (Yang et al. 2007).

Buffaloes are major producers of milk in neighbouring countries of India and Pakistan. Of the total milk produced in India, 55% is from buffaloes, 42% from cattle and 3% from goats.

Buffalo milk is high in butter fat that makes it suitable for making the true mozzarella cheese. However, in Bhutan, buffalo farming declined in the last decade, owing to less emphasis on buffalo farming (Sherpa et al. 2007). Local buffaloes are non-descript low producing animal reared by farmers in the subtropical region of Bhutan. Buffalo population has dwindled to less than 477 heads and contributes less than 1% of total milk produced in the country (Department of Livestock (DoL) 2020).

Some common issues facing buffalo farming are lack of suitable breeds, unavailability of quality breeding bull, lack of Artificial Insemination (AI) facilities, and relatively poor productivity. Buffaloes are better adapted in the subtropical region of Bhutan, and produce reasonably more milk than good quality cattle even under low input production system. The subtropical region also has abundant forages to support buffalo farming.

Improvements in livestock production and reproductive technology had contributed significantly in reduction of poverty and malnutrition globally (Hemme and Otte 2010). Genetic improvement remained as a major factor behind the dairy profitability in the last few decades (Miglior et al. 2012). Synchronization of estrus contributes to optimizing the use of time, labour and financial resources by shortening calving season and increasing uniformity of calf crop (Lamb et al. 2001). Further, there is a great potential for enhanced AI performance if problems such as non-cycling female through estrous synchronization and Fixed Time Artificial Insemination (FTAI) are addressed regularly. One of the limiting factors in the application of AI in buffaloes is difficulty in estrus detection which led to decreased efficiency when the traditional AI was employed. Estrus occurs mainly at night, and only few females exhibit proper heat behavioural signs that are discrete. Therefore, an alternative to increase the number of buffaloes to be inseminated is through use of hormonal protocols that will allow AI without the need of estrus detection, using FTAI. The use of FTAI is an advantage because it can be scheduled to pre-determined hours of the day, simplifying the management currently required in normal AI program. Further, expanding knowledge of the control of follicular wave dynamics during the buffalo estrous cycle has resulted in precisely controlling follicular and luteal dynamics and finely controlling the time of ovulation. Follicular wave development can be controlled by treatments with hormonal drugs. Thus, estrus synchronization protocols can result in synchronous onset of a new follicular wave, synchronous ovulation and acceptable pregnancy rates after FTAI in cycling buffalo during the breeding season and in anestrous buffalo during the off-breeding season. These protocols permit the use of AI throughout the year that could be utilized to increase the use of AI in buffalo herds. This makes the use of hormonal protocols (associated with FTAI) in buffaloes more advantageous and practical (Baruselli and Carvalho 2005).

However, currently, there is lack of proper scientific study to test whether the use of hormonal protocols can be effective in local buffaloes. Therefore, this study was conducted with the main objective to evaluate the responses of buffaloes to different hormonal estrus synchronization protocols, under the subtropical farming conditions of Bhutan.

2. MATERIALS AND METHODS

2.1 Study location and duration

The study covered potential buffalo rearing areas of Tashicholing, Namgaycholing and Dophuchen gewogs (sub-district) under Samtse Dzongkhag (district), Bhutan. The study was carried out between March to April and September months in 2018, corresponding to the annual seasonal anestrus and the breeding season of local buffaloes, respectively.

2.2 Description of semen and selection of animals

In 2015, Bhutan received 200 doses of Nili Ravi buffalo semen from SAARC Agriculture Centre (SAC), Dhaka. Nili Ravi buffalo originated from Pakistan. It is characterized by high milk yield, producing as high as 32 kg of milk per day and hence being propagated as a milch breed throughout Pakistan and India. It is widely used to cross with other low producing buffalo breeds to upgrade their genetic potential and enhance productivity of such breeds. The breed is stout, well adapted and can perform well despite fed with roughages viz. paddy straws, wheat straws and corn stalks. Considering the breed's production potential with minimal management care required, the germ plasm from this breed can be well utilized to upgrade the genetics and production of buffalo population in the country.

To revive buffalo farming and improve local buffalo population in the country, quality germ plasm from promising dairy buffalo breed (Nili Ravi) through estrous synchronization and FTAI was initiated for the first time in the country. All interested farmers were briefed on the semen of Nili Ravi buffalo breeds and explained the need to upgrade their buffalo population through cross breeding (estrus synchronization and FTAI). Potential buffaloes of interested farmers were per rectally examined to confirm pregnancy, reproductive status and reproductive ailments (if any) before the commencement of the study.

Animals were selected randomly irrespective of parity, age and milk yield. Buffaloes with body weight within the range 400 – 600 kg were selected since adequate body weight and body condition are important for fertility. Buffaloes below 300 kg body weight were not considered for the study. Buffaloes belonging to Murrah, Surti and non-descript breeds with body condition scores of 2.5 to 3.5 in a 1-5 scale (Edmondson et al. 1989) were also considered. Animals suffering from clinical reproductive problems were excluded from the study. A total of 38 potential buffaloes were included in the study. The selected animals were allotted in four groups, estrus synchronized and FTAI performed using four different protocols.

2.3 Experimental Design

Thirty-eight animals were divided into four groups (G): G1 (n=6), G2 (n=15), G3 (n=13) and G4 (n=4). Four different estrus synchronization and FTAI Protocol (P): P1, P2, P3, and P4 were used in the study. The animals in G1, G2, G3, and G4 were estrus synchronized and inseminated using protocols P1, P2, P3, and P4, respectively. In the design, the disparity on the allocation of animals in four treatment groups was due to logistical challenges and small breedable population base in the study location.

2.4 Description of Estrus synchronization and FTAI protocols

The details of different hormonal products used, route of administration and their doses are presented in Table 1.

Table 1: Hormonal products

Product	Commercial Name	Administration	Dose (ml/mg)
Progesterone	CIDR-B	Vaginal implant	1 implant / 1380 mg
Gonadotropin Releasing Hormone (GnRH)	Receptal	I/M injection	2.0 ml / 0.0084 mg
Prostaglandins (PGF2α)	Lutalyse	I/M injection	5 ml / 25 mg

Controlled Internal Drug Release - Bovine (CIDR-B) devices are intravaginal progesterone

Table 2: Estrus synchronization and FTAI Protocols

Protocol	Group	Description	FAIT
P1	G1	Insert CIDR-B, removal on Day 7 Observe animals for estrus 1- 2 days (24 – 48 h) after removal & FTAI	1st AI 24 h after Insert removal 2nd AI 48 h after removal
P2	G2	P1 + Inj GnRH (2 ml) on day of insert (Day 0) + Inj PGF2α (5 ml) on removal (Day 7) + Inj GnRH (2 ml) after 2nd FTAI	1stAI 24 h after Insert removal 2nd AI 48 h after removal
P3	G3	P1 + Inj GnRH (2 ml) on day of insert (Day 0) + Inj PGF2α (5 ml) on removal (Day 7) + Inj GnRH (2 ml) after 2nd FTAI	1st AI 48 h after Insert removal 2nd AI 72 h after removal
P4	G4	Inj PGF2α (5 ml) on day of examination (Day 0) after palpating CL Observe animals for estrus 2- 4 days (48 – 96 h) after PGF2α inj. & FTAI + Inj GnRH (2 ml) after 2nd FTAI	1st AI 72 h after PGF2α inj. 2nd AI within 84 h after PGF2α

insert, used alone or in conjunction with other hormones to synchronize estrous in livestock. CIDR-B is T shaped device with flexible wings that collapse to form a rod that can be inserted into the vagina with an applicator. On the end, opposite to the wings of the insert a tail is attached to facilitate removal of the device. The backbone of the CIDR is a nylon spine covered by progesterone (1.38g) impregnated silicone skin. Upon insertion, blood progesterone concentrations rise rapidly with maximal concentrations reached within an hour after insertion. Progesterone concentrations are maintained at a relatively constant level during the seven days the insert is in the vagina. Upon removal of the insert, progesterone concentrations are quickly eliminated which facilitates the animal to come to heat. Gonadotropin Releasing Hormone (GnRH) is produced naturally in the brain and causes release of other hormones which are important for follicular development and ovulation. When injected into cattle, GnRH causes ovulation of the dominant follicle. Prostaglandins (PGF2α) are naturally produced in the body to regress corpus luteum which produces progesterone during the middle of the estrous cycle. When this occurs, the dominant follicle is allowed to complete development, produce estrogen, which causes cows to come into heat and ovulate. When PG is injected at the correct stage of estrus cycle the same sequence of events will occur. Thus, the above hormonal drugs are used in buffalo estrus synchronization to precisely control follicular and luteal dynamics and time of ovulation. Table 2 summarizes different protocols used in different groups during the study.

2.5 Estrus response and Pregnancy Diagnosis

In all the groups, estrous responses to different hormonal protocols were observed by external heat signs exhibited and confirmed through rectal palpation. In G1, G2 and G3; animals were observed for heat signs 24 – 48 h after removal of CIDR-B, while in G4, heat was observed 48 - 96 h after PGF2α injection. The prominent heat signs in buffaloes like swollen, edematous vulva, frequent urination, sharp longer duration bellowing, reddish, moist and glossy vulvar mucous membrane and scanty not ropy mucus discharge were observed.

The estrus response to different estrus synchronization protocols was confirmed through uterine tone and cervix status through per rectal examination. All the animals in the four groups were per rectally examined to confirm pregnancy status after three months post FTAI by an experienced AI personnel. Pregnancies resulting from FTAI were validated by rectal palpation by a veterinarian and by calving dates. The pregnancy diagnosis was carried out to determine the conception rate and eventually calving rate of the buffaloes which were estrus synchronized and performed FTAI using four different protocols.

2.6 Data collection and Data analysis

The data collected was entered in Microsoft Excel sheet. Descriptive analysis was used to summarize the estrus and conception responses of four groups to four different protocols. The data for number of animals responded to estrus synchronization and FTAI and number of pregnant animals in the four respective protocols applied were compared.

3. RESULTS AND DISCUSSIONS

3.1 Retention rate of Intravaginal progesterone insert

The retention rate of the devices during the seven-day period was 100%. There was just one case of vaginal irritation and discomfort after inserting the device in a local buffalo. The inserts were developed for one-time use only, and after use they were collected and disposed properly.

3.2 Estrus response rate

The response rates to estrus synchronization Protocols- P1, P2, P3 and P4 were 83.3 %, 100 %, 100 % and 75%, respectively (Table 3). All the buffaloes (100%) in G2 and G3 responded to estrus synchronization, while 83.3 % of animals in G1 and 75% in G4 responded to estrus synchronization. The animals that responded to assigned protocols exhibited heat signs in some form or the other, though not as prominent as seen in cattle. Heat signs like swollen, edematous vulva, frequent urination, sharp longer duration bellowing, and reddish, moist and glossy vulvar mucous membrane and scanty not ropy mucus discharge were observed in most estrus synchronized buffaloes. The response rate was confirmed through uterine tone and cervix status through per rectal examination. Further, most animals that responded to estrus synchronization protocols were in proper heat either during 1st or 2nd FTAI. The animals were confirmed in heat as observed by external heat signs and uterine tone on rectal palpation.

The estrus response rates to four estrus synchronization protocols in buffaloes were between 75 - 100%. Unlike cattle, which mount over other cows when they come in heat and stand for mounting when they are in standing heat was not observed in buffaloes in the present study.

Table 3: Estrus response rate of buffaloes to different estrus synchronization & FTAI protocols.

Protocols	N	No. of Estrus responded	Estrus response rate (%)
P1	6	5	83.3
P2	15	15	100
P3	13	13	100
P4	4	3	75
Total	38	36	89.6

3.3 Conception rate

The conception rates of four protocols are presented in Table 4. The percentage of pregnant animals ranged from 0 to 30.8% for the four different protocols used. Out of 36 animals that responded to estrus synchronization and FTAI, 7 had conceived and the mean conception rate was recorded at 19.4%.

The proportions of pregnant buffaloes, as a result of application of protocols, were 20.0% (1/5), 13.3% (2/15), 30.8% (4/13) and 0.0% (0/3), for P1, P2, P3 and P4, respectively. The protocol P3 produced the highest number of pregnant buffaloes, compared with P1, P2 and P4. P1 also produced higher number of pregnant animals, compared with P2 and P4.

Table 4: Mean conception rate in buffaloes on application of four different estrus synchronization & FTAI protocols.

Protocols	N	No. Conceived	Conception rate (%)
P1	5	1	20.0
P2	15	2	13.3
P3	13	4	30.8
P4	3	0	0.0
Total	**36**	**7**	**19.4**

The study demonstrates that of the four protocols used; protocol P3 was observed to be effective at field level in local buffaloes with acceptable pregnancy rate (30.8 %) in buffaloes. The results obtained using protocol P3 is similar to earlier studies using protocols for synchronization during breeding season with conception rates ranging from 30% to 50% (Warriach et al. 2008; Paul and Prakash 2005). Further, the conception rate of 30.8 % in buffaloes using P3 was found promising, which is similar to results on estrus synchronization in cattle, reported by National Research and Development Centre (NDRDC), Yusipang. In this study, the average conception rate (19.4 %) in buffalo is lower than the conception rate 30 – 40 % in cattle (NDRDC 2017). The study also infers that use of estrus synchronization protocols are not very feasible in buffalo during non-breeding seasons. In order to obtain better results, the females should present good body condition score (BCS), be preferentially pluriparous and the procedure should be performed in cycling buffaloes during the breeding season. The following factors could have attributed for the lower conception rates in P1, P2 and P4 compared to P3;

3.3.1. Reproductive seasonality of buffalo

Buffaloes are polyestrous animals that breed throughout the year. However, rainfall, feed supply, ambient temperature and photoperiod influence the annual breeding pattern. The breeding pattern of buffaloes is characterized by great seasonal variation. This variation in breeding efficiency of buffaloes is termed as seasonality of buffalo breeding (seasonal breeders). The reasons for seasonal variation include physiology, nutrition, management, environmental temperature, humidity etc. Decreasing day length and cooler ambient temperature favours cyclicity in buffaloes. There is evidence that high environmental temperatures, photoperiod, relative humidity, and high levels of rainfall influence the reproductive endocrine system in buffaloes (Shah et al. 1990; Zicarelli et al. 1990). In the Indian subcontinent, most buffaloes are bred between September to February. The breeding bulls are also very active sexually and the quality and quantity of semen is very high during winters.

Buffaloes show maximum ovarian activity during this period and largest percentages of them conceive during this period. In Bhutan also, the buffaloes follow the seasonal breeding and calving trend as in most parts of the world. According to local residence, most buffaloes in the study areas are observed in oestrus during the months of September - December. In the present study, four different estrous synchronization protocols were evaluated during two different seasons; seasonal anestrus (March – April) and breeding season (September). The first study was carried out during period of seasonal anestrus using hormonal Protocols: P1 and P2; while the second study was conducted during breeding season used Protocols: P3 and P4. Even though the response rate to the four estrus synchronization protocols was encouraging and most buffaloes estrus synchronized exhibited heat signs; the lower conception rate observed in P1 and P2 might be due to seasonal breeding pattern of buffaloes. This is supported by the previous findings in which many buffaloes present prolonged periods of anestrus during the unfavourable breeding season and do not respond properly to the treatment with hormonal protocols (Baruselli et al. 2002). Further, reproductive efficiency in buffaloes depend upon the resumption of the estrus cycle and ovarian activity and is inherently susceptible to environmental stress and seasonal variation (De Rensis and Lopez-Gatius 2007; El-Wishy 2007). It has been observed by various researchers that very few buffaloes conceive during hotter period of the year due to high environmental temperature, humidity or both. In buffaloes, breeding season influenced Conception Rate (CR). Buffalos estrus synchronized during the breeding season (autumn and winter) presented higher CR than buffalos treated during the off-breeding season (Baruselli et al. 2007). This demonstrated that even with exogenous hormonal stimulation using Progesterone, prostaglandins and GnRH; buffalos continue to present marked reproductive seasonality, which had also been demonstrated by several researches (Zicarelli 1994, Baruselli 1994; Zicarelli 1997). These results suggest that buffalos in seasonal anestrous do not respond well to synchronization protocols. Thus, the seasonality in buffalo breeding might explain the lower conception rate during the seasonal anestrus using protocols P1 and P2 in the present study.

3.3.2 Buffaloes with Anestrus conditions

The buffaloes estrus synchronized and FTAI during the present study were animals which did not show normal heat signs and have not bred during the breeding season. These animals were either repeat breeders or were suffering from reproductive problems like silent heat, persistent Corpus luteum (CL) or anestrus condition (as communicated by farmers). Buffalo have relatively poor reproductive efficiency irrespective of their location throughout the world. The major limiting factor for optimum reproductive performance in buffaloes is failure to detect estrus in a timely and accurate manner. Functional disorders of the ovaries, inactive or non-functional ovaries are considered to be the important causes of anestrus in buffaloes (Rao and Sreemannarayana 1982). Buffaloes exhibit many reproductive disorders including delayed onset of puberty, poor estrus expression, longer postpartum ovarian quiescence and most importantly lowered conception rates particularly when bred artificially (Gordon 1996). Thus, it is hypothesized that these animals with anestrus conditions will have decreased fertility than the normal cycling buffaloes, which could explain the low conception rate in buffaloes in the present study.

3.3.3 Timing of FTAI

In buffaloes, ovarian follicular dynamics during the estrous cycle is similar to that in cattle. Studies from India (Taneja et al. 1996), Brazil (Baruselli et al. 1997) and Pakistan (Warriach and Ahmad 2007) have shown clearly that the majority of buffalo have two waves of follicular activity during their oestrous cycle. The interval between standing oestrous and ovulation, which is very important for AI, was 30 hours in buffaloes (Warriach et al. 2008). Under field conditions, the am - pm rule of insemination originally developed for cattle (Trimberger 1948) is generally followed in buffaloes. To apply this rule, the buffaloes should be bred 12 h after the detection of standing oestrus. However, onset of heat signs instead of onset of standing oestrus has been erroneously considered as the land mark with buffaloes often being inseminated, earlier than required. This early breeding might be potentially responsible for lowered fertility in protocols P1 and P2, and can be explained by the fact there is an interval of about 8 to 10 h between onset of heat signs and onset of standing oestrus in buffaloes. This indicates buffaloes should be inseminated 12 h after the detection of standing oestrus. Since it was the first study on estrus synchronization and FTAI in buffaloes in the country, the protocols normally used in cattle by the Centre was adopted. For all the four estrus synchronization protocols, rectal palpation was done to assess ovarian status (presence of CL). In P1 and P2; FTAI was conducted 24 h and 48 h after removal of CIDR-B, while in P3 and P4, FTAI was carried out 48 & 72 hrs after removal of CIDR-B and 72 & 84 hrs after PGF2α injection, respectively. However, in buffalo there is difficulty in identification of estrus manifestations and for application of AI at the accurate time (Pursley et al. 1995) and insemination is recommended only when buffalo is in standing heat. Thus, it is contemplated that the timing of FTAI of 24 & 48 hrs after removal of CIDR-B might be early which has resulted in low conception rate in protocols P1 and P2.

3.3.4 Different estrus synchronization protocols

Most estrus synchronization protocols for buffaloes are based on the protocols used in cattle (De Rensis and Lopez-Gatius 2007). One successful protocol in buffaloes is a combination of progesterone in a controlled internal drug release (CIDR) device along with prostaglandin F2α (Rao and Sreemannarayana 1982; De Rensis and Lopez- Gatius 2007; Barile 2012; Mehmood et al. 2012). It has been reported that administration of PGF2α after ascertaining the status of CL is economic and less laborious for achieving acceptable pregnancy rates. The ideal time of treatment with PGF2α should be established by determining ovarian activity by ultrasound (De Rensis and López-Gatius 2007). This requires trained person for detection of functional CL using ultra sound. An important requirement is the presence of functional CL, which should be confirmed by use of ultra sound. However, in the present study, CL test was determined by rectal palpation of the ovaries and animals with functional CL were administered PGF2α. The difference observed on application of protocol P4, when compared with other three protocols with respect to conception rate, might be relying solely on rectal palpation to confirm ovarian status since the current study did not use ultrasound devices.

4. CONCLUSION

Buffalo farming as an emerging dairy farming has high potential for improving rural economy through enhance milk production in the country. The buffaloes in Samtse district follows the same seasonal breeding and calving trend as in India and most parts of the world. The success on estrus synchronization with FTAI in buffaloes during the breeding season was found promising with 30.8 % conception rate. However, use of estrus synchronization protocols in buffaloes are not recommended during non-breeding seasons due to poor response. Standard estrus synchronization and FTAI protocols specifically for buffaloes has to be developed to obtain better results. For future research, an appropriate sample size is suggested to minimize the experimental error. Moreover, the use of gemplasm from superior dairy buffalo breeds like Nili Ravi and Murrah may be recommended in other potential buffalo rearing areas to revive and improve the productivity of local or non-descript buffaloes in the country.

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