

PREDICTORS OF SUCCESS OR FAILURE IN ARTIFICIALLY INSEMINATED  
BUFFALO COWS IN BAYBAY CITY, LEYTE, PHILIPPINES:  
AN UNMATCHED CASE-CONTROL STUDY

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**ABSTRACT**

A case-control study was conducted to identify the factors associated with the success or failure of artificial insemination (AI) of buffaloes in Baybay City, Leyte, Philippines. The cow-calf pair was used as the unit of interest in this study regardless of breed and number of buffalo cows the farmers own. Of the 24 selected barangays, an equal number of cases (38 failed AI) and control (38 successful AI) were selected from 78 farmer-cooperators of the Philippine Carabao Center (PCC) AI program and data were collected using a questionnaire. On the one hand, our study found that every year increment beyond the age at first breeding of the cow could predispose the animals to fail by as much as 2.5 times when compared to younger cows. On the other hand, a monthly increment in the calf weaning age may increase the likelihood of AI success by as much as 50%. These results imply that the age at first breeding must be conscientiously considered to allow optimal sexual and physical maturity of the breeding cows while ensuring that first breeding does not occur with too much delay. Moreover, while more mature calves have higher chances of success at weaning, provision of wallow during pregnancy also appears to support AI success as an effective method of cooling.

**Keywords:** *Bubalus bubalis*, buffalo, artificial insemination, PCC, Philippines

**INTRODUCTION**

Large ruminants like cattle and buffaloes are an integral part of Asian agriculture. Millions of farmers particularly in Asia rely on cattle and buffaloes as source of meat, milk and draft power in agricultural operations (Nanda and Nakao, 2003). In East and Southeast Asia, it is not uncommon to find about 1 to 5 swamp type buffaloes raised per household as source of draft power and meat; or milk from riverine-type buffaloes in South and Southwest Asia (Cruz, 2007). It is therefore necessary that these animals receive considerable attention including optimal management, good nutrition and improved breeding practices to enhance their production potential and uplift the livelihood of smallholder farmers.

Buffaloes (*Bubalus bubalis*), considered as the farmer's docile friend in agriculture and a walking fertilizer factory is inseparable among traditional farming systems in Asia (National Research Council 1981; Roxas-Lim 1998; Murtaza *et al.*, 2017). In the Philippines, a working buffalo works for about 84 to 98 days per year particularly in rice-based farming system (Alviar, 1987). Crossbred water buffaloes also provide

considerably more edible and non-edible by-products after slaughter with several characteristics of buffalo meat including crude protein, ash, fat, cholesterol comparable to the more preferred beef (Lapitan *et al.*, 2007).

The vast majority of buffaloes worldwide can be found in Asia representing nearly 2 million in 2004 (Cruz, 2007). Unfortunately, a decline in carabao inventory requires considerable attention brought about by low productivity, poor nutrition and indiscriminate slaughter among others. Unlike cattle, buffaloes only received minimal attention from the government and researchers in the past possibly due to inherent merits of this species, along with the massive introduction of farm mechanization (Nanda and Nakao, 2003).

In Pakistan, buffaloes appear to be affected more often by repeat breeding syndrome than in cattle (Khan *et al.*, 2016) causing about 3 to 6% of the herd being culled annually (Bartlett *et al.*, 1986; Yusuf *et al.*, 2010). Potential reasons for this are due to higher occurrence of silent estrus in buffalo cows as well as their docile nature which can be aggravated by several factors including breed, and agro-climatic and management conditions (Khan *et al.*, 2016). Ovulatory disturbances and reproductive tract infection (Kutty and Ramachandran, 2003) as well as hormonal aberrations alongside a combination with other factors were also identified to influence repeat breeding syndrome (Singh *et al.*, 2008). In Egypt, typical repeat breeders accounted for 7.25% of total reproductive disorders in buffalo cows (Ahmed *et al.*, 2010) and an incidence rate of 8.82% was reported in India (Kumar *et al.*, 2011).

The Philippine government through R.A. 7307 better known as the Philippine Carabao Act of 1992 established the Philippine Carabao Center (PCC) with satellite stations throughout the country. It was mandated to develop and promote

the buffalo industry though modern breeding techniques (i.e., artificial insemination or AI) and optimum management practices. The PCC dispersed Bulgarian-Murrah buffaloes to farmers including their native local stocks are direct recipients of PCC AI service. As reproductive efficiency in water buffaloes is notably lower than in cattle (*Bos taurus* and *Bos indicus*; Drost 2007), this study was aimed to identify putative factors associated with the success or failure of artificial insemination of buffalo cows in Baybay City, Leyte, Philippines.

## Methodology

### Survey design

An unmatched case-control study was conducted and administered using a written questionnaire on farmer-cooperators who are recipients of the PCC at VSU AI service as respondents. Of the 76 farmers from 24 representative barangays, 54 cow-calf pairs were qualified for the study: 27 cases and 27 controls. These respondents have had their buffalo cows bred using AI as method of breeding during the most recent breeding cycle as identified by the PCC technician.

The cow-calf pair was the unit of interest in this study regardless of what breed and number of buffalo cows the farmers owned. From the given population, cases (failed AI) and control (successful AI) were drawn as many as possible. Cases were identified as those cow-calf pairs that have any or all of the following: 1) the buffalo cow failed to get pregnant at first AI attempt within that specific breeding cycle; 2) either or both the dam and the calf died within 3 days of calving; and 3) occurrence of parturition disorders such as but not limited to fetal abnormalities, embryonic mortality, mummification, abortion, fetal death

and/or dystocia. Control cow-calf pairs included those other than the above.

### **Construction and administration of the questionnaire**

A questionnaire was formulated following extensive and thorough review of the literature and upon close coordination with assigned experts concerning the buffaloes' reproductive efficiency. The questionnaire was comprised of three main parts. The first includes the demographic background of the farmer directly in-charge of the rearing and management of the animal. This also includes the production systems employed during breeding/insemination, during pregnancy and during parturition and the reproductive management that were also employed specific to that pregnancy or AI cycle. Secondly, the insemination technique was recorded based on the interview with the PCC technician. This comprised the detailed procedure and materials used during AI including timing, number of inseminations, semen used, thawing procedure, and drugs/hormones given. Finally, the general cow-calf information was recorded which include the assessment of the cows' reproductive performance, breed, age at breeding, and whether problems were encountered during parturition. The questionnaire was written in English but translated in the local dialect during the conduct of the interview.

### **Data management and analysis**

Data from the questionnaire were consolidated using Microsoft Excel and transferred to a series of computer data files. Data entry errors were identified and outlying values were compared against the original data collection sheets.

Statistical analyses were conducted using Epi Info™ version 7.2.2.2 (Centers for

Disease Control and Prevention, U.S. DHHS). Unconditional association between the failure in AI (dependent variable) and the plausibly related independent variables were assessed for crude association using the Chi-square test for categorical independent variables and Kruskal-Wallis test for non-normally distributed independent variables ( $P \leq 0.25$ ). Variables found to have significant crude associations were examined further using the backward stepping logistic regression. All independent variables found to have unconditional association with the dependent variable were initially included in the model and the over-all  $p$  value evaluated. Thereafter, the least significant variables were identified and removed from the model one after the other and the model was run all over again. This procedure was repeated until all the remaining variables in the model and the over-all regression was significant ( $P \leq 0.05$ ). In addition, standard descriptive statistics were conducted for the farmers' demographic background, production systems used, reproductive management and AI procedure.

## **RESULTS**

Both the age of calves at weaning and the provision of cow wallow during pregnancy were found to support AI success. A monthly increment in the calf weaning age may increase the likelihood of AI success by as much as 50%. Meanwhile, every year increment beyond the age at first breeding of the cow could predispose the animals to fail in AI by as much as 2.5 times when compared to younger cows (Table 1;  $P \leq 0.05$ ).

Of the 76 farmer-respondents, 93.4% were males and 6.6% were females. Most the farmers did not complete high school (90.8%) and only 9.2%

reached college. Majority of the farmers (86.8%) were engaged in farming while the others (13.2%) were into fishing. More than half (57.9%) of the farmers spent about 2 to 3 h per day looking after their cows and 42.1% spent more than 3 h. Majority of the farmers (92.1%) had more than one-year experience in handling their cows. Interestingly, only 27.6% of the farmers had attended seminars regarding AI. Most of the farmers (89.5%) felt confident of the AI system (>60% of the rating scale).

As to the feeding management, most of the farmers practiced the tethered feeding system (89.5%) as compared to cut and carry (30.3%). One-hundred percent of the cows were fed with common grasses, half of them were given untreated straws and legumes in only 14.5%. Common grasses found in the study areas included Carabao grass, Paragrass and Napier grass. Notably, the majority of the cows were housed under trees and only about 2.6% were kept in the shed. All the animals have access to a wallow (rivers or man-made).

Most of the cows used in this study were native (81.6%) and about 18.4% were crosses. Inseminations were conducted mostly during natural onset of heat (81.6%) while 18.4% were induced. Nearly 95% of the cows were inseminated twice, 5.26% thrice and 1.32% once, conducted at

early estrus (76.3%). Cows were first bred between 2 to 9 years old with a mean of 3.77 years for control and 4.52 years for cases. Cows' age during the AI cycle when the study was conducted ranges from 2 to 15 years with a mean of 7.98 and 6.10 years for control for cases, respectively. Mostly (84.56%), farmers did not give any assistance to the cow during calving.

Semen used for insemination mainly came from frozen-thawed semen from Bulgarian Murrah buffalo (BMB) bulls. Temperature and thawing time were either at 38°C for 15 seconds or at 70°C for 4.5 seconds, respectively. Most of the PCC technicians deposited the semen at the late cervix (80.3%) and took about 1 to 3 seconds (81.6%) to completely release the full semen from the AI instrument.

## DISCUSSION

Poor breeding efficiency is often cited a major obstacle that leads to a lower production potential in water buffaloes (*Bubalus bubalis*) than in cattle (*Bos taurus* and *Bos indicus*). Nevertheless, despite many factors that hamper reproductive success in water buffaloes including seasonality, poor expression of estrus and long calving interval, its reproductive potential can be significantly

Table 1. Logistic regression showing predictor variables unconditionally associated with failure in artificial insemination of buffalo cows.

Predictor variables	Odds ratio	95% C.I.		Coefficient	S.E.	P-value
		minimum	maximum			
Cow age at first breeding	2.5384	1.1493	5.6062	0.9315	0.4043	0.0212
Calf age at weaning	0.4749	0.3219	0.7006	-0.7446	0.1984	0.0002
Cow wallow provision during pregnancy	0.0059	0.0002	0.1945	-5.1244	1.7791	0.0040

improved (Gwazdauskas *et al.*, 1981; Drost 2007). This can be accomplished following established reproductive management techniques used in cattle given the vast similarities in the anatomy and physiology of the reproductive systems between the two species (Drost, 2007). Our study demonstrated that the age of buffalo cows at first breeding must be conscientiously considered to allow optimal sexual and physical maturity of the breeding cows. Moreover, considering the feeding practiced in smallholder cow-calf operations, calves should be given sufficient time to grow and mature before weaning is initiated. The provision of cow wallow during pregnancy may also provide beneficial effects to mitigate potential impacts of heat stress on the reproductive health of buffalo cows.

The significant impact of the age of buffalo cows at first breeding puts a greater emphasis on the appropriate sexual and physical maturity of animals needed to successfully carry out pregnancy considering that buffaloes have later onset of puberty than cattle (Drost, 2007). While first calving in buffaloes is possible as early as 39 months (Tonhati *et al.*, 2000) with an average of  $53.88 \pm 0.48$  months in Murrah and  $51.51 \pm 1.18$  months in Surti buffaloes (Gogoi *et al.*, 2002), delaying first pregnancy until the animal is mature enough and have attained an optimal breeding weight would ultimately favour conception rates and thus AI success (Coleman *et al.*, 1985). Our results suggest that the older the buffalo cows, the higher is the likelihood to fail in AI by as much as 2.5 in every year increment. This amount of time at first breeding as seen in our results appear to support the report of Nanda *et al.* (2003) saying that a large proportion of buffalo heifers reach puberty at 3 to 5 years of age (Nanda and Nakao, 2003). Since age at first calving trait has a low estimate of heritability (Seno *et al.*, 2010),

our results implies that management strategies including judicious provision of balanced nutrition and the use of performance modifiers might be helpful in promoting maturity of buffalo cows as previously described both in buffaloes and Sahiwal cows (Bhatti *et al.*, 2007).

It appears that buffaloes are especially attracted to water and have been observed to wallow between 10 am and 3 pm where water is available, although buffaloes may wallow anytime in the presence of severe insect attack (Tulloch and Litchfield, 1981). It is not easily understood how the provision of wallow during pregnancy is associated with the success of artificial insemination as observed in our study but peculiar features of buffaloes such as dark skin, spare hair coat and poor sweating ability make these animals particularly susceptible to the effects of direct solar radiation (Ahmad and Tariq, 2010; Marai and Haeeb, 2010). Such conditions may predispose the buffaloes to hormonal imbalances, reduced feed efficiency and utilization and issues associated with reproductive performance (Ahmad and Tariq, 2010). It has been reported that during hot season, buffaloes may suffer from disruption in ovarian cyclicity leading 20 to 80% anoestrus (Nanda *et al.*, 2003). However, buffaloes having access to a wallow have been demonstrated to have increased feed intake and milk yield (Aggarwal and Singh, 2010). These beneficial effects may be associated with an increase in the average plasma T4 and insulin levels in buffaloes wallowing in a water pond than those provided with water showers only during hot-dry season (THI of 83.6) as well as T3 levels during the hot-humid seasons (Aggarwal and Singh, 2010). Moreover, wallowing was demonstrated to be an effective method of cooling by cutaneous evaporation (Khongdee *et al.*, 2011). It was shown that skin and rectal temperatures

were lower in the wallow group compared to those with water showers (Aggarwal and Singh, 2008) although rectal temperature didn't differ between buffaloes in wallow than those in shade (Tulloch and Litchfield, 1981).

Pre-weaning mortality in calves is directly correlated with poor nutrition in the breeding females (McDermott *et al.*, 2010). As farmer co-operators may have varying degree of management, supervision of labor and available feed supply, our results suggest that buffalo farmers need to provide enough time for calves to build sufficient body reserves before weaning since every year increment in the weaning age may reduce the likelihood of AI failure by as much as 50%. While the average weaning weight in Nili-Ravi buffaloes have been reported to be 66.12±9.16 kg at 90 days (Akhtar *et al.*, 2012), there appears to have several factors that can affect calves in attaining optimal weaning weight. These include year of birth in Swamp buffalo (Thevamanoharan *et al.*, 2001), year and season of birth in ranged Mashona cattle (Tawonezvi, 1989) and the age of dam (Akhtar *et al.*, 2012).

In conclusion, our study found that the age at first breeding must be conscientiously considered to allow optimal sexual and physical maturity of the breeding cows while ensuring that first breeding does not occur with too much delay. Calves also should be allowed enough time to grow and mature before weaning is initiated while provision of wallow during pregnancy may also provide beneficial effects on the breeding cows that would support AI success.

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