

# Follicular population, quantity and quality of cumulus-oocyte complexes of cows grazing in three seasons of the year in the Mexican tropics

## Población folicular, cantidad y calidad de complejos cúmulo-ovocitos de vacas en pastoreo en tres estaciones del año en el trópico mexicano

Bulmaro Méndez-Argüello<sup>1</sup>, Israel Martínez-Cruz<sup>1</sup>, Cristoforo Mateo Gusmán-Arcos<sup>2</sup>, Froylan Rosales-Martínez<sup>1\*</sup>, Rubén Monroy Hernández<sup>1</sup>, Jesús Benjamín Ponce-Noguez<sup>1</sup>, Juan Carlos, Torres-Ramírez<sup>1</sup>

<sup>1</sup>Universidad Autónoma de Chiapas, Facultad Maya de Estudios Agropecuarios, Catazajá, Chiapas, México.

<sup>2</sup>Colegio de postgraduados, Campus Campeche, Champotón, Campeche, México.

\*Corresponding author: [rosales.froylan@unach.mx](mailto:rosales.froylan@unach.mx) Tel: 2294201144

### ABSTRACT

The high temperatures and relative humidity of recent years have caused heat stress in cattle, which mainly affects animals in grazing systems. The objective of this research was to determine the follicular population, quantity and quality of cumulus-oocyte complexes in grazing cows during three seasons of the year in the northern regions of Chiapas, Mexico. The study was conducted in three climatic seasons: fresh-humid, hot-dry, and hot-humid. A total of 416 ovaries were used (FH=225, HD=100, and HH=91), from crossbred cows (*Bos taurus* x *Bos indicus*) from grazing systems. The number of corpus luteum, follicular population, and the quantity and quality of cumulus-oocyte complexes, which were also classified as viable and non-viable, were analyzed. The study variables were analyzed using the generalized linear models. No statistical difference was observed, by ovary, for corpora lutea ( $P \geq 0.05$ ); with means of  $0.35 \pm 0.04$  (27.3%),  $0.46 \pm 0.06$  (35.9%), and  $0.47 \pm 0.07$  (36.8%) for the fresh-humid, hot-dry, and hot-humid seasons. The season significantly affected medium follicles ( $P \leq 0.003$ ), small follicles ( $P \leq 0.001$ ), and total follicles ( $P \leq 0.001$ ), but not large follicles ( $P \geq 0.05$ ). A season effect was observed for all COCs variables ( $P \geq 0.05$ ), as well as for the cumulus-oocyte complexes viable and non-viable ( $P \leq 0.002$ ). According to the observed results, the highest fertility of crossbred bovine females occurs during the fresh-humid season, which can be used to schedule breeding and implement reproductive biotechnologies.

**Key words:** Climate change; tropical climate; heat stress; fertility; reproduction

### RESUMEN

Las altas temperaturas y humedades relativas de los últimos años, provocan estrés calórico en los bovinos, que afectan mayormente a los animales de sistemas de pastoreo. El objetivo de esta investigación fue determinar la población folicular, cantidad y calidad de complejos cúmulo-ovocitos de vacas en pastoreo en tres estaciones del año, en la región norte de Chiapas, México. El estudio se realizó en tres estaciones climatológicas, fresca-húmeda, caliente-seca y caliente-húmeda. Se utilizó un total de 416 ovarios (FH=225, HD=100 y HH= 91), provenientes de vacas mestizas (*Bos taurus* x *Bos indicus*) de sistemas de pastoreo. Se analizó el número de cuerpos lúteos, la población folicular, la cantidad y calidad de complejos cúmulo-ovocitos, que además se clasificaron en viables y no viables. Las variables de estudio fueron analizadas con el procedimiento de modelos generalizados. No se observó diferencia estadística, por ovario, para cuerpos lúteos ( $P \geq 0.05$ ), las medias fueron de  $0.35 \pm 0.04$  (27,3 %),  $0.46 \pm 0.06$  (35,9 %) y  $0.47 \pm 0.07$  (36,8 %) para las estaciones fresca-húmeda, caliente-seca y caliente-húmeda. La estación afectó significativamente folículos medianos ( $P \leq 0.003$ ), folículos pequeños ( $P \leq 0.001$ ) y total de folículos ( $P \leq 0.001$ ), pero no folículos grandes ( $P \geq 0.05$ ). Se observó efecto de estación en todas las variables de complejos cúmulo-ovocitos ( $P \geq 0.05$ ), así como en los complejos cúmulo-ovocitos viable y no viable ( $P \leq 0.002$ ). De acuerdo con los resultados observados, la mayor fertilidad de las hembras bovinas mestizas se presenta en la estación fresca-húmeda, que puede ser utilizada para programar empadres e implementar biotecnologías reproductivas.

**Palabras clave:** Cambio climático; clima tropical; estrés calórico; fertilidad; reproducción

## INTRODUCTION

In the tropical warm climate regions of America, cattle ranching is characterized by its production based on extensive grazing [1]. In these regions, animals are exposed to abrupt environmental changes, which sometimes cause stress that affect their fertility [2].

In recent years, climate elements have changed, partly attributed to greenhouse effects, and the tropical regions have been the most affected [3]. Precipitation is concentrated more abundantly over short periods, and high radiation causes prolonged droughts [4].

It has been observed that, in cattle, high temperatures and relative humidity cause heat stress in both females and males, compromising their fertility [5]. Although cattle are homeothermic animals that regulate their body temperature to maintain their functions, heat stress can cause a physiological imbalance, increasing body, vaginal, uterine, and ovarian temperatures in females [6, 7, 8, 9]. In the ovary, heat stress reduces the follicular population and affects the development of the preovulatory follicle, which requires an internal temperature up to 1.5 °C lower than in the surrounding ovarian tissues for proper functioning [10]. Peralta-Torres *et al.* [11], observed in Brahman heifers and their crosses in Mexico, maintained on pasture, the effect of the season of the year on the follicular population and the maximum size of the follicles, as well as on their cyclicity.

In cumulus-oocyte complexes (COCs), heat stress affects the communication between cumulus cells and the oocyte [12], which is essential for ensuring gestation in the female [13]. Furthermore, heat stress causes damage to the oocyte's DNA and to the zona pellucida [14].

In the northern region of Chiapas, located in the southeast of Mexico, the changing climate of the recent years, groups weather seasons with their own characteristics, where temperature and humidity are high, leading to infertility issues in cattle [2], [15]. Therefore, the objective of this study was to determine the follicular population, quantity and quality of COCs of grazing cows in three seasons of the year in the northern region of Chiapas, Mexico.

## MATERIALS AND METHODS

### Characteristics of the seasons of the year

The study was conducted in three climatic seasons: fresh-humid (FH, November-February), hot-dry (HD, March-May) and hot-humid (HH, June-October), in Palenque, Chiapas Mexico. The climate of the region corresponds to Af, warm-humid, with rainfall throughout the year [16]. During the entire study period, daily information was collected on maximum (Tmax), average (Tmed) and minimum temperature (Tmin), relative humidity (RH) and precipitation (PP), using a weather station (Vantage VUE®, Model: 6250, USA) located at the study site.

### Recovery of ovaries and cumulus-oocyte complexes

Ovaries were collected from cows (*Bos taurus* x *Bos indicus*) slaughtered at the municipal slaughterhouse in Palenque,

Chiapas. A total of 416 ovaries were used (FH=225, HD=100 and HH=91), from crossbreed females *Bos taurus* x *Bos indicus*, destined for slaughter. The period from the collection of the ovaries to their transport to the laboratory was less than half an hour (h). The ovaries were transported in a thermo with one liter of 9% saline solution (PISA®, Lab. PISA, S.A de C.V, Mexico), supplemented with 10 µg mL<sup>-1</sup> of penicillin-streptomycin (Estreptopen®, Lapisa®, Salud Animal, Mexico), at 36 °C. Upon arrival at the laboratory, the ovaries were placed in a water bath (LW Scientific®, Model: WBL-10LC-SSD1, Taiwan) and washed with the previously described solution. The presence of corpus luteum (CL) was visually verified; subsequently, the total number of follicles (5,393) was determined and classified according to their size as small (≤ 4 mm), medium (4.1 to 8 mm) and large (≥ 8.1 mm) with the help of a veterinary ultrasound equipped with a linear transducer at 7 MHz (Draminski®, iScan2, Poland) [11].

The COCs (1,798) were recovered by making two incisions in the follicles in the shape of a cross; the follicular fluid was sedimented in sterilized Falcon® tubes of 15 mL (CTR® Scientific, USA) for 15 min at 36°C. Subsequently, the supernatant was removed and the sediment containing the COCs was placed in 15 mL of Phosphate Buffered Saline (PBS; Sigma Aldrich®, Germany) at 36°C. The search and classification were carried out using a stereomicroscope (Velab™, Model: VE-S5C, USA). The PBS containing the COCs was placed in a 100 x 15 mL Petri dish (Falcon®, CTR® Scientific, USA), on a heat plate at 36°C (TED, Model: MA-3322, USA), for their search and classification.

### Classification of cumulus-oocyte complexes

The COCs were classified according to their quality into four classes: one (those with more than three compact layers of cumulus cells), two (those with a partial layer of cumulus cells and/or slightly expanded cumulus), three (those with cytoplasm and dark spots, expanded cumulus, indicative of follicular atresia), and four (naked oocytes, without any layer of cumulus cells and that did not necessarily have dark spots and cytoplasm) [17]. Additionally, once classified into classes, the COCs were divided into viable (classes 1 and 2) and non-viable (classes 3 and 4) [18].

### Response variables

The number of CL was recorded through visual observation in each ovary, before starting the collection of COCs; the follicular variables were classified according to what was described by Peralta-Torres *et al.* [11] into large follicles (FOLG) greater than 8.1 mm, medium (FOLM) from 4.1 to 8 mm, small (FOLP) less than 4 mm, total follicles (TFOL) including FOLG, FOLM and FOLP; the cumulus-oocyte complexes were classified according to what was described by Aguila *et al.* [17], class one (COCs 1), class two (COCs 2), class three (COCs 3), and class four (COCs 4); and viable (VIAB) and non-viable (UNVIAB) COCs according to what was described by [18].

### Statistical analysis

The climatic characteristics by season were determined using descriptive statistics. For follicular variables and COCs, a linear model was used, considering the fixed effect of the season (FH, HD y HH). The study variables were analyzed using the generalized models (GENMOD) procedure of SAS [19]. A Poisson distribution was used for the errors with a logistic link function.

## Fertility of grazing cows in three seasons of the year / Méndez-Argüello *et al.*

### RESULTS AND DISCUSSION

The results of this study show that, currently, the precipitation in the dry season is almost 75% less than in the rainy season and almost 50% less than in the wet season, which leads to an increase in Tmax. The climatic characteristics by season are shown in TABLE I.

**TABLE I**  
Daily climatological variables (least square means  $\pm$  standard error) from three seasons of the year, in the northern region of Chiapas

Seasons	Climatological variables				
	PP (mm d <sup>-1</sup> )	Tmax (°C)	Tmed (°C)	Tmin (°C)	RH (%)
FH	3.6 $\pm$ 1.7	27.4 $\pm$ 2.9	21.7 $\pm$ 0.7	18.4 $\pm$ 1.4	97.5 $\pm$ 0.4
HD	1.7 $\pm$ 1.5	36.4 $\pm$ 4.9	26.3 $\pm$ 1.9	23.6 $\pm$ 3.5	86.7 $\pm$ 5.2
HH	6.7 $\pm$ 1.6	34.1 $\pm$ 1.2	25.5 $\pm$ 0.9	23.7 $\pm$ 0.6	94.5 $\pm$ 2.8

FH = Fresh-humid; HD = Hot-dry; HH = Hot-humid; PP = Precipitation; Tmax = Maximum temperature; Tmed = Average temperature; Tmin = minimum temperature; RH = relative humidity.

Although the northern region of the state of Chiapas has been classified within the warm humid climates with rain throughout the year [16], in recent years, this phenomenon has been observed: the rainy seasons are becoming shorter and tropical regions have been the most affected [20]. On the other hand, the highest maximum temperatures occurred in the dry season, in the wet and rainy seasons, the values were observed above 90%. This because when the temperature decreases, the relative humidity increases, as colder air has less capacity to retain water vapor.

No statistical difference was observed, by ovary, for CL ( $p \geq 0.05$ ), with means of 0.35  $\pm$  0.04 (27.3 %), 0.46  $\pm$  0.06 (35.9 %) and 0.47  $\pm$  0.07 (36.8 %) for the FH, HD and HH seasons, respectively. The presence of a functional CL in non-pregnant female cattle is indicative of their reproductive status since a CL of at least 20 mm in diameter indicates that the females is cycling [21, 22]. In research conducted by Peralta-Torres *et al* [11] with Brahman and F1 heifers, a higher number of CL was observed in the HH season and although no statistical difference was observed in this research, a slight increase in HH was observed.

The season significantly affected FOLM ( $P \leq 0.003$ ), FOLP ( $P \leq 0.001$ ) y TFOL ( $P \leq 0.001$ ) (TABLE II), but not FOLG ( $P \geq 0.05$ ).

**TABLE II**  
Follicular population, by ovary (least square means  $\pm$  standard error), of *Bos taurus* x *Bos indicus* cows in three seasons of the year in the northern region of Chiapas

Seasons	Follicular population			
	Large follicles ( $\geq 8.1$ mm)	Medium follicles (4.1 a 8 mm)	Small follicles ( $\leq 4$ mm)	Total follicles
FH	0.70 $\pm$ 0.06 <sup>a</sup>	1.41 $\pm$ 0.08 <sup>a</sup>	9.67 $\pm$ 0.23 <sup>a</sup>	11.78 $\pm$ 0.25 <sup>a</sup>
HD	0.64 $\pm$ 0.08 <sup>a</sup>	0.92 $\pm$ 0.09 <sup>b</sup>	14.52 $\pm$ 0.38 <sup>b</sup>	16.08 $\pm$ 0.40 <sup>b</sup>
HH	0.55 $\pm$ 0.08 <sup>a</sup>	0.86 $\pm$ 0.09 <sup>b</sup>	10.86 $\pm$ 0.34 <sup>c</sup>	12.27 $\pm$ 0.37 <sup>a</sup>

<sup>a, b, c</sup> Different literal in the same column, indicates statistical difference ( $p \leq 0.05$ ) between seasons. FH = Fresh-humid; HD = Hot-dry; HH = Hot-humid

In this research, although without statistical difference, a greater number of FOLG was observed in FH; these follicles, due to their size, have a higher probability of becoming ovulatory, as they are better able to take advantage of low concentrations of FSH and the increase in LH. Additionally, the secretion of inhibin B during the follicular phase, produced by the granulosa cells of the developing follicles, decreases the amount of FSH, which helps in the selection of a single dominant follicle, keeping the remaining follicles in a subordinate status [23]. On the other hand, the greater number of FOLP was observed in HD; these follicles, being very small, have a higher probability of undergoing atresia and not continuing their development due to the dominance of the preovulatory follicle [24]. The results observed in Table 2, may be due to the presence of Tmax above 36°C in HD, as it has been reported that for each point an increase in temperature and humidity index leads to a decrease of 0.1 mm in follicle size on the day of estrus [25].

In a study conducted by Peralta-Torres *et al.* [26], using ovaries from *Bos indicus* and F1 (*Bos indicus* x *Bos taurus*) cows from Yucatan, Mexico, they observed 8.10  $\pm$  0.20, 1.82  $\pm$  0.08 and 0.53  $\pm$  0.03 FOLP, FOLM and FOLG, respectively, in the FH season and 6.81  $\pm$  0.20, 1.49  $\pm$  0.08 and 0.50  $\pm$  0.03, FOLP, FOLM and FOLG, in the HD season; these results are lower than those observed in this research (TABLE II).

An effect of season was observed in all COCs variables ( $p \geq 0.05$ ) (TABLE III). In this study, similar values of COCs 1 were observed in FH and HD with more than 60% compared to HH. COCs 2 were higher in HD, although likewise, COCs 3 and 4 were greater in the same season.

**TABLE III**  
Quality of cumulus-oocyte complexes by ovary (least square means  $\pm$  standard error) of *Bos taurus* x *Bos indicus* cows in three seasons of the year in the northern region of Chiapas

Seasons	Cumulus-oocyte complexes				
	Quality 1	Quality 2	Quality 3	Quality 4	Totals COCs
FH	1.55 $\pm$ 0.09 <sup>a</sup>	1.04 $\pm$ 0.07 <sup>a</sup>	1.00 $\pm$ 0.07 <sup>a</sup>	0.12 $\pm$ 0.02 <sup>a</sup>	3.73 $\pm$ 0.14 <sup>a</sup>
HD	1.83 $\pm$ 0.13 <sup>a</sup>	1.92 $\pm$ 0.14 <sup>b</sup>	2.64 $\pm$ 0.16 <sup>b</sup>	0.29 $\pm$ 0.05 <sup>b</sup>	6.68 $\pm$ 0.26 <sup>b</sup>
HH	0.66 $\pm$ 0.08 <sup>b</sup>	1.09 $\pm$ 0.11 <sup>a</sup>	1.79 $\pm$ 0.14 <sup>c</sup>	0.49 $\pm$ 0.07 <sup>c</sup>	4.04 $\pm$ 0.21 <sup>a</sup>

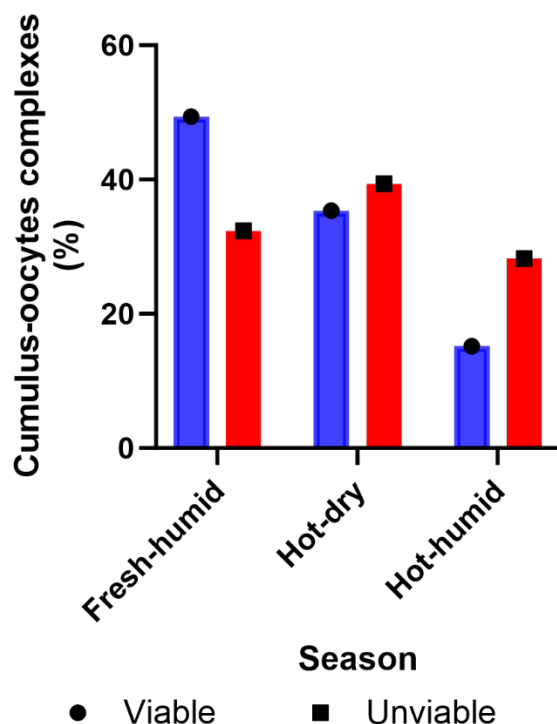
<sup>a, b, c</sup> Different literal in the same column, indicates statistical difference ( $p \leq 0.05$ ) between seasons. COCs = Cumulus-oocyte complexes. FH = Fresh-humid; HD = Hot-dry; HH = Hot-humid

The quality of the COCs is a relevant aspect when evaluating the fertility of the female bovine, as it has been observed that COCs 1 and COCs 2 have a higher probability of being fertilized [17]. A higher amount of COCs 3 and 4 may be attributed to the high temperatures present in HD, which cause damage to the cumulus cells and the oocyte [27].

There are many factors that affect the quality of COCs, *In vitro* studies, those collected in hot seasons have shown a lower capacity for development to reach the blastocyst stage [28], as high temperatures alter the expansion of cumulus cells and cytoskeletal components, which leads to increased cellular apoptosis [29].



The effect of the season was observed in VIAB COCs ( $P \leq 0.002$ ) and UNVIAB COCs ( $P \leq 0.002$ ) (FIG. 1). Out of the total COCs collected in the three seasons, 58.8% belonged to the VIAB and only 41.2% to the UNVIAB. The VIAB COCs were 36.5% more in FH compared to HH, which had the lowest count. The highest percentage of UNVIAB COCs was observed in HD, with 11% more compared to the lowest, HH.



**FIGURE 1.** Cumulus-oocyte complexes (%; viable and non-viable) of *Bos taurus* x *Bos indicus* cows in three seasons of the year in the northern region of Chiapas

In studies conducted *in vitro*, higher percentages of fertilization, embryonic division, and embryos reaching the blastocyst stage have been obtained when using COCs VIAB [17], as it is essential that cumulus cells and the oocyte are undamaged for proper communication [13]. In this study, the highest amount of VIAB was observed in FH and the lowest in HH, which may be attributed to the differences in temperature and humidity present in each season. In *in vivo* studies, Rosales-Martínez *et al.* [2, 15] with Tropical Milking criollo cows, observed higher pregnancy rates in the FH season, with a decrease from 90 to less than 25% as temperature and relative humidity increased from 20 to 35°C and 60 to 90%, respectively.

The highest percentage of UNVIAB COCs observed in HD may be due to the stress that female bovines experience when subjected to high temperatures during the dry season, since as body, vaginal [30] and ovarian temperature also increase, altering hormonal secretion and decreasing the competence of the oocytes [31]. Research has been conducted to evaluate the effect of heat stress on the viability of COCs, and it has been observed that the season has a strong impact on their quality [11]. VIAB COCs can be used for *in vitro* fertilization programs and are one of the main indicators of female fertility.

## CONCLUSION

According to the results observed in this research, the percentage of luteal bodies is similar in the fresh-humid and hot-dry seasons. However, the follicular population, with large and medium follicles that have a higher probability of ovulating, was observed in the fresh-humid season. On the other hand, the lowest number of cumulus-oocyte complexes of quality three and four was observed in the fresh-humid season, as well as the highest number of viable ones. Therefore, under the climatic conditions of the northern region of Chiapas, the highest fertility of crossbred bovine females occurs in the fresh-humid season, which can be used to Schedule mating and implement reproductive biotechnologies.

## Conflict of interest

The authors declare that they have no conflicts of interests.

## BIBLIOGRAPHIC REFERENCES

- [1] Figueroa D, Galicia L, Suárez-Lastra, M. Latin American cattle ranching sustainability debate: An approach to social-ecological systems and spatial-temporal scales. Sustainability [Internet]. 2022; 14(14):8924. doi: <https://doi.org/gv5jsx>
- [2] Rosales-Martínez F, Rosendo-Ponce A, Cortez-Romero C, Gallegos-Sánchez J, Cuca-García JM, Becerril-Pérez CM. Relation of the maximum temperature and relative humidity close to the insemination with the tropical milking criollo heifer's gestation in three seasons. Trop. Anim. Health Prod. [Internet]. 2021; 53(1):27. doi: <https://doi.org/p42n>
- [3] Byrne MP, Pendergrass AG, Rapp AD, Wodzicki KR. Response of the intertropical convergence zone to climate change: location, width, and strength. Curr. Clim. Change Rep. [Internet]. 2018; 4:355-370. doi: <https://doi.org/gj74kd>
- [4] Lyra A, Imbach P, Rodríguez D, Chan CS, Georgiou S, Garofolo L. Projections of climate change impacts on central America tropical rainforest. Clim. Change. [Internet]. 2017; 141:93-105. doi: <https://doi.org/f9xtwd>
- [5] Silva WC, Silva JAR, Camargo-Junior RNC, Silva EBR, Santos MRP, Viana RB, Silva AGM, Silva CMG, Lourenço-Júnior JB. Animal welfare and effects of per-females stress on male and cattle reproduction – A review. Front. Vet. Sci. [Internet]. 2023; 10:1083469. doi: <https://doi.org/p42p>
- [6] Chavez MI, García JE, Vélez FG, Gaytán LR, de Santiago A, Mellado M. Effects of in utero heat stress on subsequent reproduction performance of first-calf Holstein heifers. Span. J. Agric. Res. [Internet]. 2020; 18(2):e0404. doi: <https://doi.org/p42q>
- [7] Tian H, Liu J, Chen X, Li S, Li X, Mengal K, Lu Y, Wang D. Effects of ambient temperature and humidity on body temperature and activity of heifers, and a novel idea of heat stress monitoring. Anim. Prod. Sci. [Internet]. 2021; 61(15):1584-1591. doi: <https://doi.org/p42r>
- [8] Togoe D, Minca NA. The impact of heat stress on the physiological, productive and reproductive status of dairy cows. Agriculture. [Internet]. 2024; 14(8):1241. doi: <https://doi.org/p42s>

**Fertility of grazing cows in three seasons of the year / Méndez-Argüello *et al.***

- [9] Wachida N, Dawuda PM, Ate IU, Rekwot PI. Impact of environmental heat stress on ovarian function of zebu cows. *J. Anim. Health Prod.* [Internet]. 2022; 10(4):412-419. doi: <https://doi.org/p42t>
- [10] Hunter RHF, López-Gatius F. Temperature gradients in the mammalian ovary and genital tract: A clinical perspective. *Eur. J. Obstet. Gynecol. Reprod. Biol.* [Internet]. 2020; 252:382-386. doi: <https://doi.org/p42v>
- [11] Peralta-Torres JA, Aké-López JR, Centurión-Castro FG, Segura-Correa JC. Effect of season and breed group on the follicular population and cyclicity of heifers under tropical conditions. *Trop. Anim. Health Prod.* [Internet]. 2017; 49(1):207-211. doi: <https://doi.org/f9k9nd>
- [12] Campen KA, Abbott CR, Rispoli LA, Payton RR, Saxton AM, Edwards JL. Heat stress impairs gap junction communication and cumulus function of bovine oocytes. *J. Reprod. Dev.* [Internet]. 2018; 64(5): 385-392. doi: <https://doi.org/gdxtmm>
- [13] Robert C. Nurturing the egg: the essential connection between cumulus cells and the oocyte. *Reprod. Fert. Dev.* [Internet]; 2021; 34(2): 149-159. doi: <https://doi.org/p42z>
- [14] Báez F, Camargo A, Reyes AL, Márquez A, Paula-Lopes F, Viñoles C. Time-dependent effects of heat shock on the zona pellucida ultrastructure and in vitro developmental competence of bovine oocytes. *Reprod. Biol.* [Internet]. 2019; 19(2):195-203. doi: <https://doi.org/p422>
- [15] Rosales-Martínez F, Becerril-Pérez CM, Rosendo-Ponce A, Cortez-Romero C, Torres-Hernández G, Gallegos-Sánchez J. Effects of season, maximum temperature and relative humidity on the gestation success of Tropical milking criollo cows. *Agrociencia*, [Internet]. 2023; 57(5):860-881. doi: <https://doi.org/p423>
- [16] García E. Modificaciones al Sistema de clasificación climática de Köppen. [Internet]. 5th ed. Ciudad de México: Universidad Nacional Autónoma de México, Instituto de Geografía. 2004 [Accessed 15 Jan. 2025]. Available in: <https://goo.su/b6b7mc>
- [17] Aguila L, Treulen F, Therrien J, Felmer R, Valdivia M, Smith CL. Oocyte selection for in vitro embryo production in bovine species: noninvasive approaches for new challenges of oocyte competence. *Animals*. [Internet]. 2020; 10(12):2196. doi: <https://doi.org/p424>
- [18] Penitente-Filho JM, Jimenez CR, Zolini AM, Carrascal E, Azevedo JL, Silveira CO, Oliveira FA, Torres CAA. Influence of corpus luteum and ovarian volume on the number and quality of bovine oocytes. *Anim. Sci. J.* [Internet]. 2015; 86(2):148-152. doi: <https://doi.org/f6zbsg>
- [19] Statistical Analysis System (SAS). User's Guide: Statistics. Version 9.4 for Windows. Cary, NC: SAS Institute Inc. 2010.
- [20] Guo J, Hu S, Guan Y. Regime shifts of the wet and dry seasons in the tropics under global warming. *Environ. Res. Lett.* [Internet]. 2022; 17:104028. doi: <https://doi.org/gwfg4g>
- [21] Kayacik V, Salmanoglu MR, Polat B. and Özlüer A. Evaluation of the corpus luteum size throughout the cycle by ultrasonography and progesterone assay in cows. *Turk. J. Vet. Anim. Sci.* [Internet]. 2005 [cited 22 April 2025]; 29(6):1311-1316. Available in: <https://goo.su/8xNUNnB>
- [22] Vercouteren MMAA, Bittar JHJ, Pinedo PJ, Risco CA, Santos JEP, Vieira-Neto A, Galvao KN. Factors associated with early cyclicity in postpartum dairy cows. *J. Dairy Sci.* [Internet]. 2015; 98(1):229-239. doi: <https://doi.org/f6sxwq>
- [23] Verma P, Kumar AJ, Mishra A, Jesse DD, Mandal S, Gattani A, Patel P, Singh P, Jatav M. Role of inhibin hormone: An update. *Pharm. Innov. J.* [Internet]. 2023 [cited 18 april 2025]; 12(19):1162-1167. Available in: <https://goo.su/AUupK>
- [24] López-Gatius F, Llobera-Balcells M, Palacín-Chauri RJ, García-Ispuerto I, Hunter RHF. Follicular size threshold for ovulation reassessed. Insights from multiple ovulating dairy cows. *Animals*. [Internet]. 2022; 12(9):1140. doi: <https://doi.org/p427>
- [25] Schüller LK, Michaelis I, Heuwieser W. Impact of heat stress on estrus expression and follicle size in estrus under field conditions in dairy cows. *Theriogenology*, [Internet]. 2017; 102:48-53. doi: <https://doi.org/gbwz22>
- [26] Peralta-Torres JA, Aké-López JR, Segura-Correa JC, Aké-Villanueva JR. Effect of season on follicular population, quality and nuclear maturation of bovine oocytes under tropical conditions. *Anim. Reprod. Sci.* [Internet]. 2017; 187:47-53. doi: <https://doi.org/gcrkdg>
- [27] Mietkiewska K, Kordowitzki P, Pareek CS. Effects of heat stress on bovine oocytes and early embryonic development: An update. *Cells*. [Internet]. 2022; 11(24):4073. doi: <https://doi.org/p428>
- [28] Ferreira RM, Ayres H, Chiaratti MR, Ferraz ML, Araújo AB, Rodrigues CA, Watanabe YF, Vireque AA, Joaquim DC, Smith LC, Meirelles FV, Baruselli PS. The low fertility of repeated-breeder cows during summer heat stress is related to a low oocyte competence to develop into blastocysts. *J. Dairy Sci.* [Internet]. 2011; 94(5):2383-2392. doi: <https://doi.org/fppbc8>
- [29] Ahmed JA, Nashiruddullah N, Dutta D, Biswas RK, Borah P. Cumulus cell expansion and ultrastructural changes in matured bovine oocytes under heat stress. *Iran J. Vet. Res.* [Internet]. 2017 [cited 23 Feb. 2025]; 18(3):203-207. Available in: <https://goo.su/Co6XDC>
- [30] Carvalho LR, Wenceslau RR, Ribeiro LDS, de Carvalho BC, Borges AM, Camargo LSA. Daily vaginal temperature in girolando cows from three different genetic composition under natural heat stress. *Trans. Anim. Sci.* [Internet]; 2021; 5(4):txab206. doi: <https://doi.org/p429>
- [31] Roth Z. Effect of heat stress on ovarian functions and embryonic development: mechanism and potential strategies to alleviate these effects in dairy cows. *Biosci. Proc.* [Internet]. In: J.L. Juengel, A. Miyamoto, L.P. Reynolds, M.F. Smith, R. Webb (Eds.), *Reproduction in Domestic Ruminants VIII*. Packington, Leicestershire, UK: Context Publishing; 2014; pp. 193-208. Available in: <https://goo.su/0Z7mXo>