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Effect of season on follicular population, quality and nuclear maturation of bovine oocytes under tropical conditions

Jorge Alonso Peralta-Torres[a,*], Jesús Ricardo Aké-López[b], José Candelario Segura-Correa[b], Jesús Ricardo Aké-Villanueva[b]

[a] División Académica de Ciencias Agropecuarias. Universidad Juárez Autónoma de Tabasco, Carretera Villahermosa-Teapa Km. 25, R/A La Huasteca 2ª Sección, ZP 86280 Villahermosa, Tabasco, Mexico

[b] Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Yucatán, Carretera Mérida-Xmatkuil Km. 15.5, ZP, 97100 Mérida, Yucatán, Mexico

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ABSTRACT

The aim was to determine the effect of season of the year and the presence of a corpus luteum (CL) on follicular population (FP) and the quality of the oocytes, and of season on nuclear maturation of the bovine oocytes under tropical conditions. Three seasons were evaluated: hot-dry (March–June), hot-humid (July–October) and fresh-humid (November–February). In a first study, 1112 bovine ovaries were obtained from a local slaughterhouse. Follicles were classified as small (≤4 mm), middle (4.1–8 mm) and large (≥8.1 mm); and the maximum diameter of the follicle (MDF) and CL (MDCL) were also recorded. The oocytes were collected by aspiration and classified as viable (grade I and II) and damaged (grade III and IV). In the second study, 2261 viable oocytes were matured *in vitro*, and then fixed and stained with Lacmoid to classify the stage of development as mature (metaphase II), immature or degenerate. Data were analyzed using analysis of variance and chi-square procedures. The largest FP of large follicles (0.67), MDF (1.18 mm), MDCL (1.87 mm), and the highest proportion of viable oocytes (34.19%) were obtained during the hot-humid season ($P < 0.05$). The ovaries without CL had the greatest FP (10.34) with more viable oocytes (24.44%). The highest proportion of mature oocytes (76.92%) was also obtained in the hot-humid season. In conclusion, season influenced FP, MDF, MDCL, and the quality and nuclear maturation of oocytes. The presence of a CL in the ovary resulted in a decrease of FP and viability of oocytes.

1. Introduction

It is known that in tropical and subtropical regions, environmental conditions such as high temperature and high relative humidity are associated with a decrease in the reproductive efficiency of cattle (Al-Katanani et al., 1999). There are reports that indicate that environmental conditions during the hot season cause a reduction in the fertility of cows due to modifications in the estrous cycle and in endocrine secretions (Shehab-El-Deen et al., 2010). In addition, it causes changes in the follicular dynamic decreasing the population of small (3–5 mm) and middle (6–9 mm) follicles (Wilson et al., 1998; Wolfenson et al., 1995), influence the production of follicular steroids (Bilego et al., 2013) and cause a reduction of the diameter of the dominant follicle (Roth et al., 2001). It is known also that in cattle, the environmental temperature alters the ovulatory process (Siddiqui et al., 2010) and affects the formation and development of the next corpus luteum (Burke et al., 2001; Wolfenson et al., 1995).

* Corresponding author.
E-mail addresses: japt83@hotmail.com, jorge.peralta@ujat.mx (J.A. Peralta-Torres).

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Furthermore, high temperatures and humidity may influence the quality of oocytes causing premature aging (Andreu-Vázquez et al., 2010; Roth and Hansen, 2004; Roth, 2008), abnormal nuclear maturation (Maya-Soriano et al., 2013), effects on survivability, growth and development of the embryos *in vivo* or *in vitro* (Eberhardt et al., 2009; Putney et al., 1989; Silva et al., 2006). Therefore, the micro-environment of oocytes development is a cue for the success of *in vitro* maturation and posterior development of embryos (Rizos et al., 2002; Sutton et al., 2003).

Nutrition is another important factor for cattle kept under tropical conditions. Animals in the tropics are fed mainly in pasture, which quality and availability depends on the season of the year, characterized by variations in rainfall and temperature. Therefore, determining the effects of season on reproductive performance of cows is very relevant task.

The aim of this study was to determine the effect of season of the year and the presence of a corpus luteum on follicular population and the quality of oocytes, and of season on nuclear maturation of bovine oocytes under tropical conditions.

2. Materials and methods

All chemicals were purchased from Sigma Chemical Company (Sigma-Aldrich Corp., St. Louis, MO, USA), unless otherwise indicated.

2.1. Localization and study periods

Two studies were conducted, from April 2015 to January 2016 (first study) and from December 2015 to September 2016 (second study), in the laboratory of Animal Reproduction of the "Facultad de Medicina Veterinaria y Zootecnia de la Universidad Autónoma de Yucatán (FMVZ-UADY)", located in Merida, Yucatan, Mexico (20° 58′ 04″ N and 89° 37′ 18″ W). The FMVZ-UADY is 10 m above sea level and has a hot-sub humid climate with rain in summer, and average temperature and rainfall of 26.1 °C and 1022 mm, respectively (INEGI, 2015).

Based on climatological records (temperature and relative humidity) of the last 15 years in Yucatán, three seasons were established: hot-dry (March–June), hot-humid (July–October) and fresh-humid (November–February). Climatological information during the periods of study is shown in Table 1. The ambient temperature (T, °C) and relative humidity (RH, %) were used to calculate the temperature humidity index (THI) based on Mader et al. (2006) formula:

$$THI = (0.8xT) + [(RH/100)x(T-14.4) + 46.4]$$

2.2. First study: follicle population and quality of oocytes

2.2.1. Ovaries collection

One thousand one-hundred and twelve ovaries from *Bos indicus* and F1 (*Bos indicus* × *Bos taurus*) cows (364 in the hot-dry, 374 in the hot-humid and 374 in the fresh-humid seasons), were collected from a slaughterhouse in Mérida, Yucatán, México. Ovaries were transported in a thermo with 0.9% NaCl containing 70 μg mL^{-1} kanamycin at 37 °C. The period between the collection of the ovaries and their transport to the laboratory did not exceed 2 h. Once in the laboratory, ovaries were washed with a saline solution with kanamycin at 37 °C.

2.2.2. Follicle and corpus luteum diameters

According to Bó et al. (2003), follicles were counted (FP), measured and classified as small (≤4 mm), middle (4.1–8 mm) and large (≥8.1 mm) using a real time ultrasound (Mindray-DP-50Vet, USA), equipped with a 5 MHz probe. In addition, the maximum diameter of the follicle (MDF) and maximum diameter of the corpus luteum (MDCL) were obtained.

2.2.3. Collection and selection of oocytes

Oocytes were collected by puncture and aspiration of antral follicles (≥3 mm), using a 10 ml syringe (Air tite) with an 18 g needle. The extracted follicular liquid was collected in Falcon tubes of 50 ml, and let it sediment during 15 min at 37 °C. Later, the supernatant was removed and the sediment with oocytes and follicular cells was re-suspended in 10 ml of a modified Dulbecco's phosphate buffered saline solution (PBS), at 37 °C. The tube content was deposited in a Petri dish (90 × 14 mm) to next classify the

Table 1
Mean of ambient temperature, relative humidity, rainfall and temperature humidity index (THI) during periods of study (CONAGUA, 2015, 2016).

Season	Temperature (°C)	Relative humidity (%)	Rainfall (mm)	THI
Hot-dry (April–May 2015)[a]	30.1	68.0	10.6	81.1
Hot-humid (Aug–Sept 2015)[a]	28.7	86.0	342.0	81.6
Fresh-humid (Dec 2015–Jan 2016)[a,b]	25.0	91.0	26.5	76.0
Hot-dry (April–May 2016)[b]	30.0	81.5	45.8	83.1
Hot-humid (Aug–Sept 2016)[b]	28.7	89.5	221.6	82.1

[a] Seasons of the first study.
[b] Seasons of the second study.