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ESTROUS CYCLE OF BUFFALOES *Bubalus Bubalis* DETERMINED WITH A DIGITAL ESTRUS AND OVULATION DETECTOR

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SUMMARY

The research aimed to determine the estrous cycle in Buffaloes using a digital heat and ovulation detector. To do this, 30 buffalo females were studied for 40 days with an average age of 4 years and average weight of 489 kg, to which the daily measurement of vaginal mucus resistance was performed using a Draminsky (ED) heat and ovulation detection equipment. In addition, swelling of the vulva, presence of vaginal mucus and depigmentation of the heat detector patch were determined. Subsequently, measures of central tendency (range, mean, median, maximum, minimum, variance) were used, as well as graphs and histograms of frequencies through the Microsoft Excel version 365 program for the tabulation of the information. The results obtained determined that the total duration of the estrous cycle was 21.83 days, divided into estrus with an average of 1 day, metaestrus 5.43 days, right-handed 12.47 days and proestrus 2.93 days. Regarding the economic analysis, it was observed that the total cost of the experiment was 567.15 USD and a unit cost per buffalo was $18.90. It was concluded that the behavior presented by most females in the estrus stage did not demonstrate swelling and redness of the vulva, there was an absence of vaginal mucus and there was no depigmentation in the heat detector patch in most of the females analyzed.

Palabras clave: <CICLO ESTRAL>, <ESTRO>, <BÚFALAS>, <OVULACIÓN>, <DETECTOR DIGITAL>, < MOCO VAGINAL>

INTRODUCTION

Bufaline production systems, like any other production system, require clear and accurate information to be efficient. In this sense, reproductive information is key to success or failure in obtaining products and by-products of this species. The estrous cycle of buffalo females, in our environment, has been scarcely studied, which generates doubts and limitations when developing fixed-time artificial insemination programs (IATF), super ovulation protocols, embryo washing and transfer and all the methods of assisted animal reproduction that currently exist. The domestic buffalo is grouped in the subfamily Bovideg, genus bubalus and species bubalus bubalis, this species is divided into two groups the bubalus bubalis sp known as River Buffalo or Dairy Buffalo, 50 pairs of chromosomes, and the *bubalus bubalis var* called Marsh Buffalo, 48 pairs of chromosomes (Alvarado, 2012).

The breeding and production of this species has not been easy since several aspects have not been fully understood, mainly physiological, genetic and productive.

In the last 50 years, since 1968, buffalo herds have increased, globally, more than twice as much compared to cattle. This increase was originally based on the production of working animals, to later observe the obvious advantages of buffaloes in the production of milk and meat (Di Paolo, 2021).

The buffalo is a bovid with a reproductive system similar to that of the cow, observing several very marked differences that include sexual behavior, signs and symptoms of heat, duration of the estrous cycle, gestation period, puerperium, among others (Bustillos, 2016).

The buffalo species presents a predominance of two (52.4%) and three waves (19.0%) follicular during the estrous cycle, in addition a two-wave follicular growth pattern is described with 63 - 83% of cases, followed by three-wave patterns (25 to 33%) and finally with 3.3% of a follicular wave, (Sánchez, 2019).

The estrous cycle of the buffalo occurs every 19 to 23 days with an average of 21 days and is divided into four stages: estrus (24 hours), proestrus (3 days), metaestrus (3 - 4 days) and dexterous (12-15 days). The right-hander and metaestrus are grouped in the luteal phase of the estrous cycle, while the proestrus and estrus in the follicular phase (Sharma, 2014).

The ovulation process in buffaloes occurs on average at 21.41 ± 4.56 hours after the end of estrus, in addition the interval between estrus and ovulation is on average 17 hours after riding and usually occurs at night, presenting an estro-ovulation interval on average of 34 hours with a range of 24 to 48 hours (Sanchez, 2016).

The heat detection tools currently used vary widely in terms of efficiency, sensitivity and specificity and their applicability at the producer level (Fagiolo et al., 2005; Girish et al., 2022). According to Girish et al. (2013); Ravinder et al. (2016) and Shashikumar et al. (2018) it is essential to develop an easy and reliable method for accurate detection and confirmation of heat in buffaloes.

METHODOLOGY

The study was developed in La Hacienda La Victoria located at kilometer 12 via Bucay-Naranjito, Bucay canton, province of Guayas which is located at an altitude of 140 meters above sea level, has an average temperature of 24 to 32 degrees Celsius average and a rainfall of 2200 mm. To homogenize the cycles and ovulations of the buffalo females, all were subjected to oral deworming (fenbendazole) and mineralization (kirofosfan) to later perform an ovulation synchronization protocol using GnRH and Prostaglandin f2α. When subjected to this protocol, it was ensured that all females ovulate on the same day from which the measurements were initiated by the digital heat and ovulation detector.

For the measurement of vaginal mucus resistance, a digital heat and ovulation detector brand Draminsky model (ED) was used. The measurement was carried out in the morning from 07H00 for 40 days at the time of milking. The process consisted of disinfecting the probe with iodized water, inserting the probe vaginally and turning it 3 times to read the device according to the manufacturer's instructions. Following this, all the information was recorded and digitized.

The determination of swelling of the vulva was made by direct observation daily, for this we proceeded to clean the entire area of the vulva of the buffalo in addition to that it was dried with absorbent paper, it was placed next to the milking cubicle and the presence of swelling was observed and related to the day of the cycle in which they were. Then the respective registration of each of the buffalo females was made.

The observation of the presence or absence of vaginal mucus in the estrus was made at the time of measuring the resistance of the vaginal mucus, and relating it to the values emitted by the manufacturer at the time of estrus, for this the vulvar area was washed and cleaned for better observation, and the mucus was observed at the time of removing the vaginal probe.

The determination of the depigmentation of the heat detector patch was assessed according to the behavior of the females for this the observations were made at the time of milking at 07H00 and when the females were grazing from 11H00 directly in the paddocks.

All measurements obtained through the digital heat and ovulation detector were taken into account, considering the recommendations of its manufacturer, to determine the duration of the estrous cycle and its phases. For this, the manufacturer's information was considered, which mentions: *"... The scheme of the changes in the resistance of the mucosa in the vagina during the sex cycle is presented and the time of appearance of estrus is noted. If the animal is outside the estrus period, the high level of resistance is noticeable (depending on the species of the female, e.g. In cows will be approx. 300 units or more). As we approach the peak phase of estrus, the resistance decreases reaching the minimum value (e.g. In cows approx. 200 units), after which it increases again to the high level and remains at this level until the time of the next estrus. In practice, when making measurements, it is necessary to capture the minimum value and then the moment of the significant increase in results. For this reason, in the estrus period, measurements must be made with the appropriate frequency. Infrequent measurements (e.g. every few days) will cause incorrect interpretation of the results and therefore the omission of the decisive moment of estrus – ovulation time"* with this, the duration of the different phases of the estrous cycle (metaestrus, dexterous, proestrus and estrus) was determined. Through the tabulation and interpretation of the results obtained during the 40 days of measurement; Subsequently, measures of central tendency (mean, maximum, minimum, standard deviation, lower limit, upper limit, error) were used, in addition to dispersion plots were made for a better interpretation of the results obtained.

RESULTS AND DISCUSSION

Vaginal mucus resistance in buffaloes

Water buffalo (*Bubalus bubalis*) occupies an economically important place in the livestock industry (Rajanarayanan & Archunan, 2004; Albayrak et al., 2012; Mishra et al., 2021). According to data presented by FAO (2006) and Canizalez (2011) in the last 10 years, buffaloes worldwide have presented a growth rate of 9.1%. However, major problems faced by buffalo farmers include reproductive deficiency and long calving intervals (Barile, 2005; Perera, 2008). According to this, the study developed by Dhaliwal (2005) mentions that, the economic success of the buffalo cattle economy lies in the proper and optimal reproductive rhythm of each buffalo in the herd within the normal physiological range. Therefore, from this knowledge we believe that poor reproductive performance of animals leads to economic losses due to additional costs in management and reduced production.

The estrous cycle is defined as the period from one ovulation to one subsequent ovulation (Mirmahmoudi et al., 2012). Baruselli et al. (1997) stated that the estrous cycle is made up of four continuous phases: metaestrus, dexterous, proestrus and estrus. In this context, some of the studies such as those of Batra et al. (2019); Singh et al. (2020) and Chavan et al. (2023) mention that in the course of these phases a series of changes occur in ovarian structures and concentrations of hormones that interact so that buffaloes canbe cycling. Likewise, the behaviorof oxes in heat is characterized by a reduced appetite, slightly agitated and curious (Ghinet et al., 2006). For example, the study by Araujo et al. (2002) points out that the presence of vaginal mucus is a good indication of fertility and intensity of natural estrus. A second study by Sharma et al. (2013) found that estrous mucus is always transparent, while mucus one day after estrus is slightly whitish in both pregnant and non-pregnant animals. In this study, it is reported that the average values in relation to the resistance of vaginal mucus determined with the digital detector of heat and ovulation were on average of: 226.28 in the metaestrus phase, 200 in ovulation, 202.5 in estrus, 248.8 in the proestrus and 306.07 in the right-hander. So it is considered that the resistance of vaginal mucus in buffaloes varies according to the hormonal status of the estrous cycle.

Phase of the estrous cycle and behavior in the estrus stage in buffaloes

Understanding the factors that regulate the estrous cycle in dairy animals is an indispensable component in reproductive management on livestock farms (Driancourt, 2001). Several studies, such as those by Verma et al. (2014); Ravinder et al. (2016) and Singha et al. (2022) note that estrus detection is a major problem in buffalo farming due to inconsistent expression of estrus signs in different seasons and a high prevalence of silent estrus and postpartum anestrus in this species leading to low reproductive efficiency. In the present research, several evaluation parameters were taken into account for the behavior of females in the estrus stage, through direct observations it was determined: swelling of the vulva, presence of vaginal mucus and depigmentation of heat detector patch.

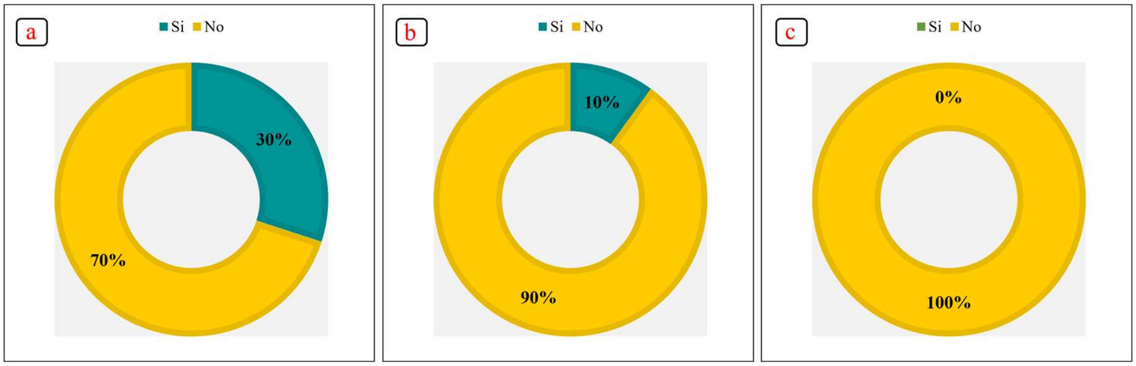

Figure 1. Signs of estrus *in buffaloes: Hinchazon of the vulva* (***a***), presence of *vaginal mucus* (***b***), *presence of depigmentation of estrus detector patch (**c**).*

Under the influence of increased estrogen, the blood supply to the genital organs in the female in estrus increases, which causes swelling of the vulva and redness of the mucous membrane of it (Verma et al., 2014 and Shashikumar et al., 2018). In this sense, in this study it was observed that most buffaloes (21 – 70%) did not show swelling of the vulvar lips while intense vulvar swelling was found in a very small number of animals (9 – 30%) (Figure 1a). A study by Mohan et al. (2010) shows that swollen vulva was the main symptom of estrus and was observed in all buffaloes. A second study currently developed by Singh et al. (2022) shows that among the main signs of estrus exhibited by buffaloes, vulvar swelling was represented in 72.2% of animals. The variations observed in the present research could probably be attributed to the different diet, environmental conditions, management and breed of buffaloes. Additionally, authors such as Singh et al. (1984); Sharma et al. (2012); Verma et al. (2014); Mahalingam et al. (2019) and Kumar et al. (2021) indicated that among other behavioral signs of heat shown in buffaloes after the onset of spontaneous estrus are frequent urination, uterine tone, mucus discharge, bull chasing, chin rest, tail elevation and bellowing.

It was also evaluated that 27 – 90% buffalo females did not present vaginal mucus discharge, while there was the presence of vaginal mucus in only 3 – 10% buffaloes (Graph 1b). The findings of this observed study are inferior to those reported by Verma et al. (2014), which revealed that 56.38% of buffaloes in heat had abundant mucous secretion. Similarly, Sharma et al. (2013) point out that the mucus in buffalo females studied during the luteal phase was abundant, quite fluid and adhesive. Research by Singh et al. (2022) shows that among the main signs of estrus exhibited by buffaloes, cervicovaginal mucus secretion was represented in 89.9% of animals. Our data provide evidence that the buffalin females studied did not show an increase in vaginal mucus secretion as a result of the increase in estrogen level.

Patches are devices that help detect heat in real time, improving pregnancy times, conception rates and minimizing the interval between births; however, they may be ineffective as the paint may rub or the patch may peel off (Kumar et al., 2013 and Chandra et al., 2016). In this sense, the results obtained denote that all buffalo females (30 – 100%) did not show some degree of depigmentation in the heat detector patch (Graph 1c). Madkar et al. (2022) in their study used between the hip and tail of buffalo females, at an angle of 90 degrees patches for the identification of estrus, revealing that there is a low depigmentation of the patch. This is likely attributed to crowded conditions that could cause inferior mounting behavior and high-voltage environments.

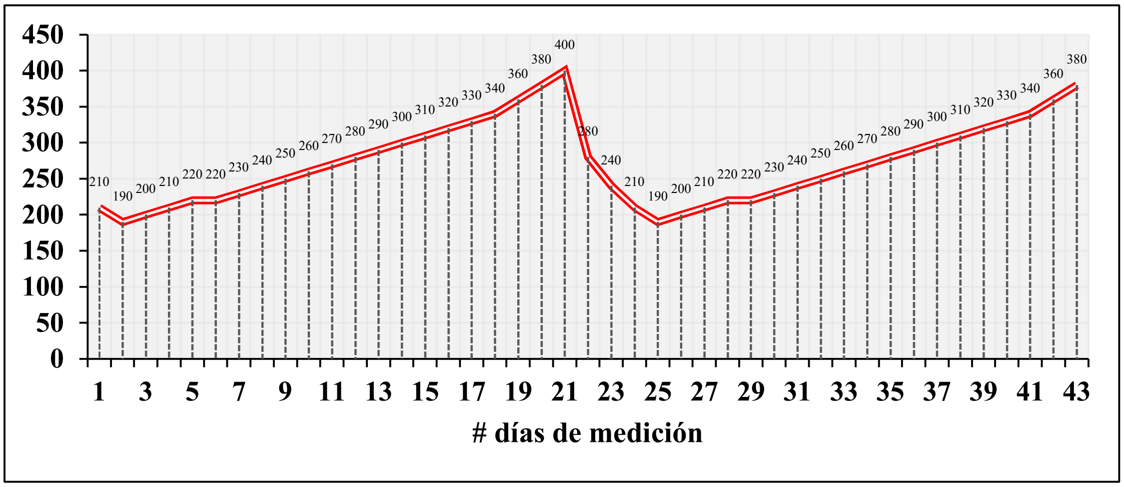

Figure 2. Curve of variation in the measurement of vaginal mucus during the different days of the estrous cycle in búfalas

Thedetermination of estrus at the right time is considered an important component within successful artificial insemination programs and for the improvement of conception rates in buffaloes (Gaikwad et al., 2023). Thus, the duration of the estrous cycle in buffaloes is similar to that of cattle, ranging from 17 to 26 days with an average of about 21 days (Jainudeen and Hafez, 1993).

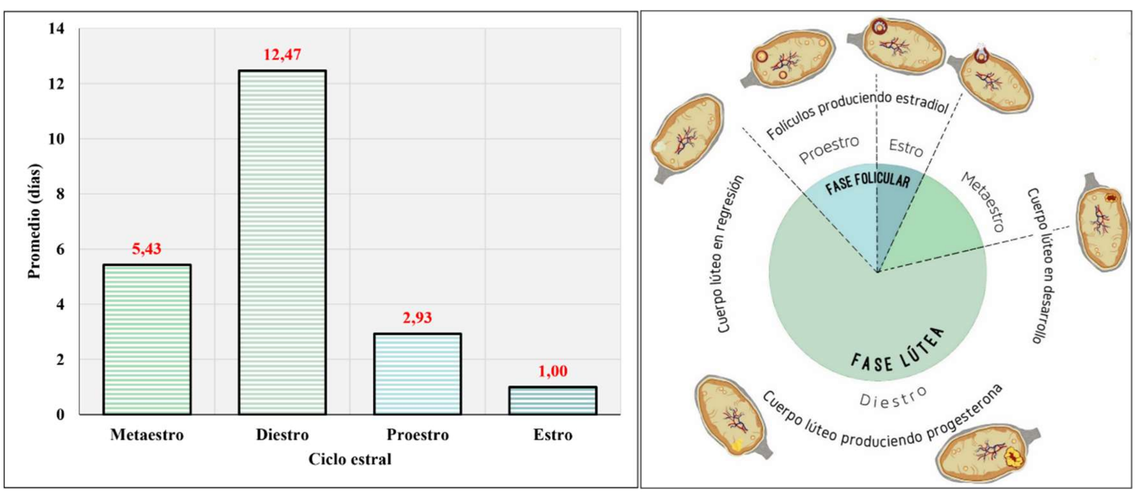

Figure 3. Duration of the estrous cycle in buffaloes

The mean value of the estrous cycle in the buffalo females analyzed was 21.83 days. The average for each of the four stages is as follows: metaestrus (5.43 days), right-handed (12.47 days), proestrus (2.93 days) and estrus (1 day) (Graph 2 and 3). Given the above, similar studies show that an average period of an estrous cycle in their experimental analyses of *Bubalus bubalis* is 21 days (Perera, 2011), 22 to 24 days (Sagar et al. , 2012) and 21.7 days (Ravinder et al. , 2016). Consequently, it can be mentioned thatthere is a greater variability in the duration of the estrous cycle in buffaloes, with a higher incidence of abnormally short and long estrous cycles, attributed to several factors, includingadverse environmental conditions, nutrition and irregularities in the secretion of ovarian steroid hormones.

Economic analysis of the requirements used in determining the estrous cycle in buffaloes

For the determination of the estrous cycle in buffaloes, a total budget of $567.15 was invested, with 74% - $420 of the budget allocated for equipment and 26% - $147. 15 for materials and inputs (Table 1).

Table 1: Requirements used in the determination of the estrous cycle

Requirements	Photography	Quantity	Unit	Unit price ($)	Total price ($)
Equipment					
Digital heat and ovulation detector		1	In the	420	420
Subtotal					**420**
Materials and supplies					
Latex gloves		80	In the	0.25	20
Quaternary ammonium		2	lt	4.5	9
Absorbent paper		1	In the	3.9	3.9
Rope		5	m	1.1	5.5
Valde 10 lt		1	In the	4.75	4.75
Heat detection patch		30	In the	3.47	104
Subtotal					**$147.15**
Total					**$567.15**

CONCLUSIONS

By measuring vaginal mucus resistance with the use of a digital heat and ovulation detector, it was determined that the estrous cycle of buffalo females lasted an average of 21.83 days ; divided into 5.43 days for metaestrus, 12.47 days for right-hander, 2.93 days for proestrus and 1 day for estrus.

The signs presented by the females in estrus did not report depigmentation of the heat detector patch, a very low number presented swelling of the vulva and discharge of vaginal mucus. These findings highlight the difference in buffalo behavior compared to dairy cows subjected to the same management.

The use of this information may contribute to developing strategies to efficiently apply fixed-time Artificial Insemination (IATF) and embryo transfer programs in buffalo females, the duration of each of the phases of the estrous cycle represents a tool to understand the reproductive behavior of this species.

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