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EFFECT OF PHOTOPERIOD AND OTHER ENVIRONMENTAL FACTORS ON THE OOCYTE POPULATIONS OF WATER BUFFALO (*Bubalus bubalis*) IN VERACRUZ STATE, MEXICO

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ABSTRACT

The objective of this study was to investigate the effect of photoperiod and other environmental factors on the population of Water buffalo oocytes in Veracruz, Mexico. Oocytes (n=123) were obtained by follicular aspiration and graded according to morphological characteristics. Data regarding regional environmental factors (temperature, humidity, and daylight) were collected from a historic database of a metrological station. Statistical analysis was performed through Person correlation, one-way ANOVA and linear regression analysis, $P<0.05$ was considered significant. There was a correlation ($P<0.05$) between oocyte populations (total and viable) with temperature, humidity, and duration of daylight. ITH (index of temperature and humidity) and oocyte viability did not correlate ($P>0.05$) with other variables. Oocyte populations (total and viable) and environmental variables changed ($P<0.05$) during all the sampled months. Oocyte populations (total and viable) were affected ($P<0.05$) by temperature ($r=-0.63$; $r=-0.57$), humidity ($r=0.46$; $r=0.39$) and daylight ($r=-0.86$; $r=-0.87$), no association ($P>0.05$) with ITH or oocyte viability were identified. In conclusion, temperature, humidity, and daylight affected the oocyte populations (total and viable). Photoperiod seemed to be the most important factor affecting oocyte populations of water buffalos in Veracruz state, Mexico.

Keywords: *Bubalus bubalis*, buffaloes, oocytes, photoperiod, Veracruz state, Mexico

INTRODUCTION

For the last 5,000 years, the buffalos (*Bubalus bubalis*) have been a key piece in the productive systems of Asian farms. Currently, the buffalo has an important niche in the dairy and meat industries of several countries (Nanda *et al*., 2003). In Mexico, the buffalo arrived from Central America 30 years ago. Thanks to the specie characteristic (rusticity, productive efficiency, and resistance to some diseases), buffalo herds can be found in most Mexican states, especially in those with tropical and subtropical environments such as Veracruz state (Hernández-Herrera *et al*., 2018).

There is low reproductive efficiency in the buffalo specie due to their reproductive biology as late puberty age, long gestation length, low primordial oocytes population at birth, low response

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to exogenous hormones, and environmental factors (Jainudeen and Hafez, 2000; Gasparrini, 2019). Photoperiod is the most important factor affecting reproduction, hence, ovarian activity, calving cycles, and milk production. Therefore, the photoperiod impact on the economy of buffalo production systems (Campanile *et al*., 2010; Zicarelli, 2016).

The buffaloes are considered short-day breeders, which means that have a major reproductive activity in seasons with less daylight when they improve ovarian activity, and even fertility (Campanile *et al*., 2010; Di Francesco *et al*., 2012; Campanile *et al*., 2013; Phogat *et al*., 2016; Zicarelli, 2017; Gasparrini, 2019; Wankhade *et al*., 2019; D'Occhio *et al*., 2020). The effect of photoperiod on the reproductive biology of buffaloes is more evident at high-latitude places (Campanile *et al*., 2016), but can also affect tropical and subtropical countries (de Carvalho *et al*., 2016; Monteiro *et al*., 2018). In those places, other environmental factors (temperature and humidity), along with the photoperiod, play a very important role in reproductive performance, leading even to seasonal anestrous (Das and Khan, 2010; Perera, 2011; Khan *et al*., 2012; Abdoon *et al*., 2014; Dash *et al*., 2016; Ramadan, 2018; Phogat *et al*., 2016; Aksoy *et al*., 2022). In summer, due to the high temperatures and humidity (especially in tropical and subtropical environments), there is a lack in the amount and quality of forage, resulting in low nutrition (Perera, 2011; Phogat *et al*., 2016), affecting, even more, the reproductive efficiency of buffalo herds. In places near the equatorial line, where the duration of the days is almost the same throughout the year, the major factors affecting buffalo reproduction are nutrition and health (Vale *et al*., 1996).

Has been shown that in those places where the photoperiod has a variation between different seasons, the biology of ovarian function is affected (Gasparrini, 2019). These studies demonstrated that the follicle and oocyte populations increase during the seasons of short days (breeding season) compared with seasons of long days (no-breading season; Shahzad *et al*., 2020). Additionally, it has been demonstrated that environmental factors such as temperature and humidity affect the oocyte recuperation and quality in abattoir-sourced ovaries (Nandi *et al*., 2001). This reduction in the quality and population of oocytes during the no-breeding season can lead to low *in vitro* embryo production (Manjunatha *et al*., 2009), which is the reproductive biotechnology with the best results for buffalo specie.

In Mexico, Avalos Rosario *et al*. (2022) made possible the first buffalo embryos produced by *in vitro* fertilization, but there are no studies about the impact of photoperiod and other environmental factors on the reproductive biology of water buffalo. Thus, this study aimed to investigate whether the total and viable oocyte populations and oocyte viability of water buffalos were affected by photoperiod and other environmental factors such as temperature, humidity and ITH in Veracruz state, Mexico.

MATERIALS AND MEDTHODS

This study was performed during April, May, June, July, August, September and October 2021. The ovaries were collected at TIF 647 slaughterhouse, Highway Road of Golfo, Km. 221.5, Acayucan, Veracruz state, Mexico (17°56′32″N, 94°54′37″W).

Collect of oocytes

After the slaughter of Murrah buffaloes, ovaries were obtained, washed with a saline solution (0.9% NaCl), and stored in an isothermal container with a saline solution at 38.5 °C. Follicles between 2 to 8 mm were aspirated with sterile needles connected to plastic syringes. The follicular liquid was collected and deposited in Petri plates with PBS solution for oocyte searching (Figure 1). Total oocytes (n=123) were separated and classified into grades A, B and C according to their cumulus oophorous, corona radiata, zona pellucida, plasma membrane and cytoplasm characteristics (Avalos Rosario *et al*., 2021). Grade A oocytes are lined with a round plasmatic membrane, surrounded by at least three cumulus cell layers, with a dark and homogeneous cytoplasm. Grade B oocytes are round, with less than three layers of cumulus cells, with a regular and homogeneous cytoplasm. Grade C oocytes have no cumulus cells, and irregular plasmatic membrane and cytoplasm. Only grade A and B oocytes were classified as viable oocytes (Figure 2). The viability percentage was calculated following the next formula:

$$\text{Viability (\%)} = \frac{\text{Viable oocytes/buffalo (n)}}{\text{Total oocytes/buffalo (n)}} \text{x}100$$

Environmental factors

Daily data regarding temperature (T; °C), humidity (H; %), and duration of the day (daylight; h) were obtained from the historic database of the meteorological station of the National Meteorological Service (SMN; available in smn.cna.gob.mx) in the city of Acayucan, state of Veracruz, Mexico. The index of temperature and humidity (ITH) was calculated following the next formula:

$$\text{ITH} = (0.8\, x\, T) + \frac{H}{100} x\, (T - 14.3) + 46.5$$

Statistical analysis

Data were analyzed using the Statistica v.10 (StatSoft) software. One-way ANOVA analyses were performed to examine the variation of total oocytes number per buffalo, viable oocytes number per buffalo, viability, and environmental factors by month with the Least Square Differences (LSD) of Fisher. Relation between total oocytes, viable oocytes and viability with environmental factors was performed with Person correlation analysis, and significant relations were followed by an analysis of lineal regression between continuous variables. Values of P<0.05 were considered significant.

RESULTS

Total oocytes/buffalo and viable oocytes/buffalo populations had significant (P<0.05) correlations with temperature, humidity and duration of daylight; but no significant (P>0.05) correlations with ITH. Oocyte viability had no significant (P>0.05) correlations with environmental factors (Table 1).

The temperature of June and July was similar (P>0.05) to the rest of the months, but the temperatures of April, August, September and October were lower (P<0.05) compared with the temperature of May. The humidity of June, July and August was higher (P<0.05) than the humidity of April, May, September, and October. Only April had an ITH lower (P<0.05) compared to the rest of the months. All the months had different (P<0.05) daylight. The number of total oocytes recovered per buffalo in September and October was higher (P<0.05) compared to the rest of the months. The number of total oocytes recovered per buffalo in August was higher (P<0.05) compared to April,

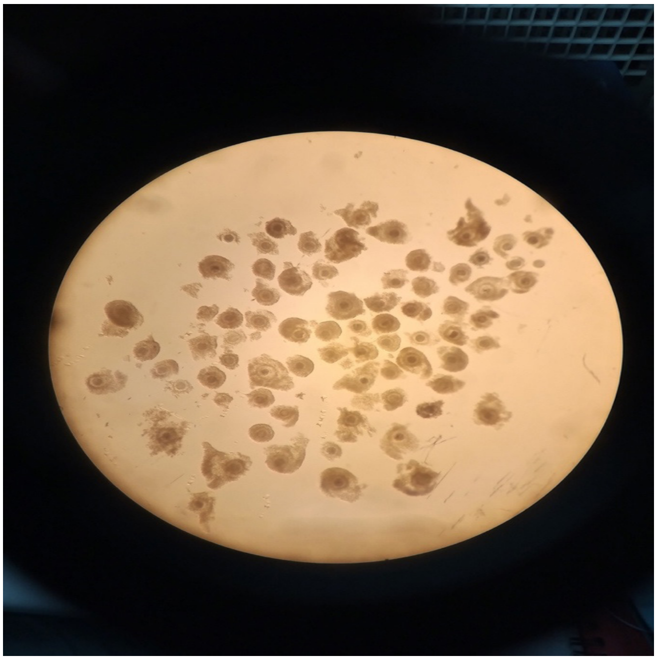

Figure 1. Water buffalo (*Bubalus bubalis*) oocytes obtained by follicular aspiration of abattoir-sourced ovaries in Veracruz, Mexico.

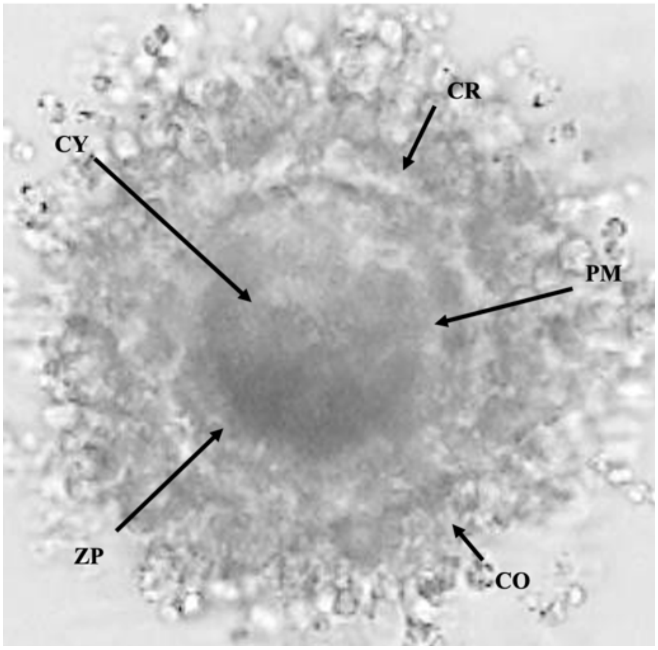

Figure 2. Water buffalo (*Bubalus bubalis*) oocyte characterized as viable (CO: Cumulus oophorous; CR: Corona radiata; ZP: Zona pellucida; PM: Plasma membrane; CY: Cytoplasm).

Table 1. Person correlation analysis between oocyte populations of water buffaloes and environmental factors in Veracruz, Mexico.

Item	Total oocytes/buffalo (n)	Viable oocytes/buffalo (n)	Oocyte viability (%)
Temperature (ºC)	-0.634625 *	-0.577918 *	0.027640
Humidity (%)	0.463702 *	0.390320 *	-0.082201
ITH	0.048715	0.004976	-0.028138
Daylight (h)	-0.860926 *	-0.870109 *	-0.131913

Values with * are significant (P<0.05).

May and June, but similar (P>0.05) to July. The number of total oocytes recovered per buffalo in May was similar (P>0.05) to April and June, but different (P<0.05) to the rest of the months. The number of viable oocytes per buffalo recovered in September and October was higher (P<0.05) compared to the rest of the months. April, July and August had lower oocyte viability than May, June, September and October (Table 2).

There is a negative relation (P<0.05) between temperature with total oocytes (r= -0.63) and viable oocytes (r= -0.57). As the temperature increases, the number of recovered total and viable oocytes reduces (Figure 3).

There was a positive effect (P<0.05) of the humidity on the total oocytes (r=0.46) and viable oocytes (r=0.39) populations. As the humidity increases, the number of recovered total and viable oocytes increases (Figure 4).

There was a strong negative relation (P<0.05) between daylight with total oocytes (r=-0.86) and viable oocytes (r=-0.87). As daylight increases, the number of recovered total and viable oocytes reduces (Figure 5).

There was a strong influence of daylight on oocyte populations. Daylight changes by month and is the most favourable factor (P<0.05) affecting the number of total and viable oocytes of Water buffalo in Veracruz, Mexico. Viability was constant and was not affected (P>0.05) by daylight (Figure 6).

Temperature and humidity change during the months and affect (P<0.05) the oocyte populations. ITH changes (P<0.05) during the months but did not affect (P>0.05) the oocyte populations of water buffalo in Veracruz, Mexico (Figure 7).

DISCUSSION

Several articles have discussed the role of environmental factors in the reproductive biology of buffaloes. Shahzad *et al*. (2020) observed differences in the temperature and daylight length between breeding and no breeding season in China. In that study, lower temperatures were registered from January to March, higher temperatures were registered from July to September, and humidity remained constant throughout the year, similar to our registers. High temperatures and long days were present in no breeding season. These factors were associated with a low number of recovered oocytes and poor-quality oocytes (Abdoon *et al*., 2014). The opposite effect was found during the breeding season (lower temperature and short

Table 2. Variation of environmental factors and oocytes population of water buffalos by month in Veracruz, Mexico.

Item	Month						
	April	May	June	July	August	September	October
Temperature (ºC)	29.03 ± 0.32^{a}	30.06 ± 0.31^{b}	29.42 ± 0.32^{ab}	29.23 ± 0.31^{ab}	29.05 ± 0.31^{a}	29.02 ± 0.32^{a}	28.77 ± 0.31^{a}
Humidity (%)	69.08 ± 1.53^{a}	69.10 ± 1.51^{a}	80.73 ± 1.53^{b}	81.23 ± 1.51^{b}	82.64 ± 1.51^{b}	82.49 ± 1.53^{b}	81.77 ± 1.51^{b}
ITH	79.54 ± 0.40^{a}	81.24 ± 0.39^{b}	82.03 ± 0.40^{b}	81.88 ± 0.39^{b}	81.80 ± 0.39^{b}	81.69 ± 0.40^{b}	81.22 ± 0.39^{b}
Daylight (h)	12.52 ± 0.02^{a}	12.94 ± 0.02^{b}	13.15 ± 0.02^{c}	13.03 ± 0.02^{d}	12.67 ± 0.02^{e}	12.18 ± 0.02^{f}	11.68 ± 0.02^{g}
Total oocytes per buffalo (n)	7.80 ± 0.40^{a}	7.20 ± 0.40^{ab}	6.60 ± 0.40^{b}	8.00 ± 0.40^{ac}	9.00 ± 0.40^{c}	11.40 ± 0.40^{d}	12.20 ± 0.40^{d}
Viable oocytes per buffalo (n)	6.40 ± 0.32^{a}	6.00 ± 0.32^{a}	5.60 ± 0.32^{a}	6.40 ± 0.32^{a}	6.40 ± 0.32^{a}	9.40 ± 0.32^{b}	10.20 ± 0.32^{b}
Oocyte viability (%)	82.34 ± 3.90^{b}	83.57 ± 3.91^{a}	85.24 ± 3.90^{a}	80.40 ± 3.91^{b}	71.34 ± 3.90^{b}	82.78 ± 3.91^{a}	83.82 ± 3.90^{a}

Values (LSM ± MSE) within a row with different superscripts differ ($P<0.05$).

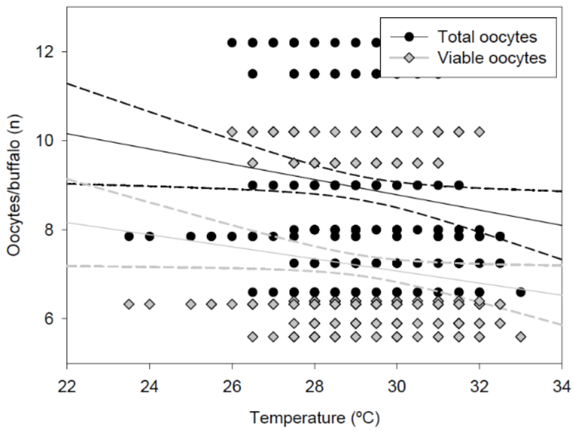

Figure 3. Relation between temperature and oocyte populations of water buffalo in Veracruz, Mexico (Total oocytes: r=-0.63, r^2=0.40, P<0.05; Viable oocytes: r=-0.57, r^2=0.33; P<0.05).

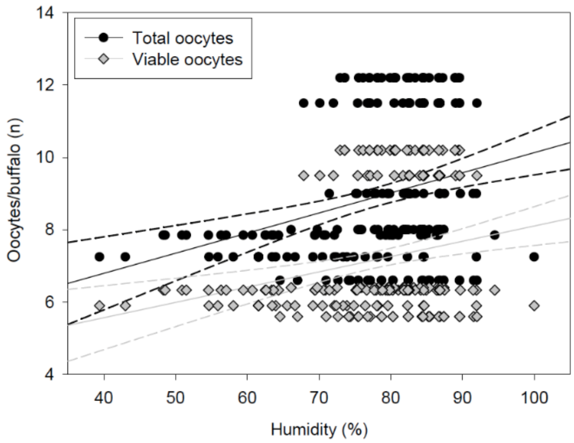

Figure 4. Relation between humidity and oocyte populations of water buffalo in Veracruz, Mexico (Total oocytes: r= 0.46, r^2= 0.22, P<0.05; Viable oocytes: r=0.39, r^2=0.15, P<0.05).

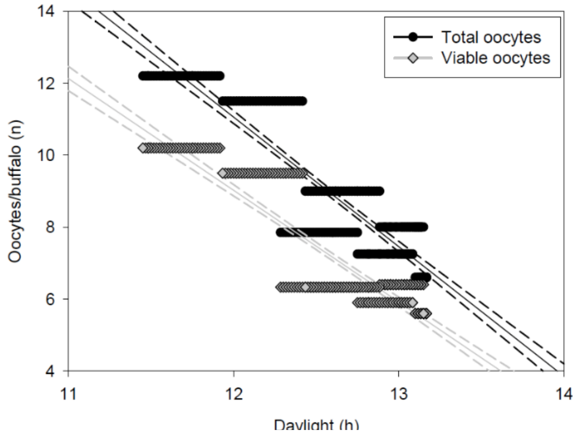

Figure 5. Relation between daylight and oocyte populations of water buffalo in Veracruz, Mexico (Total oocytes: r=-0.86, r^2=0.74, P<0.05; Viable oocytes: r=-0.87, r^2=0.76, P<0.05).

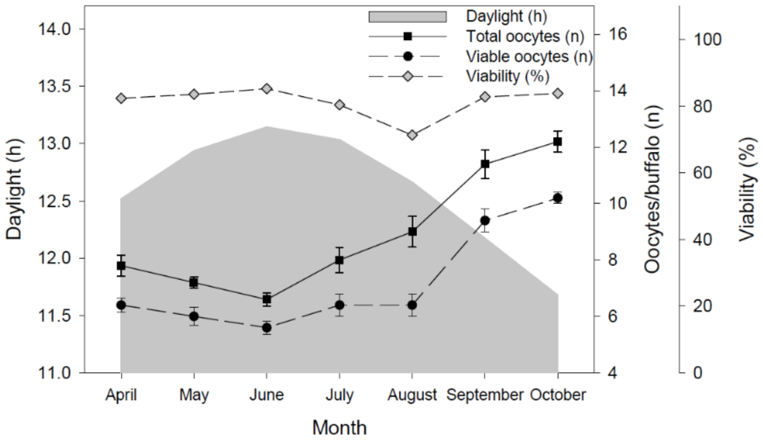

Figure 6. Association between daylight and oocyte populations of water buffalo by month in Veracruz, Mexico.

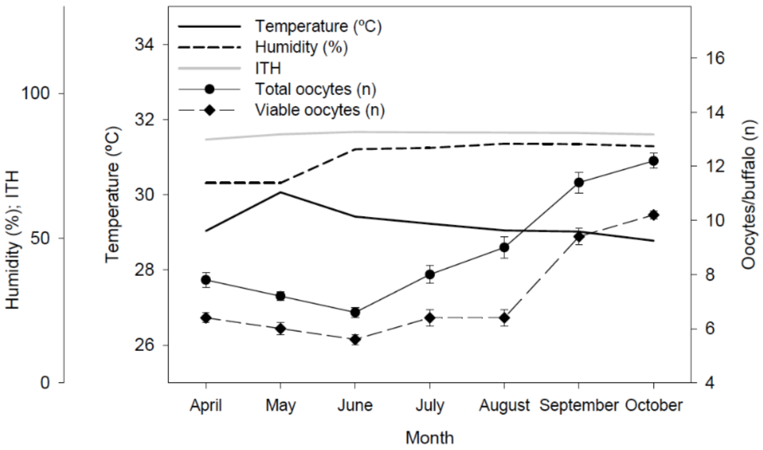

Figure 7. Association between environmental factors and oocyte populations of water buffalo by month in Veracruz, Mexico.

days). The poor-quality oocytes found during no breeding season lead a lower oocyte competence and therefore, low embryo quality and number (Di Francesco *et al*., 2012; Gasparrini, 2019). Opposite to the breeding season finding a large number of high-quality oocytes were recovered, hence, reaching higher maturation rates, and improving the embryo quality and number. Other studies have shown that despite no found differences in the oocyte viability (assessed by morphological criteria), oocyte developmental competence (assessed by *in vitro* maturation) or follicular population between seasons (Di Francesco *et al*., 2011), the *in vitro* embryo development during the breeding season was higher compared with the no-breeding season (Di Francesco *et al*., 2012).

Despite the photoperiod has been described as the most important factor affecting reproductive biology in buffaloes in most countries, other environmental factors have been related to seasonal anestrous. In Italia, where the temperature and humidity remain constant during the year, the photoperiod is the factor controlling the reproductive activity in buffaloes (Gasparrini, 2019). But in tropical regions, where the photoperiod is constant, anestrous may be due mainly to heat stress (Egypt, India, Pakistan) and low humidity levels (Venezuela; Marai and Haeeb, 2010).

The oocyte viability differences between seasons respond to endocrine, biochemistry and even genetic factors led by changes in the photoperiod and environmental factors. In 2011, Payton *et al*. (2011) identified that the expression of HSP70 (a proapoptotic cellular factor) in cumulus oophorous cells and oocytes complex (COC) was superior at high temperatures in bovines. Later, the same results were found in bufalin COC during no breeding seasons (Abdoon *et al*., 2014), as well as seasonal changes in transcriptomic profile and mRNA (Capra *et al*., 2020).

Another substance important in the regulation of bubaline reproduction is prolactin. High levels of prolactin have been found in no breeding season (high temperature and long days periods; Sheth *et al*., 1978), inhibiting the secretion of gonadotropins, and leading to an anestrous (Roy *et al*., 2009; Roy and Prakash, 2007).

Substances related to nutrition, such as the intrafollicular insulin-like growth factor (IGF1), have a big impact on reproductive function (Khan *et al*., 2012; D'Occhio *et al*., 2019a; D'Occhio *et al*., 2019b). In a study conducted by Salzano *et al*. (2019) in Brazil, low (intrafollicular and plasmatic) levels of IGF1 and estradiol were associated with a low-quality population of oocytes during no breeding seasons.

Melatonin seemed to be the most important substance (related to photoperiod) affecting the reproductive biology of buffaloes (D'Occhio *et al*., 2020). Melatonin is a hormone produced by the pineal gland. Depending on the response of the animal to certain stimuli, melatonin is produced more in either short or long days periods (D'Occhio and Suttie, 1992). Talking strictly about buffaloes, melatonin is released in short day periods (Parmeggiani *et al*., 1992). Due to the influence of photoperiod on the production and release of melatonin, this has an important function in the reproduction biology of buffaloes (Ghuman *et al*., 2010; Kumar *et al*., 2016; Emet *et al*., 2016; Ramadan, 2017; Zhao *et al*., 2019). The melatonin released by the pineal gland has a positive effect on the hypothalamus, stimulating the production and release of GnRH. This hormone act on the hypophysis (pituitary gland), which produces a release of gonadotrophin hormones (FSH and LH) into the bloodstream. This stimulus results in higher ovarian activity (Misztal *et al*., 2002; Reiter *et al*., 2009; Weems *et al*., 2015) during the breeding season.

Lipids have an important role in ovarian function. During the non-breeding season, triglycerides, cholesterol, and phospholipids levels are high on the follicular fluid and follicular cells. Moreover, lower levels of non-esterified fatty acids can be found. These differences lead to low oocyte competence during the non-breeding season, due to the importance of lipids on ovarian activity (Kosior *et al*., 2022).

CONCLUSION

Veracruz state has a wide range of temperature, humidity, and daylight throughout the year. These changes seemed to affect the total oocyte and viable oocyte populations of water buffalo, but not the oocyte viability, which was constant throughout all the sampled months. Temperature and humidity affected the number of oocytes (total and viable) recovered, but photoperiod seemed to be the most important factor regarding oocyte populations. Breeding strategies must be established to take advantage of the seasonal viability of the oocytes of water buffalos in Veracruz state, Mexico.

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