Effect of Dominant Follicle Size and GnRH Administration at Fixed-Time Artificial Insemination on Pregnancy Rates in Female Buffaloes on Marajó Island, Brazil

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Abstract: This study aimed to verify the effect of GnRH at the time of artificial insemination and Dominant follicle (DF) diameter on the Pregnancy rate (PR). 384 multiparous upgraded Murrah breed buffalo within 30 to 60 days postpartum with a body condition score of 3.4±0.6 were used. The synchronization protocol consisted of a day 0 (D0) intravaginal device of 1g of P4, application of 2.0mg of BE, and administration of 10 ml of injectable mineral supplementation at 4 pm. On D9, at 4 pm, the device was removed, and injected 0.265 mg of PGF2α and 300 IU of eCG Intramuscular (IM). On day 11, females were categorized based on the size of their dominant follicles. Females with ≥ 13 mm DF were divided into two groups: group 1 received GnRH, while group 2 did not. Similarly, females with < 13 mm DF were split into Group 3, which received GnRH, and Group 4, which did not. At 4 pm, 0.1 mg of GnRH was administered IM according to their groups. On D12, 16 hours after the injection of GnRH (at 8 am), Fixed-time artificial insemination (FTAI) was performed. The pregnancy was diagnosed after 30 days of FTAI by ultrasound. The overall PR was 53.9% (207/384). No significant difference was observed between the groups and follicular size. More pregnancies were observed when the follicle size was around 13mm. It was concluded the administration of GnRH could enhance the efficiency of FTAI in buffaloes.

Keywords: Amazon region, Follicle size, Timed artificial insemination, Water buffaloes.

INTRODUCTION

The domestic buffalo (Bubalus bubalis) originates from Asia, and it is found between the parallels of 2° South and 31° North, adapting well to various tropical and temperate climates [1]. The first importation of buffaloes to Brazil occurred in the late 19th century when animals were brought from East Asia, traversing French Guiana and Suriname to Marajó Island and the Amazon valley, particularly in the states of Amapá, Pará, and Amazonas [2]. Marajó Island, located at the mouth of the Amazon River delta in eastern Pará, is the world's largest fluviomarine island, measuring 295 km long and 200 km wide, with an area of 47,964 square km. It has a hot, humid climate (Köppen climate classification) characterized by annual rainfall of 3,000 mm, 80% relative humidity, and an average temperature of 27°C. The rainy season spans January to June, with flooding peaking in March, April, and May, while the driest months are from July to December. The predominant vegetation consists of natural grasslands with herbaceous layers of grasses and sedges, along with areas of primary forest, floodplain pastures, degraded areas ("capoeira"), and savanna [3]. The native pastures in the archipelago cover 2.3 million hectares, part of the 75 million hectares of native pastures in the Brazilian Amazon [4]. Cattle and buffalo are raised extensively in these native pastures for breeding and fattening [3].

Unlike buffaloes raised in other regions of Brazil, the principal cause of anoestrus in the Amazon is irregular feed supply rather than seasonal effects. Buffaloes in this region show continuous polyoestrous reproductive activity [4]. As a result, breeding and parturition occur throughout the year, provided there is no nutritional anoestrus [5].

In order to address these environmental and species-specific difficulties, reproductive biotech-nologies such as artificial insemination (AI) and fixed-time artificial insemination (FTAI) were developed [6]. However, conventional AI faces challenges, including oestrus detection due to subtle behavior and seasonal nutritional anoestrus, which reduces reproductive activity [7]. Protocols have been developed to eliminate the need for oestrus detection, synchronizing the luteal phase, follicular growth, and ovulation to enable FTAI in animals not displaying oestrus signs. Proper nutrition

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and management are essential to maintain favorable body condition scores (BCS), especially during the 90 days postpartum. Improved reproductive management and nutrition, supplemented with minerals, have significantly enhanced the application of these protocols, facilitating the adoption of AI in various herds throughout the Amazon, Brazil, and beyond [8]. It has led to genetic improvement and increased meat and milk production [9]. Studies using synthetic GnRH analogs and reproductive hormones 60 days postpartum have shown positive effects on oestrus cycle control and fertility in buffaloes. Females who exhibit oestrus during FTAI protocols tend to have higher ovarian response and fertility, with higher pregnancy rates compared to those who do not show oestrus [10]. However, literature on this subject remains limited, particularly in the Amazon region. Therefore, this study aimed to verify the effect of dominant follicle size and GnRH administration at fixed-time artificial insemination on pregnancy rates in female buffaloes.

MATERIALS AND METHODS

Ethical Statement

This work was approved by the Committee on Ethics and Use of Animals of the Federal Rural University of the Amazon, with protocol No. 066/2016 from CEUA and 23084.019476/2016-11 - UFRA.

Period and Location of the Experiment

The experiment was conducted from June to September 2019 on a commercial buffalo farm in Salvaterra, a municipality located on Marajó Island in Pará State. This area is approximately 85 km from the capital city, Belém. The geographic coordinates of the municipal headquarters are 00° 45' 21" S and 48° 45' 54" W.

This region has a rainy season from December to May (flooded pasture from February to March, Figure **1-A**), followed by less rain from June to November (dry season from September to October, Figure **1-B**). Between these two seasons, there is a transitional phase in which the rains may be delayed or start early, with varying intensity (between June and July, Figure **1-C**).

Experimental Animals

A total of 384 multiparous buffalo cows (*Bubalus bubalis*) upgraded with Murrah breed, aged 4 to 10 years and 30 to 60 days postpartum, were utilized. The

Figure 1: Different periods (**A**) flooded pasture through February-March, (**B**) dry season, September-October, (**C**) transitional period through June-July, after the flooding period at Salvaterra, Marajó island., Brazil.

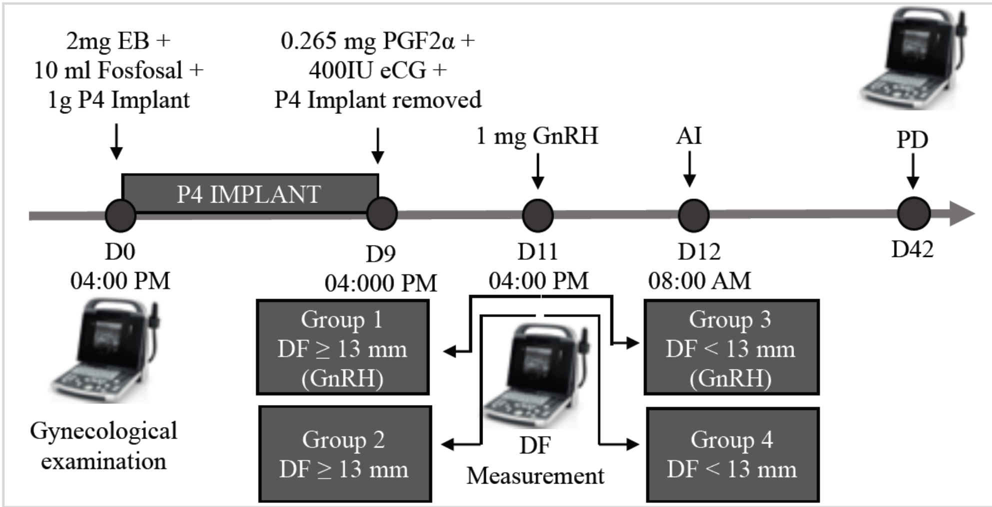

Figure 2: A schematic representation of estrus synchronization protocol and grouping of animals.

average body score of the animals was 3.4±0.6 (scale from 1 to 5) [11]. The buffaloes were kept on native pastures of Panicum, Echinochloa repens, Paspalum virgatum, and cultivated pasture of Brachiaria humidicola, with free access to commercial mineral mixture and water.

Synchronization Protocol for FTAI, Grouping and Pregnancy Diagnosis of Females

All buffaloes were submitted to the same estrus synchronization protocol for 12 days (Figure **2**) [9]. On a random day during the estrus cycle (D0), the buffaloes received a new intravaginal device containing 1 g of progesterone (P4) (Primer®, Agener União Química, Brazil). Additionally, 2.0 mg of buserelin (BE) (Fertilcare Sincronização®, MSD Saúde Animal, Brazil) was administered intramuscularly (i.m) along with 10 ml of injectable mineral supplementation (Fosfosal®, Virbac Saúde Animal, Brazil) via intramuscular injection at 4:00 pm. On day 9, the primer was removed, and 0.265 mg of cloprostenol sodium (Ciosin®, MSD Saúde Animal, Brazil) and 300 IU of eCG (Folligon®, Intervet International, Germany) were administered i.m at 04:00 pm. On day 11, at 4:00 pm, 0.1 mg of GnRH (Fertagyl®, Intervet International, Germany) was administered intramuscularly according to their groups. Then, on day 12 (D12), a deep cervical artificial insemination was performed 16 hours after the GnRH application, specifically at 8:00 am.

On day 11, animals were categorized based on the size of their dominant follicles (DF) (Figure **2**): those with ≥ 13 mm DF and those with < 13 mm DF. Females with ≥ 13 mm DF were divided into two groups: group 1 received GnRH while group 2 did not. Similarly, females with < 13 mm DF were split into Group 3, which received GnRH, and Group 4, which did not. The females had < 7 mm follicular size were excluded from the study.

The buffaloes were submitted to ultrasound evaluation (7.5 MHz linear transducer, Mindray DP10 Vet, Shenzhen, China) on D42 for pregnancy diagnosis (Figure **2**).

Statistical Analysis

Data were analyzed using the SAS OnDemand 2021. The chi-square statistical test was used, with 5% significance ($P<0.05$).

RESULTS

The overall pregnancy rate was 53.9%. The pregnancy rates of different groups are presented in Table **1**. The follicular size and pregnancy rate curve of the females are presented in Figure **3**. The more pregnancies were observed when the size of follicle around 13mm (Figure **3**).

Table 1: Effect of GnRH Administration on Pregnancy Rates During FTAI in Buffaloes on Marajó Island

Groups	Pregnancy rate (%)
Group 1 (DF ≥ 13 mm, GnRH)	64.2 (27/42)
Group 2 (DF ≥ 13 mm)	58.3 (84/144)
Group 3 (DF < 13 mm, GnRH)	53.1 (51/96)
Group 4 (DF < 13 mm)	44.1 (45/102)
overall	53.9 (207/384)

DF, Dominant Follicle.

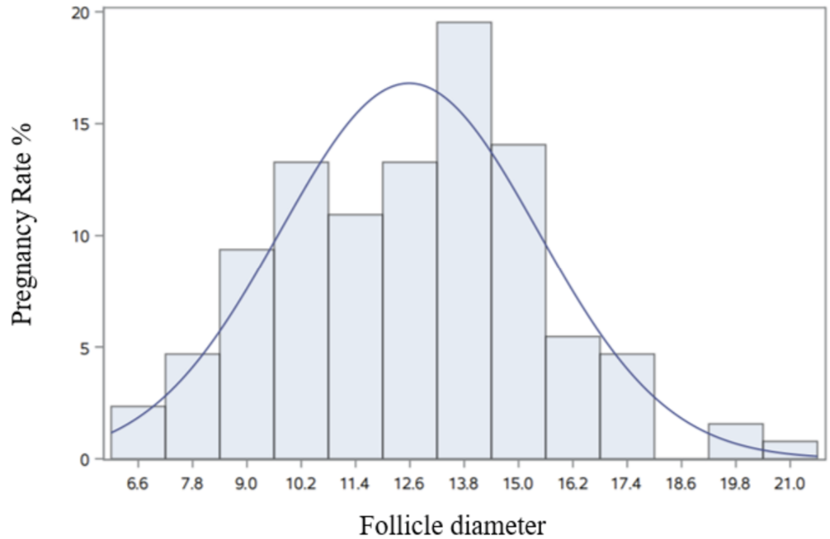

Figure 3: General distribution of the follicular size and pregnancy in the buffalo population used in the present experiment.

DISCUSSION

Globally, the popularity and population of buffalo are increasing due to the demand for A2A2 milk and its high-quality milk and meat products [12, 13]. Buffalo is the last domesticated dairy animal [12], and all breeding techniques have been adapted from bovines with modifications [14]. Currently, assisted reproductive technology yields satisfactory results compared to the past because we now have sufficient literature on the reproductive physiology of buffalo [15]. Historically, buffaloes showed excellent breeding performance during natural mating. The results of this study will provide additional information on how to enhance breeding and production strategies within bubaline species.

The overall pregnancy rate in our study was satisfactory, approximately similar to Ribeiro *et al*. [16], who reported a 55.88% pregnancy rate in buffaloes using the Ovsynch Plus protocol. These results indicate that FTAI in buffalo is satisfactory. The same group also suggested that, apart from nutrition, environmental or climatic conditions affect FTAI performance in buffaloes. Weiller [15] obtained an overall pregnancy rate of 60.9% in research using crossbred buffaloes raised in a mixed nutritional management system (floodplain and area of artificial pasture) in the state of Amazonas, where the work consisted of testing different types of ovulation inducers in the FTAI protocol, a result superior to that obtained in the present research.

Corroborating our present work, previous studies in Nelore or crossbred cattle also did not observe any significant difference with or without using of GnRH during the FTAI program [17, 18]. In contrast, different studies observed better results with GnRH [19, 20]. Burnett *et al*. [21] observed that GnRH increased the estrus expression but did not link directly with the ovulation rate in cows. Our results were better for GnRH groups compared to those without it ($P>0.05$). This fact may relate to the improved luteinization process using GnRH, thereby enhancing progesterone production in the corpus luteum and promoting a more favorable maternal environment for maintaining a pregnancy [22].

Our study did not observe any significant difference when comparing follicular size during FTAI. Different studies have observed a positive correlation between follicular size and pregnancy rate in dairy cattle [23, 24]. Females that ovulate from small follicles have lower estrogen levels during ovulation compared to cows with larger follicles [25]. A higher physiological concentration of estradiol positively influences fertilization by reducing uterine pH, modifying sperm transportation, and increasing sperm longevity until ovulation [26]. Sá Filho *et al*. [27] observed that the presence of larger diameter follicles at the time of P4 device removal in a synchronization protocol for FTAI is associated with a higher occurrence of estrous signs, as well as high ovulatory capacity and a greater probability of pregnancy in Zebu cows.

Our follicle diameter curve on day 11 of the protocol behaves similarly to the Gaussian curve, where animals with dominant follicles situated more to the right of the curve have greater probabilities of achieving pregnancy. The follicle diameter curve is supported by the findings of Echternkamp *et al*. [28]. Studies have observed that oocytes ovulated from smaller or excessively large follicles are less physiologically competent [23, 28]. Perry *et al*. [29] reported that a diameter between 12.5 and 13.5 mm is ideal for achieving pregnancy in estrus-synchronized beef heifers using GnRH. Follicles with diameters <11.5 mm or >16 mm were less likely to result in pregnancy [29]. In buffaloes, it has been observed that dominant follicles larger than 12 mm [30] or 13 mm that ovulate provide better results after the FTAI protocol [31, 32]. An optimal-size follicle has sufficient LH receptors and a better response to gonadotropin and follicular maturity, which may increase fertility rates [33].

CONCLUSION

A preovulatory follicle measuring around 13mm in diameter yields better results, and the administration of GnRH could enhance the efficiency of the protocol during FTAI in buffalo, with limited benefits in buffaloes with smaller dominant follicles. These findings are based on specific environmental conditions in Marajo Island, with limits on results generalizability.

CONFLICT OF INTEREST

We hereby declare that there is no conflict of interest with respect to the publication of this manuscript.

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