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Environment and donor management affect production amount and viability of *cumulus-oocyte complexes* (COCs) from *Bos Indicus* cows in the tropics

Ambiente y manejo modulan la cantidad y viabilidad del *complejo cumulus oophorus* (COCs) de vacas donadoras Cebú en el trópico

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ABSTRACT

In order to correlate environmental and management factors that influence the quantity and viability of the *cumulus-oocyte* complexes (COCs) in donor zebu females (N = 205) under tropical conditions. A total of (N = 5896) COCs were collected through ovum-pick up across different livestock production units. The COCs were evaluated under a stereomicroscope, and the dependent variables were: 1) the total number of recovered COCs and 2) the percentage of viable COCs. Environmental variables included: Temperature-Humidity Index (THI; classified as comfort, alert, danger, and emergency); seasons by rainfall: dry (December-May) and rainy (June-November); and season by month. Management-related variables included: technological index (TI: low, medium, high), diet (grazing only or grazing + supplementation), and acclimation (<30, 30-90, and > 90 days). One-way ANOVA, factorial ANOVA, and multivariate analyses were conducted. THI levels in the comfort and alert categories were associated with higher viability rates (68.86 ± 2.00 and 74.10 ± 2.59, respectively) compared to danger and emergency indexes (62.40 ± 2.01 and 56.52 ± 5.51; $p < 0.05$). Additionally, during summer and winter seasons (56.88 ± 3.20 and 61.07 ± 2.25) showed lower viability than spring and autumn (67.90 ± 2.19 and 76.25 ± 1.92). A high TI (35.54 ± 5.16) was associated with a greater number of COCs compared to low and medium TI (27.31 ± 1.72 and 28.20 ± 1.84, respectively), while grazing plus supplementation increased COC yield (30.96 ± 1.77 *vs.* 25.25 ± 2.09; $p < 0.05$) compared to grazing alone. Acclimation also influenced COC quantity, being higher in < 30 and 30–90 days (37.62 ± 5.24 and 32.65 ± 2.45) than in > 90 days (24.21 ± 1.50; $p < 0.05$).

KEY WORDS: Thermal comfort, bovine, Thermal stress, Oocytes embryos *in vitro*.

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RESUMEN

Con la finalidad de correlacionar factores ambientales y de manejo que modulan la cantidad y viabilidad del *complejo cumulus oophorus* (COCs) en donadoras (N = 205) cebú en el trópico, fueron obtenidos COCs (N = 5896) por aspiración folicular guiada por ultrasonografía en distintas unidades de producción pecuaria. Los COCs se evaluaron por Microscopio-estereoscópico y las variables dependientes fueron: 1) cantidad de COCs y 2) porciento de COCs viables. Variables ambientales fueron: Índice de Temperatura-Humedad (THI, *confort*, alerta, peligro y emergencia); épocas: seca (diciembre-mayo) y lluvia (junio-noviembre), y estaciones. Manejo incluyó: Índice tecnológico (TI: bajo, medio, alto), alimentación (pastoreo o pastoreo + suplementación), preparación (<30, 30–90 y > 90 días). Se realizó ANOVA de una vía, factorial y análisis multivariados. THI en Comfort y alerta mostraron viabilidad más alta (68.86 ± 2.00 y 74.10 ± 2.59) que peligro y emergencia (62.40 ± 2.01 y 56.52 ± 5.51; $p < 0.05$); verano e invierno (56.88 ± 3.20 y 61.07 ± 2.25) muestran menor viabilidad que primavera y otoño (67.90 ± 2.19 y 76.25 ± 1.92). El TI alto (35.54 ± 5.16) mostró mayores cantidades de COCs que el bajo y medio (27.31 ± 1.72 y 28.20 ± 1.84), pastoreo más suplementación aumentan la cantidad (30.96 ± 1.77 *vs* 25.25 ± 2.09; $p < 0.05$) que solo pastoreo. La preparación afectó la cantidad de COCs siendo mayor en <30 y 30-90 días (37.62 ± 5.24 y 32.65 ± 2.45) que > 90 (24.21 ± 1.50; $p < 0.05$).

PALABRAS CLAVE: Confort térmico, bovino, Estrés térmico, ovocitos, embriones *in vitro*.

Introduction

Bovine embryo transfer (ET) is a reproductive biotechnology that involves transplanting embryos from donor cows or heifers into the uterus of recipient females, which will carry the pregnancy to term. This technique is a tool for increasing the offspring of genetically superior individuals and for disseminating desirable genetic traits within cattle populations (Baruselli *et al.,* 2006). To implement an efficient ET program, reproductive technologies must be employed to enable the multiple production of embryos (Narváez *et al.,* 2022). Oocytes can be collected from ovaries obtained at slaughterhouses or *in vivo* from donor females; the former method presents the disadvantage of lacking information on the genetic background and health status of the animals; in contrast, using bovine donor females (BDs) with ultrasound-guided follicular aspiration, commonly known as Ovum Pick-Up (OPU), allows for detailed knowledge of donor characteristics, environmental conditions, and prior management (Morera *et al.,* 2022).

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In vitro bovine embryo production remains a challenge across livestock production systems, where the quality and viability of the *cumulus-oophorus* complex (COC) play a crucial role; the COC consists of an oocyte surrounded by a cumulus cell mass, which is essential for oocyte development and competence for *in vitro* fertilization (IVF) (Bó & Pincay, 2017). The quality and viability of the COC are key indicators, as they influence the embryo's developmental capacity; recent studies have shown that factors such as management practices in the livestock production unit (LPU), nutritional strategies (Pérez & Castillo, 2021), and environmental conditions significantly affect both the quantity and quality of the BD's COCs (Cao & Jiang, 2020). COC viability is not solely determined by the animal's genetic background; it is also closely linked to physiological homeostasis; environmental variables such as average temperature, relative humidity, rainfall, and wind speed can induce stress in tropical regions, which in turn may compromise COC quality, reduce IVF success rates, and impair embryo development (González & Salas, 2019). Thermal stress in BDs causes reproductive physiological alterations (Roth, 2020; Cardone *et al*., 2022), interfering with oocyte maturation, fertilization, and embryo development (Roth *et al*., 2001; Gendelman & Roth, 2012). The negative effects of heat stress on BDs can impair oocyte quality and may persist for up to three estrous cycles after a heatwave (Roth & Hansen, 2004; Zavaleta-Martínez *et al.,* 2024). Oocytes from tertiary and pre-ovulatory (antral) follicles are considered the most susceptible to thermal stress (Roth *et al*., 2001; Kawano *et al.,* 2022). Moreover, heat stress has been associated with the induction of oocyte apoptosis during follicular development, as well as apoptosis of cumulus cells (Ahmed *et al.,* 2017). COC quality has also been linked to the availability of key nutrients in BD's diet, including vitamins, minerals, and antioxidants that come from dietary management and that can influence the quantity and quality of COCs, so a balanced diet with sufficient essential nutrients can improve the quantity and quality of COCs (Lucy, 2001).

Therefore, it is necessary to perform an integral analysis of these factors (management and environment) in the tropics where BDs management and climatic variability are highly dynamic, and with this, to find procedures, times, or seasons that may improve COC yield and quality, thereby optimizing their use in *in vitro* embryo production.

Materials and Methods

Study area

The study was conducted in the Mexican tropics, within livestock production units (LPUs) located in the states of Chiapas, Guerrero, Oaxaca, and Veracruz.

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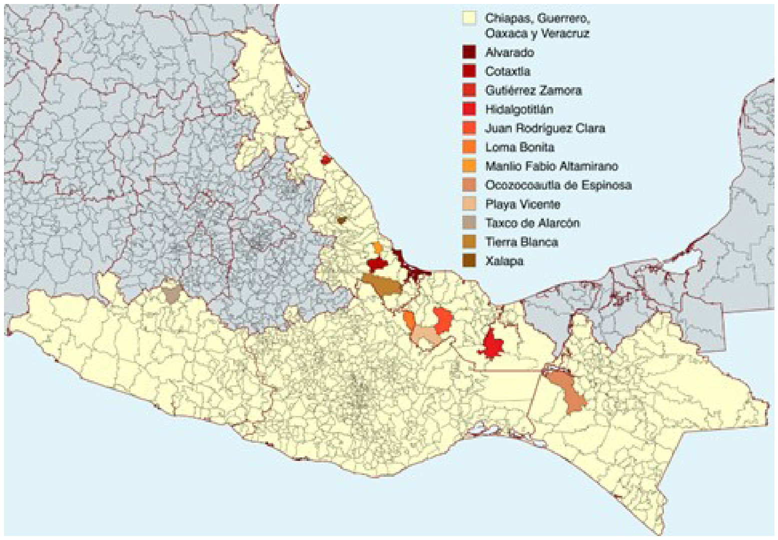

Figure 1. Location of Livestock Production Units in the different municipalities of the Mexican states analyzed.

The states of Chiapas, Guerrero, Oaxaca, and Veracruz are shown.

Experimental animals

COC data were obtained from 205 Zebu BDs without superovulation, using ultrasound-guided follicular aspiration (Ovum Pick-Up, OPU).

Nutritional and health management of donor cows

All DBs were maintained under an extensive grazing system on native pastures of *Cynodon nlemfuensis* and *Brachiaria humidicola*; in some LPUs, a commercial feed containing 16 % crude protein was also provided. The nutritional plan included mineral salts offered *ad libitum* and bypass fat supplementation (300 g/animal; Percutrin Energy, Bayer; Leverkusen, Germany). The health management plan included acaricide bathing (Amitraz 12.5 %, BOVITRAZ®, Bayer; Leverkusen, Germany), internal deworming (Ivermectin 1 %, BAYMEC® Prolong, Bayer; Leverkusen, Germany), and vaccination against viral diseases (BOVILIS® VISTA 5 L5 SQ, MSD Animal Health; Rahway, New Jersey, USA) and clostridial diseases (CLOSTRIGEN® 9 + T, Virbac; Westlake, Texas, USA).

Collection of *cumulus-oocyte* complexes (COCs)

COCs were obtained via ultrasound-guided follicular aspiration (Ovum Pick Up, OPU); which allows the collection of COCs from BDs from ovarian follicles with a diameter of 2 - 8mm; a 19 “G” disposable catheter (Punzocat) was used, coupled to a Teflon line with a negative pressure of 70 mm/Hg. Before aspiration, epidural anesthesia was administered (0.2 mg/kg of 2 % lidocaine; Logymed, Logistic & Medicine, Colombia) between the last sacral and first coccygeal vertebrae.

Once *in situ*, both ovaries were identified with the vaginal transducer, which was manipulated transrectally to subsequently aspirate the follicles (Mindray ultrasound machine, DP 10 VET). This ultrasound machine is equipped with a microconvex transducer of 7.5 MHz frequency, featuring a transvaginal guide for follicular aspiration (Morera *et al*., 2022; Narváez *et al.,* 2022). A 50 ml conical tube was used with 5 ml of D-PBS supplemented with 10UI/ml (0.1%) of heparin (Hep-Tec®; Heparin 10000UII/10ml) in each BD; the follicular contents were collected and then washed with 50 ml of D-PBS with a 75-micron EmCon filter (Agtech, USA) and placed in a Petri dish (60 x 15mm).

Recovered structures were counted, evaluated, and classified based on granulosa cell layer coverage, uniform cytoplasmic appearance, and integrity of the zona pellucida, following the criteria of Loss *et al*. (1989) and the guidelines of the International Embryo Transfer Society (IETS, 1998); viable oocytes were defined as Grade I and II (classification I–IV).

Evaluated factors

The evaluated factors were grouped into two categories: environmental factors related to the LPU location, and management-related factors within the LPU.

Environment: ambient temperature (°C), relative humidity (%), dominant wind speed (km/h), and monthly accumulated rainfall (mm). Some measurements were taken *in situ* using portable digital thermo-hygrometers (AcuRite, Model 01083M, Lake Geneva, Wisconsin, USA), while others were obtained from nearby meteorological stations. To assess climatic safety for livestock, the temperature-humidity index (THI) was calculated using Equation 1:

$$\boldsymbol{THI = 1.8 * T + 32 - \left(0.55 - (0.55 * RH)\right) * \left(1.8 * (T - 26)\right)} \quad \text{(Equation 1)}$$

Where: T = ambient temperature in °C, and RH= relative humidity (%).

Based on the calculated THI values, results were categorized as follows: *Comfort* <74, *Alert* 75–78, *Danger* 79–83, and *Emergency* ≥84 (Saizi *et al.,* 2019).

Management: The Technological Index was calculated using data obtained through surveys based on the methodology proposed by De Freitas & Pinheiro (2013) and Juárez-Barrientos (2015), which considers 23 technological practices across five zootechnical areas (nutritional management, genetic and reproductive management, health management, administrative/ commercial management, and pasture management). The zootechnical area TI was calculated using Equation 2:

$$TI_{area} = \frac{1}{n} * \sum_{i=1}^{n} Practices\ performed \quad \text{(Equation 2).}$$

Where: n= number of practices in the zootechnical area; i= i-th practice in the zootechnical area.

The Total Technological Index was calculated with Equation 3:

$$TI_{total} = \frac{1}{N} * \sum_{j=1}^{n} area\ \sum_{i=1}^{n} Practices\ performed \quad \text{(Equation 3).}$$

Where: N = total number of practices (N = 23), j = j-th zootechnical area, and i = i-th practice in the j-th area (n).

The Total Technological Index (TI_{total}) was then multiplied by 100 to express it as a percentage of implemented practices in the LPUs. Based on quartiles: Q_1 (<70) was considered low, Q_2 (70-75) medium, and Q_3 (>75) high (Arrieta-González *et al.,* 2022). Feeding strategy: DBs were grouped based on year-round nutritional management into two categories: grazing only (DBs exclusively on LPU pastures) and grazing with supplementation (commercial feed containing 16 % protein). The preparation time of DBs refers to the number of days prior to OPU when DBs were selected for oocyte donation. This included the general feeding plan plus implementation of the nutritional program, consisting of mineral salts *ad libitum* and bypass fat (300 g/animal), starting <30, 30–90, or >90 days before OPU.

Statistical analysis

Statistical analyses were conducted using STATISTICA v10.0 for Windows, StatSoft, Inc. (2011). One-way ANOVA was applied to analyze main effects, and factorial ANOVA was used to assess interactions; mean comparisons were performed using Tukey's test ($p < 0.05$). Additionally, multivariate analyses (Cluster Analysis and Principal Component Analysis, PCA) were used to explore associations between dependent variables (COC count and viability) and independent variables (environmental and management factors). Graphs were generated using SigmaPlot v11.0 (Systat Software, 2008).

Results and Discussion

As an initial step, a general climatological analysis was conducted using meteorological data from the study area, collected via digital thermo-hygrometers and nearby meteorological stations. This allowed for the construction of a general climograph (Figure 2A). Based on this, the year was subsequently divided into seasons, climatic periods, and THI categories (Figure 2B) to assess their association with the quantity and viability of the COCs.

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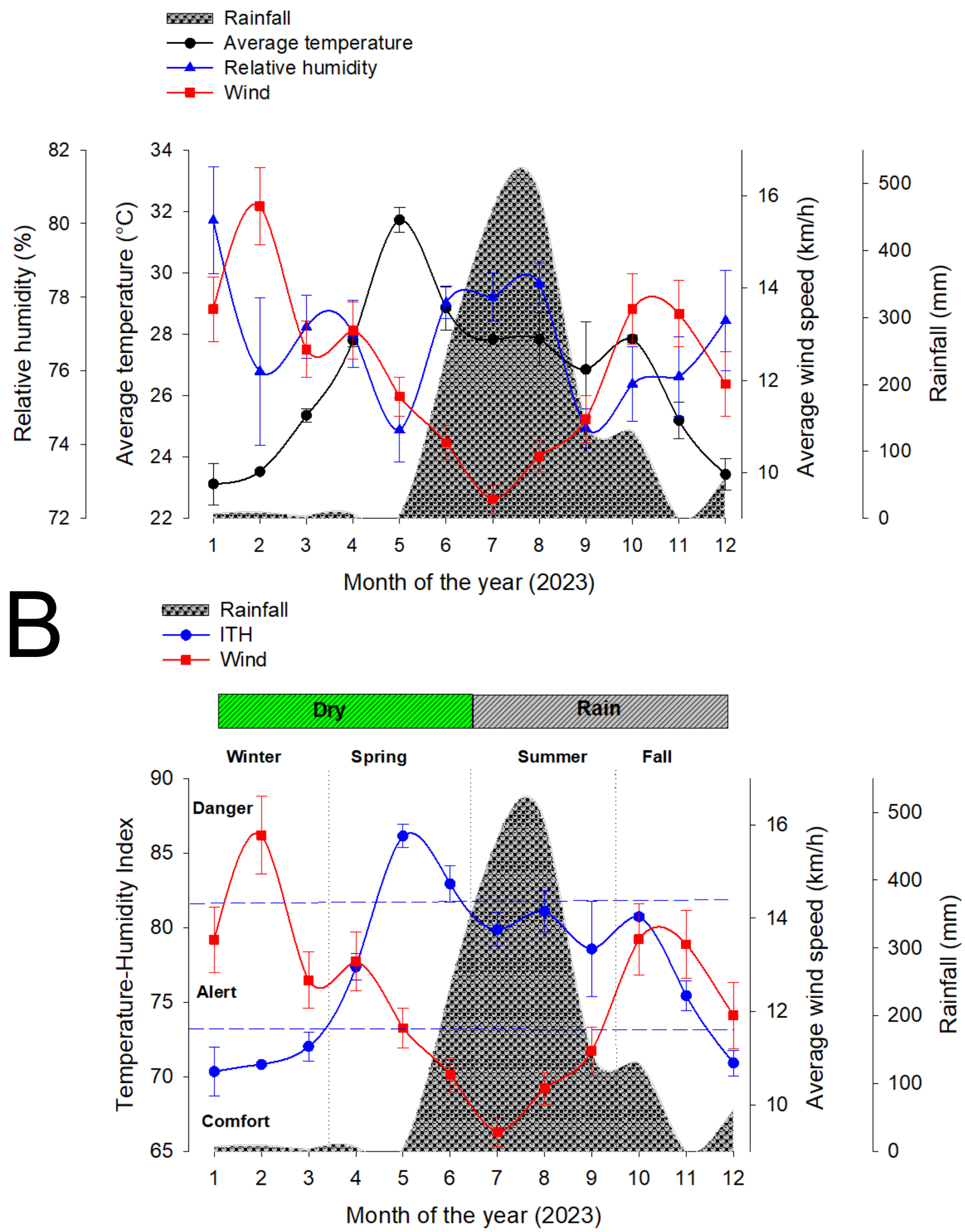

Figure 2. General climatological analysis in the study area.

A. General Climatogram. The accumulated monthly rainfall (▒, mm), the average temperature (●, °C), the relative humidity (▲, %) and the average speed of the dominant wind (■, km/h) are shown. **B.** Categorized climatogram, where the divisions of the dry season (February-June, ▒), rainfall (July-November, ▒), the seasons, (vertical dotted lines) and the THI (●), horizontal dashed blue lines; as well as the speed of the dominant wind (■, km/h) can be seen.

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The humid tropical region of Mexico accounts for more than half of the land area used for livestock production. In this region, the dual-purpose cattle system is primarily based on extensive grazing (Vilaboa & Díaz, 2009) and represents one of the main productive activities in the agricultural sector for both milk and meat production at the national level (Orantes *et al.,* 2010). These regions are predominantly covered with native grasses of the genera *Cynodon*, *Paspalum*, *Brachiaria*, and *Axonopus*, which constitute the primary forage for grazing Zebu BDs; among the most notable characteristics of these grasses, we can reference their resistance to pests, their adaptation to acid soils of low to medium fertility, and temporary flooding; despite their low protein quality, native grasses are the most important forage source in cattle production systems due to their adaptation to the changing tropical environment (Aguiar *et al.,* 2014; Cruz *et al.,* 2017). In the humid tropics of southeastern Mexico, where the analysis was conducted, there are well-defined periods of the year: the rainy season from June to October, the period of the dominant north wind “nortes” in October to February, which is coupled with the dry season from February to May (Vidal-Zepeda *et al.,* 2005), which affect the amount and duration of the dry season (Vidal-Zepeda *et al.,* 2005), which affect the quantity and quality of forage consumed by DBs, as well as the reproductive behavior of cattle (Galina & Geffroy, 2023), for example, age at first calving of 36 months and fertility of 43 percent (Ríos-Utrera *et al.,* 2020). Previous studies reported that regardless of the forage season, cows have energy deficits in dry periods (Absalón-Medina *et al.,* 2012).

Based on the climograph, COC variables (quantity and viability) were plotted by month of collection to observe their behavior throughout the year 2023 (Figure 3). The data reveal that as the THI increases (from *comfort* to *alert* to *danger*), COC viability decreases, and vice versa. Additionally, variations in COC quantity can be observed in relation to environmental conditions during the study period.

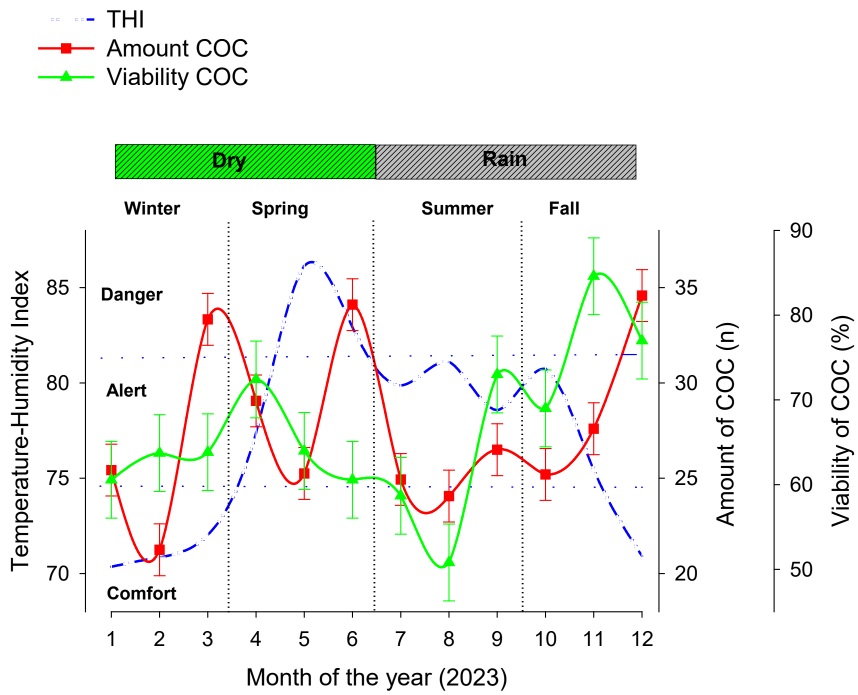

Figure 3. Distribution of Cumulus Oocyte Complex (Amount and Viability) throughout the year of study.

The year has been divided into seasons (dry ░ and rainy ▒, upper box), seasons (vertical dashed lines) and THI categories (horizontal dashed blue lines). The viability (%, ▲) and amount (■, n) of COC can be seen as a function of time (months of the year).

Based on the divisions made throughout the year (THI, climatic period, and season) and the management categories for the BDs (Technological Index, feeding strategy, and acclimation), inferential analyses were conducted to evaluate the main effects (factors). Subsequently, interaction effects were analyzed to determine whether the observed changes in environmental and management conditions were statistically significant. The results of these analyses are presented in Tables (1-4).

Table 1. Analysis of extrinsic factors that modulate the amount and viability of COC in zebu bovine donors in the tropics.

Extrinsic factors		***Cumulus-oocyte* Complex COC**				
Environment		N = 205	Amount (n)	***p*-value**	Viability	***p*-value**
THI	Comfort	61	31.09 ± 2.74	0.4071	68.86 ± 2.00[b]	0.0003
	Alert	44	30.77 ± 3.62		74.10 ± 2.59[b]	
	Danger	78	26.91 ± 1.89		62.40 ± 2.01[a]	
	Emergency	22	24.81 ± 2.67		56.52 ± 5.51[a]	
Period	Dry	93	27.98 ± 2.04	0.6087	64.67 ± 1.67	0.2904
	Rain	112	29.40 ± 1.85		67.47 ± 1.95	
Season	Spring	40	30.70 ± 2.57	0.7734	67.90 ± 2.19[b]	0.0001
	Summer	58	29.03 ± 2.75		56.88 ± 3.20[a]	
	Fall	50	27.92 ± 2.90		76.25 ± 1.92[c]	
	Winter	57	26.65 ± 2.67		61.07 ± 2.25[ab]	
Management						
Technology index	Low	54	27.31 ± 1.72	0.2204	67.10 ± 2.09	0.4412
	Medium	129	28.20 ± 1.84		65.11 ± 1.83	
	High	22	35.54 ± 5.16		70.39 ± 2.78	
Feeding	Grazing	79	25.25 ± 2.09[a]	0.0422	66.93 ± 2.57	0.6625
	Grazing+supplementation	126	30.96 ± 1.77[b]		65.75 ± 1.41	
Preparation	< 30	16	37.62 ± 5.24[b]	0.0019	63.84 ± 3.57	0.2214
Days	30 - 90	85	32.65 ± 2.45[b]		68.91 ± 1.72	
	> 90	104	24.21 ± 1.50[a]		64.35 ± 2.08	

[a,b]Different superscript between rows of the same column are significant (Fisher LSD, $p < 0.05$).

The effects of environmental and management factors on cattle are variable and complex, as they shape the conditions under which animals grow and reproduce; their influence on animal welfare, productivity, and reproduction has long been recognized and studied. Climate and its variability affect cattle both directly and indirectly by altering forage quality and/or availability, water supply, energy intake, and its utilization. As a result, cattle must respond to adverse environmental conditions by adjusting physiological and behavioral mechanisms to maintain their core body temperature within a normal range. These adjustments often lead to changes in feed intake, behavior, and productivity, typically reflected in poor performance indicators. Such effects are exacerbated under extreme tropical conditions, resulting in reduced productivity and reproductive efficiency (Grossi *et al*., 2018; Galina & Geffroy, 2023). In addition, management (performed practices) within the LPUs play a critical role in tropical regions, where the adoption of technology tends to be limited (Galina *et al.,* 2016; Arrieta-Aguirre *et al.,* 2022). Consequently, the implementation of reproductive biotechnologies aimed at improving LPU profitability is not straightforward and often yields suboptimal results (**Table 1**), particularly in terms of the quantity and viability of COCs necessary for successful *in vitro* maturation, fertilization, and embryo development prior to transfer into recipient cows (Wu & Zan, 2012; Pérez-Mora *et al*., 2020).

The following tables present the factorial analyses of the effects of the Technological Index (**Table 2**), feeding strategy (**Table 3**), and preparation time of the Zebu donor cows (**Table 4**), in interaction with environmental factors.

Table 2. Factor analysis of the effects of the technological index with the environmental factors that modulate the amount and viability of COC in Zebu bovine donors in the tropics.

Factor 1		Factor 2		Cumulus-oocyte Complex COC				
				N=205	**Amount (n)**	***p*-value**	**Viability (%)**	***p*-value**
Technology index	Low	THI	Comfort	10	37.50 ± 5.5	0.1627	74.94 ± 4.4[c]	0.0001
			Alert	3	25.33 ± 7.4		51.04 ± 4.5[b]	
			Danger	25	24.12 ± 1.8		64.49 ± 3.0[b]	
			Emergency	16	26.31 ± 2.9		69.29 ± 3.8[c]	
	Medium		Comfort	45	29.53 ± 3.1		67.91 ± 2.4b[c]	
			Alert	34	27.14 ± 3.8		75.97 ± 3.0[c]	
			Danger	44	28.68 ± 3.1		59.67 ± 2.9[b]	
			Emergency	6	20.83 ± 5.9		22.48 ± 5.3[a]	
	High		Comfort	6	32.16 ± 12.1		65.80 ± 5.2[b]	
			Alert	7	50.71 ± 10.2		74.92 ± 3.5[c]	
			Danger	9	26.00 ± 3.7		69.93 ± 5.1[c]	
			Emergency	***	***		***	
	Low	Period	Dry	29	25.31 ± 2.3	0.1520	62.43 ± 2.8	0.2290
			Rain	25	29.64 ± 2.5		72.52 ± 2.8	
	Medium		Dry	59	29.89 ± 2.9		65.97 ± 2.2	
			Rain	70	26.78 ± 2.3		64.39 ± 2.8	
	High		Dry	5	21.00 ± 5.9		62.40 ± 4.9	
			Rain	17	39.82 ± 6.1		72.74 ± 3.14	

[a,b] Different literals between rows of the same column are significant (Fisher LSD, $p < 0.05$).

*** No bovine donors were obtained in this interaction.

Continuation

Table 2. Factor analysis of the effects of the technological index with the environmental factors that modulate the amount and viability of COC in Zebu bovine donors in the tropics.

Factor 1		Factor 2		Cumulus-oocyte Complex COC				
				N=205	**Amount (n)**	***p*-value**	**Viability (%)**	***p*-value**
Technology index	Low	Season	Winter	10	29.60 ± 4.7	0.3338	52.03 ± 2.7[a]	0.0001
			Spring	24	24.37 ± 2.2		69.03 ± 2.9[c]	
			Summer	5	27.00 ± 4.3		71.81 ± 3.9[c]	
			Fall	15	30.60 ± 3.6		72.50 ± 4.3[c]	
	Medium		Winter	25	26.60 ± 3.6		64.42 ± 3.0[bc]	
			Spring	34	32.32 ± 4.3		67.11 ± 3.1[c]	
			Summer	45	28.02 ± 3.2		55.22 ± 3.4[ab]	
			Fall	25	24.56 ± 3.0		80.89 ± 2.6[d]	
	High		Winter	5	21.00 ± 5.9		62.40 ± 4.9[ab]	
			Spring	***	***		***	
			Summer	***	***		***	
			Fall	17	39.82 ± 6.1		72.74 ± 3.1[c]	

[a,b] Different literals between rows of the same column are significant (Fisher LSD, $p < 0.05$).

*** No bovine donors were obtained in this interaction.

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Table 3. Factor analysis of the effects of the type of Feeding with the environmental factors that modulate the amount and the viability of the COC in Zebu bovine donors in the tropics.

Factor 1		Factor 2		***Cumulus-oocyte* Complex COC**				
				N=205	**Amount (n)**	***p*-value**	Viability (%)	***p*-value**
Feeding	Grazing	THI	Comfort	30	28.66 ± 3.6	0.1519	74.34 ± 2.7[c]	0.0001
			Alert	14	20.07 ± 3.3		80.67 ± 4.1[c]	
			Danger	29	25.13 ± 3.7		61.82 ± 4.0[b]	
			Emergency	6	20.83 ± 5.9		22.48 ± 5.3[a]	
	Grazing+ supplementation		Comfort	31	33.45 ± 4.0		63.55 ± 2.6[b]	
			Alert	30	35.76 ± 4.8		71.03 ± 3.1[c]	
			Danger	49	27.95 ± 2.0		62.74 ± 2.1[b]	
			Emergency	16	26.31 ± 2.9		69.29 ± 3.8[bc]	
	Grazing	Period	Dry	24	18.75 ± 1.8[a]	0.0461	67.85 ± 3.8	0.5123
			Rain	55	28.09 ± 2.8[b]		66.53 ± 3.3	
	Grazing+ supplementation		Dry	69	31.20 ± 2.5[b]		63.57 ± 1.8	
			Rain	57	30.66 ± 2.4[b]		68.38 ± 2.1	
Feeding	Grazing	Season	Winter	13	17.23 ± 2.3	0.2746	67.09 ± 4.8[b]	0.0001
			Spring	11	20.54 ± 3.0		68.75 ± 6.4[b]	
			Summer	24	26.16 ± 4.5		48.81 ± 5.1[a]	
			Fall	31	29.58 ± 3.6		80.24 ± 2.2[c]	
	Grazing+ supplementation		Winter	27	31.18 ± 3.4		58.17 ± 2.2[a]	
			Spring	47	31.02 ± 3.2		67.70 ± 2.2[b]	
			Summer	26	29.53 ± 3.7		64.33 ± 3.4[b]	
			Fall	26	32.03 ± 3.7		71.49 ± 3.0[c]	

[a,b] Different literals between rows of the same column are significant (Fisher LSD, $p < 0.05$).

*** No bovine donors were obtained in this interaction.

Table 4. Factor analysis of the effects of bovine donor preparation time on environmental factors that modulate the amount and viability of COC in Zebu bovine donors in the tropics.

Factor 1		Factor 2		***Cumulus-oocyte* Complex COC**				
				N=205	Amount (n)	***p*-value**	**Viability**	***p*-value**
Preparation Days	< 30	THI	Comfort	8	45.37 ± 8.8[c]	0.0126	72.26 ± 4.3[c]	0.0001
			Alert	2	48.00 ± 2.0[c]		65.40 ± 2.2[bc]	
			Danger	6	23.83 ± 3.6[ab]		52.10 ± 4.4[b]	
			Emergency	***	***		***	
	30-90		Comfort	23	35.21 ± 5.0[b]		63.28 ± 3.0[bc]	
			Alert	26	36.50 ± 5.5[b]		74.69 ± 3.3[c]	
			Danger	25	28.64 ± 3.6[b]		65.03 ± 3.1[c]	
			Emergency	11	27.36 ± 2.9[b]		75.87 ± 2.8[c]	
	> 90		Comfort	30	24.13 ± 2.7[ab]		72.22 ± 2.9[c]	
			Alert	16	19.31 ± 2.6[a]		74.24 ± 4.6[c]	
			Danger	47	26.38 ± 2.4[ab]		62.32 ± 2.8[bc]	
			Emergency	11	22.27 ± 4.4[ab]		37.17 ± 6.6[a]	
	< 30	Period	Dry	9	37.44 ± 8.1[b]	0.0201	54.62 ± 3.4	0.1065
			Rain	7	37.85 ± 6.6[b]		75.70 ± 3.3	
	30-90		Dry	42	30.88 ± 3.5[ab]		69.40 ± 2.3	
			Rain	43	34.39 ± 3.3[b]		68.44 ± 2.5	
	> 90		Dry	42	23.07 ± 1.9[a]		62.10 ± 2.6	
			Rain	62	24.98 ± 2.1[a]		65.87 ± 3.0	
	< 30	Season	Winter	7	34.42 ± 10.2	0.0871	51.55 ± 3.5[a]	0.0001
			Spring	2	48.00 ± 2.0		65.40 ± 2.2[b]	
			Summer	1	26.00 ± ***		68.42 ± ***	
			Fall	6	39.83 ± 7.5		76.91 ± 3.7[c]	
	30-90		Winter	13	30.07 ± 3.6		63.08 ± 3.5[b]	
			Spring	34	30.97 ± 4.2		72.38 ± 2.5[bc]	
			Summer	20	30.35 ± 4.7		62.26 ± 4.3[b]	
			Fall	18	40.27 ± 5.8		73.96 ± 3.2[bc]	
	> 90		Winter	20	21.70 ± 3.0		63.10 ± 3.5[b]	
			Spring	22	24.31 ± 2.6		61.20 ± 3.9[b]	
			Summer	29	26.31 ± 3.8		52.77 ± 4.5[a]	
			Fall	33	23.81 ± 2.2		77.38 ± 2.7[c]	

[a,b] Different literals between rows of the same column are significant (Fisher LSD, $p < 0.05$).

*** No bovine donors were obtained in this interaction.

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Oocyte retrieval through OPU is a commonly used technique for *in vitro* embryo production (Hernández-Ignacio *et al.,* 2023), and the viability of the COC is determined based on morphological classification criteria (Calvo, 2004). The present study demonstrates that management factors, analyzed through the Technological Index, feeding strategy, and preparation time, have a significant effect on the number of COCs obtained, whereas environmental factors primarily influence their viability. In this context, abiotic factors such as temperature, humidity, wind, and rainfall affect the reproductive activity of donor cows and, consequently, the implementation and success of reproductive biotechnologies (Díaz-Rivera *et al.,* 2011; Torres-Armas & Huayama, 2020; Kayser *et al.,* 2023; Thoriya *et al*., 2024).

Finally, to determine the weight of correlation coefficients or associations between the study variables (environmental and management) and the COC outcomes (quantity and viability), a multivariate analysis was conducted. The first was a cluster analysis (**Figure 4A**), designed to display, via dendrogram (linkage), the association strength among variables. Cluster grouping was performed using the City-Block (Manhattan) distance method and unweighted pair-group average linkage, normalized by the equation: ([Linkage Distance / Maximum Distance]*100). The weighted association coefficient between management variables and COC quantity was r = 0.65 ($p < 0.05$), while the association between environmental variables and COC viability was r = 0.75 ($p < 0.05$). **Figure 4B** presents the second multivariate analysis: Principal Component Analysis (PCA), used to reduce dimensionality (number of variables) and group those with the strongest associations. The variability explained by the two main components in this model was 38.77 + 29.26 = 68.03 %, for Factor 1 (environmental) and for Factor 2 (management), respectively.

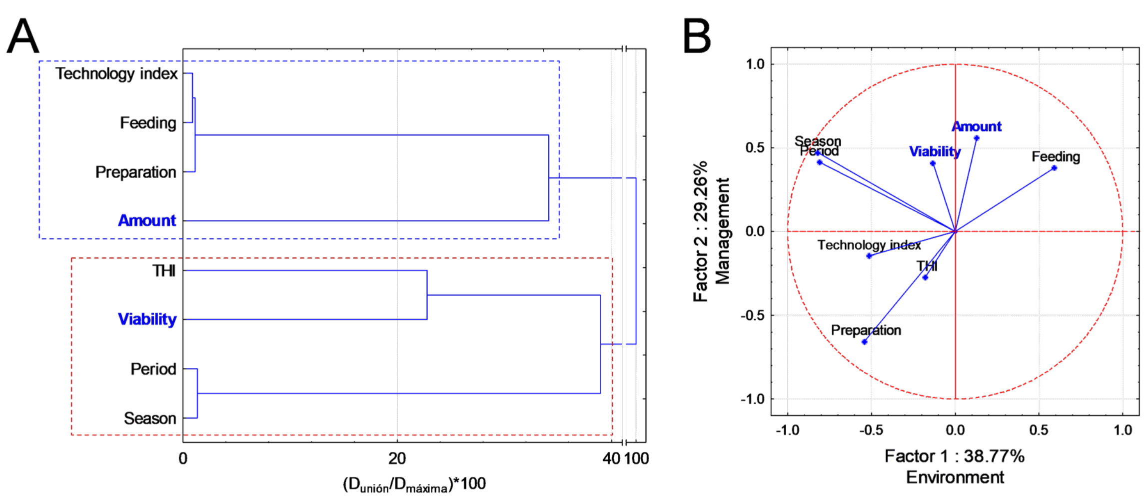

Figure 4. Multivariate analysis of association between environmental and management variables with the COC variables (amount and viability).

A. Union dendrogram, showing the clusters (boxes) of management and environmental variables with the COC variables. **B.** Principal component analysis (PCA), where the vectors (lines) with direction and magnitude of each variable and its association value can be seen; in addition, the total value of variability 38.77 + 29.26 = 68.03%, Factor 1 (environment), Factor 2 (management), respectively, is shown.

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The high temperatures observed in this study (**Figure 1**) may cause hyperthermia, thereby reducing cellular activity in the reproductive system of DBs, manifesting in various impairments to its function; these alterations may result in direct damage to the oocyte. According to Gutiérrez (2018), such impairments are associated with increased apoptotic activity in the COC and the production of metabolites, substances, or reactive oxygen species in both cytoplasmic and nuclear compartments of the cells. These oxidative compounds compromise oocyte quality by damaging DNA due to increased levels of radicals, thus reducing oocyte quality (Silva & Baruselli, 2012). This damage has been associated with the generation of lower-quality embryos during the summer when compared to those evaluated in winter (Ferreira *et al.,* 2009; Hernández-Ignacio *et al.,* 2023). The present study found higher COC viability values during autumn, when the THI falls within the *comfort* zone, and lower quality during winter (**Table 1**), likely due to the presence of northern winds that dehydrate pasture and reduce its nutritional value, as also reported by Hernández-Ignacio *et al.* (2023).

In this regard, environmental variability (heat and cold) may cause disruptions in follicular development, thereby altering embryonic development (Vélez & Uribe, 2010). It has been reported that oocyte growth and maturation are negatively affected by increased oxidative stress and undergo apoptosis when DBs are subjected to thermal stress (Hansen, 2009). Moreover, heat tolerance has been associated with specific biological markers such as the heat shock transcription factor (HSF) and heat shock proteins (HSP70, HSP90, and HSP27) (Saravanan *et al.,* 2021). Both HSF and HSPs function as cytoprotective agents, preventing the formation of non-functional proteins (Srikanth, 2017). Hernández-Ignacio *et al.* (2023) also report an increase in the production of lower-quality embryos in DBs exposed to thermal stress during or after *in vivo* fertilization.

To achieve optimal results in a reproductive program, oocytes should be obtained from DBs with an adequate body condition score, which is dependent on nutrition and feeding practices, as well as proper pasture management (Tinco-Salcedo *et al.,* 2021). It has been reported that DBs with a body condition score of 3 (on a scale of 1 to 5) present a more favorable metabolic microenvironment within their follicles, facilitating the formation of high-quality embryos (Velázquez, 2023). A balanced diet with sufficient essential nutrients can improve oocyte quality and increase ovulation rates; whereas protein deficiency in the diet negatively impacts ovulation, zygote formation, and embryo development (Restrepo-Mesa *et al.,* 2021). In this regard, supplementation with rumen-protected fats prior to OPU may help protect oocytes from oxidative damage (Lucy, 2001). The present study showed better COC recovery when DBs were prepared for 90 days or less prior to OPU. In contrast, when preparation periods exceeded 90 days, the number of retrieved COCs was lower, likely due to an increase in body condition score (Kasimanickam *et al.,* 2020; Tinco-Salcedo *et al.,* 2021; Velázquez, 2023). It has been reported that the number of viable structures (COCs) and transferable embryos is greater in DBs with a body condition score between 2 and 4 compared to scores of 1 (emaciated) or 5 (obese), due to changes in biomarkers such as insulin that influence oocyte viability (Kasimanickam *et al.,* 2020). Furthermore, DBs experiencing negative energy balance have been shown to have prolonged anovulatory periods and produce lower-quality oocytes (Turk *et al.,* 2015; Ninabanda, 2018). The findings of this study confirm that management practices influence COC yield, while environmental conditions affect COC viability. Environmental variability is an abiotic stressor; especially temperature (Das *et al.,* 2016; Jaya *et* *al.,* 2016), which affects cattle at the productive and reproductive levels by decreasing nutrient intake and reducing energy levels (Velez & Uribe, 2010; Das *et al.,* 2016; Thoriya *et al*., 2024). The combination of abiotic stressors and inadequate nutrition can have deleterious effects on COC quality (LeBlanc, 2004). In this sense, dietary supplementation with vitamins, concentrates, minerals, fats, etc., for periods of 30 to 90 days or more can function as homeostasis regulators, particularly in terms of thermoregulation. Thermal stress also leads to mineral loss through body fluids (sweating), which increases the demand for sodium and potassium to maintain thermal balance (Das *et al*., 2016), thereby inducing changes in cellular homeostasis.

Conclusions

The environment affects COC viability; autumn and thermal *comfort* conditions exhibit the highest viability percentages. The number of COCs is influenced by management practices, feeding, and the preparation period of the DBs; a preparation period of 30 to 90 days yields the best results.

Author contributions

Work conceptualization: AZM, BDM, and MBM; Methodology development: BDM, MBM, and AHB; Software management: AZM, BDM; Experimental validation: MBM, VAM, AHB, and BDM; Data analysis: AZM, BDM, MBM, ARA, and PCA; Data management: AZM, BDM, MBM, ARA, and JMMH; Manuscript writing and preparation: AZM, BDM, AHB, and VAM; Drafting, reviewing, and editing: AZM, BDM, MBM, VAM, AHB, PCA, ARA, and JMMH; Project administration: BDM, MBM; Funding acquisition: MBM, BDM.

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Ethical statement

The handling and restraint procedures applied to the donor bovines (DBs) were reviewed and approved by the Bioethics Committee of the Faculty of Veterinary Medicine and Animal Science at the Universidad Veracruzana, under the reference number: COBIBA011/2021.

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Conflict of Interest

The authors declare no conflict of interest.

References

Absalón-Medina, V., Blake, R., Fox, D. Juárez-Lagunes, F., Nicholson. C., Canudas-Lara, E., & Rueda-Maldonado, B. (2012). Limitations and potentials of dual-purpose cow herds in Central Coastal Veracruz, Mexico. *Tropical Animal Health Production*. 44, 1131-1142. https://doi.org/10.1007/s11250-011-0049-1

Aguiar, A., Vendramini, J., Arthington, J., Sollenberger, L., Sánchez, J., da Silva, W, Valente, A., & Salvo, P. (2014). Stocking rate effects on 'Jiggs' bermudagrass pastures grazed by heifers receiving supplementation. *Crop Science*, 54(6), 2872-2879. https://doi.org/10.2135/cropsci2014.02.0135

Ahmed, J. A., Nashiruddullah, N., Dutta, D., Biswas, R. K., & Borah, P. (2017). Cumulus cell expansion and ultrastructural changes in in vitro matured bovine oocytes under heat stress. *Iranian journal of veterinary research*, 18(3), 203–207. https://pubmed.ncbi.nlm.nih.gov/29163650/

Arrieta-González, A., Hernández-Beltrán, A., Barrientos-Morales, M., Martínez-Herrera, D.I., Cervantes-Acosta, A., Rodríguez-Andrade, A., & Domínguez-Mancera, B. (2022). Characterization and technological typification of bovine dual-purpose system of the Huasteca Veracruzana Mexico. *Revista MVZ Córdoba*, 27(2), e2444. https://doi.org/10.21897/rmvz.2444

Calvo, J., Pérez, V., Fila, D., & Campos, E. (2004). Evaluación de la viabilidad de ovocitos bovinos mediante la luteinización' 3-(4-5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazoliuln bromid. *Veterinaria*, (Montevideo), 39(154), 7-10. https://www.revistasmvu.com.uy/index.php/smvu/article/view/474

Baruselli, P. S., de Sá Filho, M. F., Martins, C. M., Nasser, L. F., Nogueira, M. F., Barros, C. M., & Bó, G. A. (2006). Superovulation and embryo transfer in Bos indicus cattle. *Theriogenology*, 65(1), 77–88. https://doi.org/10.1016/j.theriogenology.2005.10.006

Bó, G. A., & Pincay, J. (2017). The role of oocyte competence in the reproductive success of cattle. *Theriogenology*, 87, 26-36. https://doi.org/10.1016/j.theriogenology.2016.09.009

Cao, L., & Jiang, X. (2020). Environmental effects on oocyte quality and fertility in dairy cows. *Animal Reproduction Science*, 218, 106400. https://doi.org/10.1016/j.anireprosci.2020.106400

Cardone, A., Cáceres, R., Sanhueza, A., Bruna, A., & Laconi, R. (2022). Effects of short-term in vitro heat stress on bovine preantral follicles. *Livestock Science.* 254. https://doi.

org/10.1016/j.livsci.2022.105076.

Cruz, H. A., Hernández, G. A., Chay, C. A. J., Mendoza, P. S. I., Ramírez, V. S., Rojas, G., Adelaido, R., & Ventura, R. J. (2017). Componentes del rendimiento y valor nutritivo de Brachiaria humidicola cv Chetumal a diferentes estrategias de pastoreo. *Revista Mexicana de Ciencias Agrícolas*, 8(3), 599–610. https://doi.org/10.29312/remexca.v8i3.34

Das, R., Sailo, L., Verma, N., Bharti, P., Saikia, J., Imtiwati., & Kumar, R. (2016). Impact of heat stress on health and performance of dairy animals: A review. *Veterinary world*, 9(3), 260–268. https://doi.org/10.14202/vetworld.2016.260-268

De Freitas W., & Pinheiro E. (2013). Nível tecnológico e seus determinantes na apicultura cearense. RPA. 22(3):32–47. https://seer.sede.embrapa.br/index.php/RPA/article/view/764/721

Díaz-Rivera, P., Oros-Noyola, V., Vilaboa-Arroniz, J., Martínez-Dávila, J. P., & Torres-Hernández, G. (2011). Dinámica del desarrollo de la ganadería doble propósito en las Choapas, Veracruz, México. *Tropical and Subtropical Agroecosystems*, 14(1), 191-199. https://www.redalyc.org/articulo.oa?id=93915703018

Ferreira, F., Pires, M., & Martinez, M. (2009). Parâmetros clínicos, hematológicos, bioquímicos e hormonais de bovinos submetidos ao estresse calórico. *Arq. Bras. Med. Vert. Zootec*. 61(4), 769-776. https://doi.org/10.1590/S0102-09352009000400002

Galina, C., Turnbull, F., & Noguez-Ortiz, A. (2016) Factors Affecting Technology Adoption in Small Community Farmers in Relation to Reproductive Events in Tropical Cattle Raised under Dual Purpose Systems. *Open Journal of Veterinary Medicine*, 6, 15-21. http://doi:10.4236/ojvm.2016.61003.

Galina, C.S., & Geffroy, M. (2023). Dual-Purpose Cattle Raised in Tropical Conditions: What Are Their Shortcomings in Sound Productive and Reproductive Function? *Animals*, 13, 2224. https://doi.org/10.3390/ani13132224

Gendelman, M., & Roth, Z. (2012). Seasonal effect on germinal vesicle-stage bovine oocytes is further expressed by alterations in transcript levels in the developing embryos associated with reduced developmental competence. *Biology of reproduction*, 86(1), 1–9. https://doi.org/10.1095/biolreprod.111.092882

González, F., & Salas, R. (2019). Impact of climatic factors on reproductive performance in tropical cattle. *Tropical Animal Health and* Production, 51(4), 657-664. https://doi.org/10.1007/s11250-019-01926-5

Grossi, G., Goglio, P., Vitali, A., & Williams, A. G. (2018). Livestock and climate change: impact of livestock on climate and mitigation strategies. *Animal frontiers: the review magazine of animal agriculture*, 9(1), 69–76. https://doi.org/10.1093/af/vfy034

Gutiérrez, A. M. (2018). Estrés calórico en la hembra bovina: cambios fisiológicos in vivo y modelo de estudio in vitro de ovocitos. [Tesis de Doctorado, Universidad de la republica uruguay, Doctor en ciencias veterinarias]. https://bibliotecadigital.fvet.edu.uy/handle/123456789/1386

Hansen, P. (2009). Effects of heat stress on mammalian reproduction. *Phil. Trans. R. Soc.* 3341–3350. https://doi.org/10.1098/rstb.2009.0131

Hernández-Ignacio, J., Gonzalez-Gómez, R., & Mejia-Flores, I. (2023). Effect of climate on superovulatory response, quality and stage of embryonic development in tropical cattle. *Archivos Latinoamericanos de Producción Animal.* 31, 57-60. https://doi.org/10.53588/alpa.310511

International Embryo Transfer [IETS]. (1998). Manual of the International Embryo Transfer

Society. Stringfellow DA., Seidel SM (eds). USA: Ed. Savoy. 170 p

Jaya, B., Kumar, S., Sinha, B., Sinha, S., & Paswan, J. (2016). Focusing biotic stress in livestock, 3(11), 812-814. http://dx.doi.org/10.13140/RG.2.2.23341.82409

Juárez-Barrientos, J. M., Herman-Lara, E., Soto-Estrada, A., Avalos-de la Cruz, D. A., Vilaboa, A. J., & Díaz-Rivera P. (2015). Tipificación de sistemas de doble propósito para producción de leche en el distrito de desarrollo rural 008, Veracruz, México. *Revista Científica*. 25(4):317-323. https://www.redalyc.org/articulo.oa?id=95941173007

Kayser, Y., Montiel, F., Severino, V., Canseco, R., Ahuja, C., Barrientos, M., & Molina, O., (2023). Caracterización tecnológica de ganaderos y su percepción sobre la transferencia de embriones en Guerrero, México. *Acta universitaria*, 33, e3745. https://doi.org/10.15174/au.2023.3745

Kasimanickam, R., Kasimanickam, V., Kastelic, J. P., & Ramsey, K. (2020). Metabolic biomarkers, body condition, uterine inflammation and response to superovulation in lactating Holstein cows. *Theriogenology*, 146, 71–79. https://doi.org/10.1016/j.theriogenology.2020.02.006

Kawano, K., Sakaguchi, K., Madalitso, C., Ninpetch, N., Kobayashi, S., Furukawa, E., Yanagawa, Y., & Katagiri, S. (2022). Effect of heat exposure on the growth and developmental competence of bovine oocytes derived from early antral follicles. *Scientific reports*, 12(1), 8857. https://doi.org/10.1038/s41598-022-12785-2

LeBlanc, S. J. (2004). Heat stress in dairy cattle. *Journal of Dairy Science*, 87(7), 2175-2189.

Loss, F., Van Vliet, C., Van Maurik, P., & Kruip Th. A.M. (1989). Morphology of immature bovine oocytes. *Gameto Res*, 24, 197-204. https://doi.org/10.1002/mrd.1120240207

Lucy, M. C. (2001). The role of nutrition in controlling ovulation rate in cattle. *Journal of Animal Science*, 79(1), 300-311.

Morera, A., Velasco, E., Herán, S., Romero, J., & Ruiz, S. (2022). Respuesta a la estimulación ovárica mediante fsh (folltropin®) y rendimiento de OPU en vacas adultas obtenidas por diferentes técnicas de reproducción asistida. *Anales de Veterinaria Murcia*, 36, 1-17. https://doi.org/10.6018/analesvet.538651

Narváez, H., Fontes, R. da S., Campos de carcalho, B., Varella, R., Slade, C., & Dos reis, A. (2022). Efecto de la progesterona plasmática en la competencia para el desarrollo embrionario in vitro de vacas Bos taurus taurus y Bos taurus indicus. *Ciencia y Tecnología Agropecuaria*, 23(2). DOI: https://doi.org/10.21930/rcta.vol23_num2_art:2003

Ninabanda, J.J. (2018). Impacto del balance energético negativo en vacas lecheras tratadas con somatotropina recombinante bovina. *Revista veterinaria*, *29*(1), 68-72. https://dx.doi.org/10.30972/vet.2912794

Orantes, Z. M. A., Vilaboa, A. J., Ortega, J. E., & Córdova, A. V. (2010). Comportamiento de los comercializadores de ganado bovino en la región centro del estado de Chiapas. *Revista que hacer científico,* 1(9), 51-56. https://www.dgip.unach.mx/images/pdf-REVISTA-QUEHACERCIENTIFICO/QUEHACER-CIENTIFICO-2010-ener-jun/5_QCCH_9_Comportamiento_de_los_comercial.pdf

Pérez, J. A., & Castillo, F. (2021). Nutritional management of bovine reproductive health in tropical regions. *Veterinary Clinics of North America: Food Animal Practice*, 37(2), 307-321. https://doi.org/10.1016/j.vcfa.2021.02.006

Pérez-Mora, A., Segura-Correa, J. C., & Peralta-Torres, J. A. (2020). Factors associated with pregnancy rate in fixed-time embryo transfer in cattle under humid-tropical conditions of México. *Animal reproduction*, 17(2), e20200007. https://doi.org/10.1590/1984-3143-AR2020-0007

Restrepo-Mesa, S., Manjarres-Cor, I. & Parra-sosa B. (2021). *Alimentación y nutrición de la mujer en etapas de gestación y lactancia: De lo básico a lo aplicado*. 1 ed. Universidad de Antioquia. https://libros.udea.edu.co/index.php/editorial_udea/catalog/book/33

Ríos-Utrera, A., Villagómez-Amezcua, M. E., Zárate-Martínez, J. P., Calderón-Robles, R. C. & Vega-Murillo, V. E. Análisis reproductivo de vacas Suizo Pardo x Cebú y Simmental x Cebú en condiciones tropicales. *Rev MVZ Cordoba.* 2020; 25(1):e1637. https://doi.org/10.21897/rmvz.1637

Roth, Z., Arav, A., Bor, A., Zeron, Y., Braw-Tal, R., & Wolfenson, D. (2001). Improvement of quality of oocytes collected in the autumn by enhanced removal of impaired follicles from previously heat-stressed cows. *Reproduction* (Cambridge, England), 122(5), 737–744. https://pubmed.ncbi.nlm.nih.gov/11690534/

Roth, Z., & Hansen, P. J. (2004). Involvement of apoptosis in disruption of developmental competence of bovine oocytes by heat shock during maturation. *Biology of reproduction*, 71(6), 1898–1906. https://doi.org/10.1095/biolreprod.104.031690

Roth, Z. (2020). Reproductive physiology and endocrinology responses of cows exposed to environmental heat stress - Experiences from the past and lessons for the present. *Theriogenology*, 155, 150-156. https://doi.org/10.1016/j.theriogenology.2020.05.040

Saizi, T., Mpayipheli, M., & Idowu, P. (2019). Heat tolerance level in dairy herds: a review on coping strategies to heat stress and ways of measuring heat tolerance. *Journal of Animal Behaviour and Biometeorology*, 7, 39–51. http://doi.org/10.31893/2318-1265jabb.v7n2p39-51

Saravanan, K., Panigrahi, M., Kumar, H., Parida, S., Bhushan, B., Gaur, G., Dutt, T., Mishra, B., Singh, R. (2021). Genomic scans for selection signatures revealed candidate genes for adaptation and production traits in a variety of cattle breeds. *Genomics*, 113 (3), 955-963. https://doi.org/10.1016/j.ygeno.2021.02.009

Silva, L. O., & Baruselli, P. S. (2012). Effects of heat stress on reproductive function in dairy cattle. *Journal of Dairy Science*, 95(3), 861-875.

Srikanth, K., Kwon, A., Lee, E., & Chung, H. (2017). Characterization of genes and pathways that respond to heat stress in Holstein terneros through transcriptome analysis. *Chaper de estrés celular*. 22, 29–42. https://doi.org/10.1007/S12192-016-0739-8

StatSoft, Inc. (2011) STATISTICA (Data Analysis Software System), Version 10. http://www.statsoft.com

Systat Software (2008). SigmaPlot V11, San Jose, CA. www.systatsoftware.com

Torres-Armas, E., & Huayama, P. (2021). Factores estructurales y funcionales de la ganadería de bovinos doble propósito de Molinopampa, Amazonas. *Revista de investigación Agropecuaria Science and biotechnology*. 1(1), 23-24. https://doi.org/10.25127/riagrop.20211.661

Turk, R., Podpecan, O., Mrkun, J., Flegar-Mestric, Z., Perkov, S., & Zrimsek, P. (2015). The Effect of Seasonal Thermal Stress on Lipid Mobilisation, Antioxidant Status and Reproductive *Performance in Dairy Cows. Reproduction in domestic animals*, 50, 595-603. https://doi.org/10.1111/rda.12534

Thoriya, A., Bhoi, D., Patel, M., Kumar, A., & Raval, K. (2024). Effect of stress on dairy animal reproduction. *Journal of livestock science*, 15, 276-284. https://doi.org/10.33259/JLivestSci.2024.276-284

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Tinco-Salcedo, J., Quispe-Gutiérrez, U., & Zea-Gonzales, D. (2021). Asociación entre calidad de ovocitos recuperados y condición corporal en vacas criollas. *Revista de Investigaciones Altoandinas*, 23(3), 133-138. https://dx.doi.org/10.18271/ria.2021.294

Velázquez, M. A. (2023) Nutritional Strategies to Promote Bovine Oocyte Quality for In Vitro Embryo Production: Do They Really Work?. Vet. Sci. 10(10), 604. https://doi.org/10.3390/vetsci10100604

Vidal-Zepeda, R. (2005). Las Regiones Climáticas de México. Instituo de Geografía UNAM. 210

Vilaboa, A.J., & Díaz, R.P. (2009) Caracterización socioeconómica de los sistemas ganaderos en siete municipios del estado de Veracruz, México. *Zootecnia Tropical* 27(4): 427-436. https://ve.scielo.org/scielo.php?script=sci_arttext&pid=S0798-72692009000400008

Vélez, M. M., & Uribe, V. L. F. (2010). ¿Cómo afecta el estrés calórico la reproducción? *Biosalud*, 9(2), 83–95. https://revistasojs.ucaldas.edu.co/index.php/biosalud/article/view/5505

Wu, B., & Zan, L. (2012). Enhance beef cattle improvement by embryo biotechnologies. *Reproduction in domestic animals Zuchthygiene*, 47(5), 865–871. https://doi.org/10.1111/j.1439-0531.2011.01945.x

Zavaleta-Martínez, A., Barrientos-Morales, M., Alpirez-Mendoza, M., Rodríguez-Andrade, A., Cervantes-Acosta, P., Hernández-Beltrán, A., Avedaño-Reyes, L., & Dominguez-Mancera, B. (2024). Effect of heatwaves on the pregnancy rate of dual-purpose recipient cows transferred with produced in-vitro embryos in tropical locations. *Multidisciplinary Science Journal*, 6(7), 2024103. https://doi.org/10.31893/multiscience.2024103