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Research Article

Variability impact weather of Mexico humid tropical regions on pregnancy rate of in vitro produced bos indicus embryos

Impacto da variabilidade meteorológica das regiões tropicais úmidas do México na taxa de concepção de embriões bos indicus produzidos in vitro

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ABSTRACT

Livestock production in the humid tropical regions of Mexico faces several changes derived from weather variability, particularly heat stress, measured by the temperature humidity index (THI), which compromises reproductive efficiency. The aim of this study was to evaluate the impact of environmental variables on the pregnancy rate (PR) of embryo recipient cows (RC) to which Bos indicus embryos, in vitro produced (IVP), were transferred. 3610 embryo transfers were analyzed (2021-2024) in the humid tropics. Temperature, relative humidity, rainfall and prevailing winds were measured, and the THI was calculated and categorized (comfort, alert, danger, emergency). Pregnancy was verified through ultrasound scan (45-50th day) after the embryo transfer. χ^2 tests, logistic regression and multivariate analyses (Principal Component Analysis, PCA) were applied. Global PR was 40%. According to the annual analysis, 2023 had the highest rate (47.9 %) and the lowest were observed in 2021-2022 (37-38 %). In the seasonal analysis, winter and fall registered the best results (43-44 %), while the least favorable season was summer (34.9 %). Dry period showed better results than rainy (42.7 % vs. 37.8 %; $p<0.05$). Big corpus luteum (CL3 ≥25 mm) were associated with greater successful PR ($p<0.05$). The logistic regression indicated a reduction from 56.8 % (THI 59.1) to 32.2 % (THI 87.4) in the possibility of a successful pregnancy. PCA explained 61.6 % of the variation, showing a negative correlation between PR and Temperature. Weather variability in the humid tropics of Mexico reduces the efficiency of in vitro embryo transfers under extreme heat and humidity conditions. These findings provide a basis for seasonal management and reproductive biotechnology adaptation strategies under tropical climates.

KEYWORDS: Embryo transfer. Heat stress. Temperature-humidity index. Corpus luteum. Embryo recipient cows.

RESUMO

A produção pecuária nas regiões tropicais úmidas do México enfrenta diversas mudanças derivadas da variação climática, particularmente o estresse térmico, medido pelo índice de temperatura e umidade (ITU), que compromete a eficiência reprodutiva. O objetivo deste estudo foi avaliar o impacto de variáveis meteorológica na taxa de prenhez (TP) de vacas receptoras de embriões (VR) para as quais foram transferidos embriões de Bos indicus produzidos in vitro (PIV). Foram analisadas 3610 transferências de embriões (2021-2024) nos trópicos úmidos. Foram mensurados a temperatura do ar, a umidade relativa do ar, a precipitação pluviométrica e os ventos predominantes, e o ITU foi calculado e categorizado (conforto,

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alerta, perigo, emergência). A prenhez foi confirmada por ultrassonografia trans retal (45º-50º dia) após a transferência de embriões. Foram aplicados testes χ^2, regressão logística e análises multivariadas (Análise de Componentes Principais, ACP). A TP global foi de 40%. De acordo com a análise anual, 2023 apresentou a maior taxa (47,9%) e as menores foram observadas em 2021-2022 (37-38%). Na análise sazonal, o inverno e o outono registraram os melhores resultados (43-44%), enquanto a estação menos favorável foi o verão (34,9%). O período seco apresentou melhores resultados do que o chuvoso (42,7% vs. 37,8%; $p<0,05$). Corpos lúteos grandes (CL3 ≥25 mm) estiveram associados a maior sucesso na concepção ($p<0,05$). A regressão logística indicou uma redução de 56,8% (THI 59,1) para 32,2% (THI 87,4) na possibilidade de uma concepção bem-sucedida. A análise de componentes principais (ACP) explicou 61,6% da variação, mostrando uma correlação negativa entre a taxa de concepção e a temperatura. A variabilidade meteorológica nos trópicos úmidos do México reduz a eficiência das transferências de embriões in vitro em condições extremas de calor e umidade. Essas descobertas fornecem uma base para o manejo sazonal e estratégias de adaptação da biotecnologia reprodutiva em climas tropicais.
PALAVRAS-CHAVE: Transferência de embriões. Estresse térmico. Índice de temperatura e umidade. Corpo lúteo. Vacas receptoras de embriões.

INTRODUCTION

Livestock production in humid tropical regions of Mexico faces multiple challenges related to adverse environmental conditions. Among the most relevant are weather variability, characterized by elevated temperature-humidity index (THI) values, as well as high rainfall and a marked seasonality of fodder availability (GODDE et al. 2021, GALINA & GEFFROY 2023, SILVA-NETO et al. 2025). Such factors have a direct impact on animal welfare, reproductive efficiency, the adoption of reproductive biotechnologies (RB) and, above all, on the sustainability of dual-purpose production systems present in these regions (HANSEN 2004).

In this context, RB, such as Artificial insemination at detected estrus (AIE), Fixed-Time Artificial Insemination (FTAI) and transfer of in vitro produced (IVP) embryos, have been conceived as strategic tools to improve genetics and reproductive efficiency of adapted cattle, or cattle in the process of adapting to these environmental conditions (BARUSELLI et al. 2018, DE ALMEIDA et al. 2022).

The transfer of embryos IVP has consolidated as an RB that allows the acceleration of genetic progress in the humid tropics of Mexico, because it multiplies the number of bovines with high genetic value, especially Bos *indicus* and synthetic breeds with *Bos taurus*, to adapt them to the humid and sub-humid tropical environment (PÉREZ-MORA et al. 2020, ZAVALETA-MARTINEZ et al. 2024a). However, the success of this biotechnology depends on physiological, nutritional, genetic, technological and environmental factors of the embryo recipient cow (RC), with weather variations at the moment of transfer, a crucial factor for the reproductive yield of RC and thus for embryo survival and successful pregnancy rate (PALACIOS et al. 2023, SMITH et al. 2018, MARTINEZ et al. 2021).

Continuing exposure of livestock to extreme climate variations (humidity, temperature, rainfall, wind) causes environmental stress, especially associated with temperature and humidity, which reduces fertility (BOVA et al. 2014, WACHIDA et al. 2021, KHAN et al. 2023). Several studies have demonstrated that the livestock safety weather index, also known as the temperature-humidity index (THI) (THOM 1959), is an indicator of heat stress in cattle. When it is >74 units, it compromises animal welfare and alters the reproductive physiology of RC, causing the alteration of ovarian function,

luteinization of follicular cells, the endometrial environment and the embryonic capacity for adequate interaction with the cow (KHAN et al. 2023, DÍAZ et al. 2020, NARANJO-GOMEZ et al. 2021). High relative humidity (≥70%) limits heat dissipation mechanisms of heat dissipation, such as sweat and panting, prolongs the periods of hyperthermia and worsens the negative effects on the welfare of the RC and reproductive physiology (DÍAZ et al. 2018, GÓMEZ-GUZMÁN et al. 2024, COSTAMAGNA et al. 2024).

These conditions represent an obstacle for embryo transfer programs that depend on the estrus cycle of the RC and allow efficient synchronization between the post-ovulation moment of the cows and the developmental stage of the embryo being transferred (BARUSELLI et al. 2010, HANSEN 2020, SARTORI et al. 2025). These functional alterations include, among others, reduced oocyte quality, changes in progesterone secretion by smaller corpus luteum (CL) and failure of the maternal recognition of pregnancy (GÓMEZ-GUZMÁN et al. 2024, SAKATANI 2017, ZHOU et al. 2024).

In the case of IVP embryos, even when this RB allows the selection and cultivation of viable embryos of optimal quality in controlled lab conditions, the critical stage begins after the transfer to cows immersed in environments that may cause stress, especially heat stress (BOVA et al. 2014, HANSEN 2020, ZAVALETA-MARTINEZ et al. 2024a) Heat stress increases the production of reactive oxygen species (ROS) and affects the uterine environment, generating inflammatory conditions that reduce the capacity for embryonic implantation (HANSEN 2004, SAKATANI 2017, GÓMEZ-GUZMÁN et al. 2024).

It has been documented that the pregnancy establishment rate can be reduced 40% under these conditions, in comparison with favorable temperate environments (MARTINEZ et al. 2021). Moreover, environmental conditions not only compromise uterine receptivity, but also the final success of the transfer, reducing reproductive efficiency of genetic improvement programs in the humid tropics of Mexico (TAKAHASHI 2011, WACHIDA et al. 2021, QU et al. 2024).

Weather variability in the humid tropics of Mexico is intensified by global warming, which poses a challenge for livestock reproduction and RB implementation (GODDE et al. 2021, TOLEDO-ALVARADO & OROZCO-LUCERO 2025, MUZZO et al. 2025). However, it is also an area of opportunity for basic and applied research oriented to the optimization of ovulation synchronization protocols, the selection of donor cows and adapted RC, as well as the recognition of favorable periods or seasons for RB implementation (CASANOVA-PEREZ et al. 2018, GODDE et al. 2021, TOLEDO-ALVARADO & OROZCO-LUCERO 2025). Thus, the aim of this study was to associate environmental variables such as temperature, humidity, rainfall and wind with the pregnancy rate (PR) of embryos IVP *Bos indicus* in humid tropical conditions with the purpose of finding seasons or periods that favor successful gestation.

MATERIAL AND METHODS

Location

The study was performed in 75 livestock production units (LPU), located in the tropical region of Mexico, within the States of Chiapas, Hidalgo, Oaxaca, Puebla, San Luis Potosi, Tabasco, Tamaulipas and Veracruz (Figure 1), under temperatures (°C)

that oscillated between 11.14±2.34 - 30.12±2.07 °C (min and max) and Relative humidity (%) between 60±5.0 and 85.0±5.0 (National Weather Service, National Water Distribution Commission; SMN Conagua, 2021-2024) (SMN-CONAGUA 2021-2024). In total, 3610 transfers of Bos *indicus* embryos (Gyr, Black Sardinian, Brahman, Nellore), to dual-purpose receptor cows (Bos *taurus* x Bos *indicus*) at different breed percentages, were evaluated between January 2021 and December 2024.

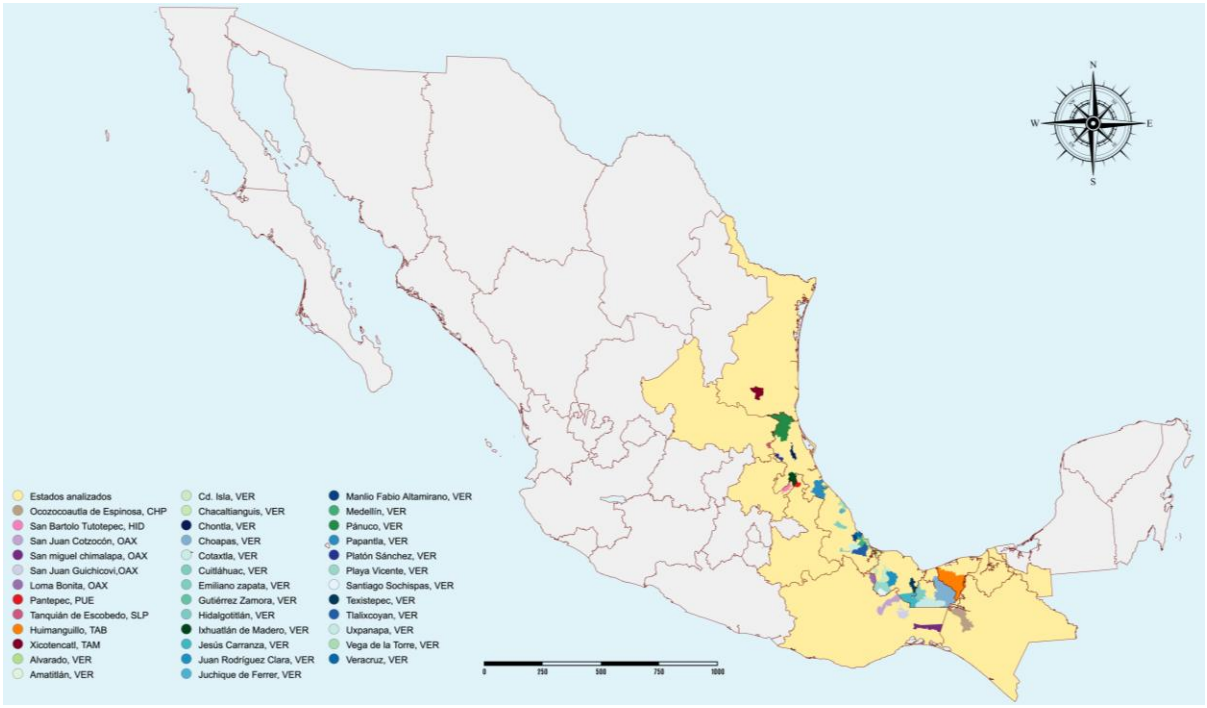


Figure 1. Map of Mexico; the states are shown in yellow and, within them, the municipalities where the Livestock Production Units where the embryo transfers were performed. The scale bar shows 250 km segments (Created in www.mapchart.net)

Weather variables

The weather data (average air temperature in °C, relative humidity in %, monthly accumulated rainfall in mm, speed and direction of the prevailing wind, km/h) were obtained from the weather stations of the National Weather Service, a division of the National Water Commission (Conagua), which were closest to the units where the transfers were performed (SMN-Conagua stations: 7123, 20117, 20237, 24085, 27018, 28120, 30009, 30202, 30464, 30037, 30195, 30371, 30064, 30072, 30224, 30143, 30073, 30101, 30124, 30125, 30130, 30131, 30184, 30270, 30192); additionally, other weather data were recorded in situ on transfer days, with the help of portable digital hygrothermographs (AcuRite, Model:01083M).

The equation used to calculate the Tem-perature-Humidity Index (THI) was derived from NIENABER & HAHN (2007), THI=1.8×Te+32-(0.55-0.55×RH)×(1.8×Te-26), where: Te = Daily average temperature (°C) and RH = Relative Humidity (%). NIENABER & HAHN (2007), consider four (4) categories of THI to evaluate the impact of the thermal conditions of the environment associated with the livestock respiratory rate p/minute; the rise of respiratory rate is a proportional compensatory response to heat stress. THI values of ≤ 74 were considered as comfort levels (10-30 breaths per

minute/ bpm, without signs of stress); THI values between 75 and 78 were considered alert values (30-60 bpm, moderate increase, stress starts), THI values between 79 and 83 were considered danger values (60-90 bpm, severe thermal stress) and THI values ≥ 84 were considered emergency levels (>90 bpm, risk of collapse) (SAIZI et al. 2019).

Characteristics and handling of embryo recipient cow (RC)

The handling and immobilization procedures used on the embryo recipient cows were reviewed and approved by the bioethics committee of the Universidad Veracruzana School of Veterinary Medicine and Animal Sciences, folio n°: COBIBA011/2021. The RC were from an indistinct breed, crossbreeds of Bos *indicus* x Bos *taurus*, with Body condition score ranged from 3 to 4 on a 1-5 scale, where 1 = emaciated and 5 = obese (medium condition considered optimal for transfer) (AYRES et al. 2009) and ages between 4 and 7 years; all RC had given birth at least and were in non-lactating (dry) cow condition at the time of embryo transfer.

The RC were under an extensive grazing system with native *Cynodon nlemfuensis*, *Brachiaria humidicola* and *Digitaria decumbens* grasses, and for ~30 days before the transfers, had a nutritional plan which consisted in adding mineral salts ad libitum and 300 g/cow of bypass fat (Percutrin Energy, Bayer; Leverkusen, Germany). Additionally, a zoosanitary plan was implemented, which consisted in a tick bath (Amitraz 12.5%. BOVITRAZ®, Bayer; Leverkusen, Germany) and internal deworming (Ivermectin 1%, BAYMEC® Prolong, Bayer; Leverkusen, Germany), as well as immunization against viral (BOVILIS® VISTA 5 L5 SQ, MSD Animal Health; Rahway, New Jersey, USA) and clostridial diseases (CLOSTRIGEN® 9 + T, Virbac; Westlake, Texas, USA).

Estrus synchronization and embryo transfer

The RC estrous synchronization was performed using a progesterone-based protocol following the next schedule: Day 0, an intravaginal device containing progesterone (1.9 g, CIDR, Zoetis, USA) + intramuscular (IM) administration of 2 mg of estradiol benzoate (Bio Estrogen®, Biogenesis Bagó). Day 7, an IM administration of 0.15 mg of prostaglandin-cloprostenol (Bio Estrogen®, Biogenesis Bagó). Day 9, progesterone implant withdrawal; an IM administration of 400 IU of equine chorionic gonadotrophin (eCG) (Ecegon®, Biogenesis Bagó) + IM administration of 0.075 mg of prostaglandin-cloprostenol (Bio Estrogen®, Biogenesis Bagó). Day 10, an IM administration of 1 mg of estradiol benzoate (Bio Estrogen®, Biogenesis Bagó). Day 18, Embryo Transfer.

The transferred embryos were produced *in vitro* and preserved in fresh. At the moment of the transfer, they were in the blastocyst stage (earlator expanded), their quality went from good (Grade II) to excellent (Grade I) (IETS 1998, STRINGFELLOW & GIVENS 2010). And they were transferred using a transcervical non-surgical technique (PALMA 2008). Summarizing, 5 ml of an epidural anesthetic (2% lidocaine, Logymed, Logistic & Medicine, Colombia) were administered, the vulva was cleaned with water (without soap) and dried with a paper towel.

The RC were examined by transrectal ultrasound evaluation to identify the corpus luteum (CL) on the ovary before transfer (Ibex Pro/r L7HD ultrasound, iL7 5-9 MHz broadband rectal linear for reproductive exams, E.I. Medical Imaging, Loveland, Colorado USA). In this examination the RC with corpus luteum measuring (CL) ≥ 10

mm were identified as potential receptors, based on the classification proposed by ALKAN et al. (2020); where corpus luteum size was classified in 3 categories, CL1: <15 mm, CL2: 15-25 mm; CL3: >25 mm diameter. The ultrasound evaluation of CL size was performed independently of any meteorological data, and the technician was blinded to environmental conditions and subsequent pregnancy outcomes, minimizing the risk of observational bias.

The embryo was placed on a 0.25 ml straw and then this straw was placed on the transfer pipette. The embryo was transferred to the cranial portion of the uterine cornu, on the side of the corpus luteum. Pregnancy was determined at the 45th-50th day after the transfer via ultrasound (Ibex Pro/r L7HD transducer, E.I. Medical Imaging, Loveland, Colorado, USA), and the response variable was dichotomous, where 0 = non-pregnant and 1= pregnant.

Statistical analysis

The STATISTICA V10 (STATSOFT INC 2011) program was used for all statistical analysis, and the figures were edited by Sigma Plot V11 software (SYSTAT 2008). First, an exploratory data analysis (EDA) was done to find patterns, atypical values and correlations between meteorological variables, temperature, relative humidity, monthly accumulated rainfall and speed of the prevailing wind throughout the months of the year. This allowed the elaboration of a climograph of the study region and to associate it with pregnancy variable.

The THI was categorized and quantified in accordance with the proportion of days in the year (2021-2024) that fit into each thermal comfort category. A Generalized Linear Mixed Model (GLMM) with THI (categories), rainfall (months), wind (norths or souths) and age (data missing, recipient cow) as fixed effects and LPU as a random effect. Then, 2^k contingency tables, with the help of the χ^2, were used for the analysis of the main effects (year, month, season and period) with the pregnancy variable. Frequency was analyzed using a χ^2 distribution of corpus luteum size with the different factors (THI categories, year, period and season), A p-value < 0.05 was considered statistically significant ($p< 0.05$). The possibility of successful pregnancy at the different THI values reported in the study was determined by the use of logistic regression, the following model was used:

$$log\left(\frac{p}{1-p}\right) = \beta_0 + \beta_1 X_1 \quad (1)$$

Where:

P= probability of the event occurring (pregnancy).

$\left(\frac{p}{1-p}\right)$ =odds or probability ratio.

β_0, β_1 = model coefficients, β_0 (intercept), β_1 (slope)

Finally, a multivariate cluster analysis and principal component analysis (PCA) were used to find the degree of association between the environmental factors and the pregnancy variable. Clustering was done through a simple Euclidean distance matrix, and the resulting data were combined through an unweighted pair clustering procedure and normalized with the Pearson 1-r correlation coefficient ([Correlation distance/maximum distance] * 100). The PCA used the correlation method.

Importantly, the inclusion of livestock production unit as a random effect did not eliminate the significance of meteorological predictors, confirming that the observed effects are robust and not driven by inter-farm management differences.

RESULTS

An EDA was done with the meteorological variables of the embryo transfer sites with the purpose of showing a climograph (Figure 2) of the environment of the RC at the moment of the transfer.

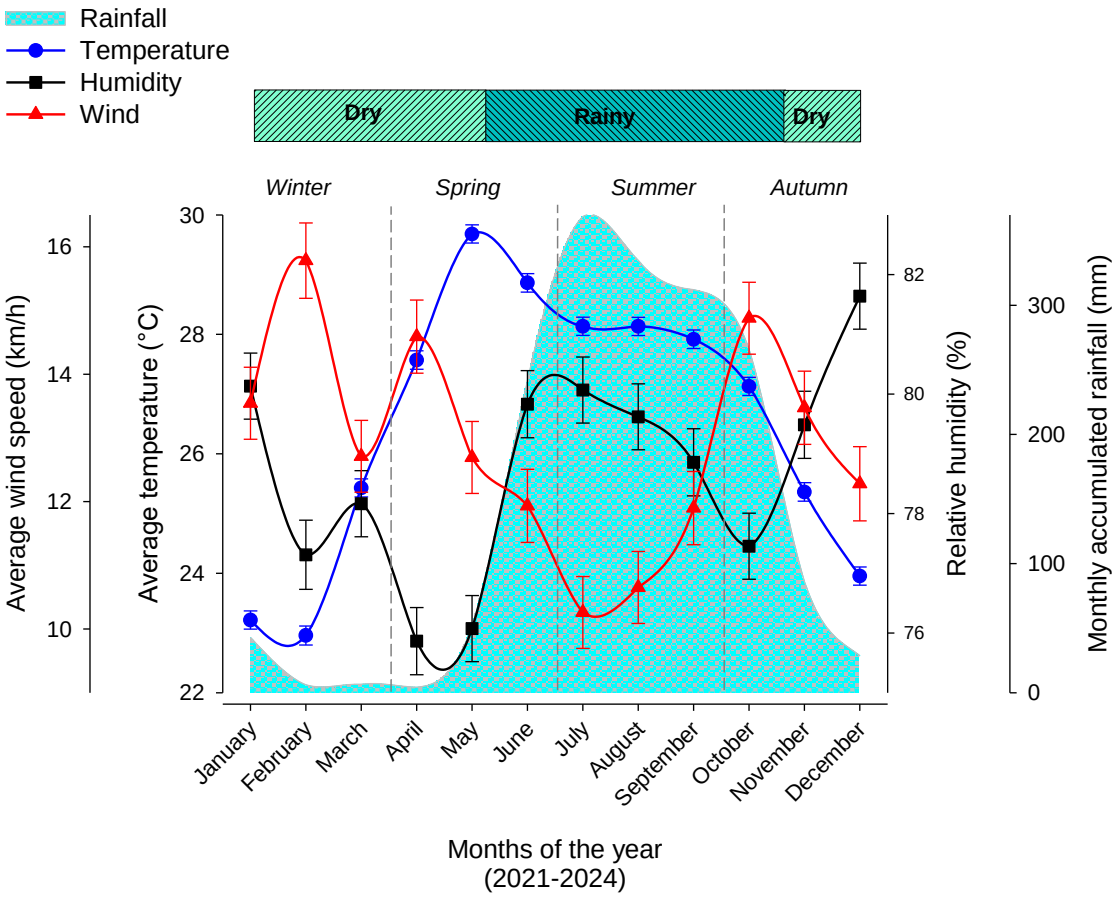


Figure 2. General climograph of the study region. It shows the environmental variables in the 2021-2024 period for the regions of the Mexican tropics where the RC are located. (▒) Monthly accumulated rainfall in mm, (●) Average temperature in °C, (■) % of relative humidity, (▲) Average wind speed in km/h. The vertical dotted lines (⁞) show season changes. The upper dotted bar (▒) shows the Dry and Rainy period of the year.

As shown in the climograph (Figure 2), weather conditions change constantly throughout the year in the Mexican humid tropics. Two important periods of the year are shown, the dry (December-May) and rainy (June-November). Rain is concentrated in summer, temperature is high in spring and summer, relative humidity remains high throughout the year, >75% and wind is most present in fall and winter.

Temperature-Humidity Index categories (comfort, alert, danger, emergency) were defined according to NIENABER & HAHN (2007), based on respiratory rate thresholds (30-90 bpm). THI were obtained from the meteorological data and the climograph environmental variables; with this, it was decided to analyze the yearly

magnitude and percentage of every category of thermal comfort within the THI (Figure 3).

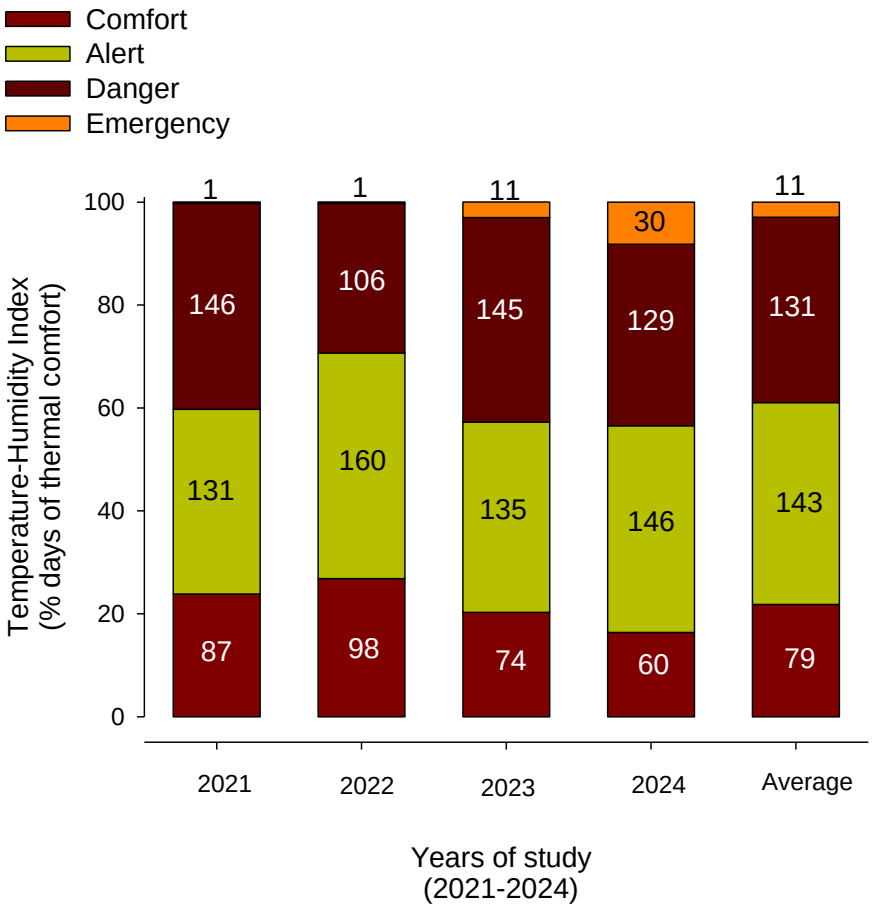


Figure 3. Percentage and number of days that fell within the different categories of thermal comfort in the Temperature-Humidity Index during the years of the study. The numbers in each column represent the number of days in the year that fell within each category; the total is 365 days (100%).

The results of the analysis of Figure 3, demonstrate that the percentage of days when the RC were found to differ every year. 2021 and 2022 show the highest percentage of days with comfort and alert conditions ($p < 0.05$), while 2023 and 2024 show the largest proportion of days with danger and emergency conditions ($p < 0.05$).

Pregnancy rate was analyzed with the results of the environmental variables in different years, months and seasons to determine the differences between each other. These results are shown in Table 1.

The higher PR observed in 2023 coincided with a lower number of days in the "alert" category of THI than in the previous year (2022) or the following year (2024). Furthermore, in 2024, a greater number of days were observed in the "emergency" category. This difference may indicate a more benign climate profile for RC. In subsequent analyses, the data were standardized for each environmental variable to remove the year effect, ensuring the equitable contribution of all years.

The pregnancy variable was plotted using monthly percentages, according to a time scale (months) and associated with each one of the environmental variables (Figure 4). THI, monthly rainfall accumulated, prevailing north and south wind (Figures 4A,B,C,D), respectively.

Table 1. Analysis of the pregnancy variable in the embryo transfers to recipient cows at different years, months, seasons, periods and THI category of the year in the humid tropics of Mexico.

Factor	Level	Pregnancy (%)	N (3610)	P-level
Year	2021	38.48 ± 1.91[a]	647	0.001
	2022	37.12 ± 1.23[a]	1554	
	2023	47.92 ± 1.73[b]	795	
	2024	38.59 ± 1.97[a]	614	
Month	January	35.00 ± 3.56[ab]	180	0.001
	February	43.57 ± 3.66[ac]	218	
	March	48.88 ± 2.82[c]	315	
	April	41.17 ± 2.18[a]	510	
	May	36.90 ± 3.04[ab]	252	
	June	39.34 ± 2.28[a]	460	
	July	31.59 ± 2.57[b]	326	
	August	31.38 ± 2.80[b]	274	
	September	42.22 ± 3.01[ac]	270	
	October	41.83 ± 3.53[ac]	196	
	November	35.76 ± 2.89[ab]	288	
	December	49.84 ± 2.79[c]	321	
Season	Spring	39.64 ± 1.46[a]	1115	0.001
	Summer	34.91 ± 1.56[b]	925	
	Autumn	42.74 ± 1.67[a]	875	
	Winter	43.88 ± 1.88[a]	695	
Period	Rainy	42.75 ± 1.24[a]	1574	0.002
	Dry	37.86 ± 1.07[b]	2036	
THI	Comfort	45.35 ± 0.02[a]	579	0.005
	Alert	37.77 ± 0.01[b]	1292	
	Danger	37.96 ± 0.01[b]	1671	
	Emergency	41.17 ± 0.06[ab]	68	
	Total	40.00 ± 0.01	3610	

[a,b,c] the difference of the superindices of rows of the pregnancy column is significant ($p < 0.05$).

As illustrated in Figure 4, the PR of RC does not remain constant throughout the year (months), but it fluctuates due to the effect of environmental variables such as temperature and humidity (Figure 4A, THI). When THI is high, >78, the pregnancy percentage lowers. Moreover, monthly accumulated rainfall has a negative effect on pregnancy percentage (Figure 4B), judging by the fact that PR was lowest in peak levels of rainfall (July-August). Regarding average wind speed, the prevailing north wind is cool and less humid (November-March), and it has a positive ($r = 0.6376$ $p < 0.05$) effect on PR (Figure 4C); in contrast with the prevailing south wind, which is hot and humid and shows a negative effect although the value is low, (April-June, $r = -0.2370$ $p = 0.05$) on PR of the RC (Figure 4D).

A physiological way to explain the impact of weather variability on the percentage of successful pregnancies was the indirect measurement of the levels of progesterone produced by the CL formed after RC synchronization for transfer, given that bigger CL produce greater quantities of progesterone to support pregnancy. Such measurement was done through ultrasound on transfer day (Only CL size was assessed as an indirect indicator of progesterone competence, not plasma concentrations). The results are shown in Figure 5.

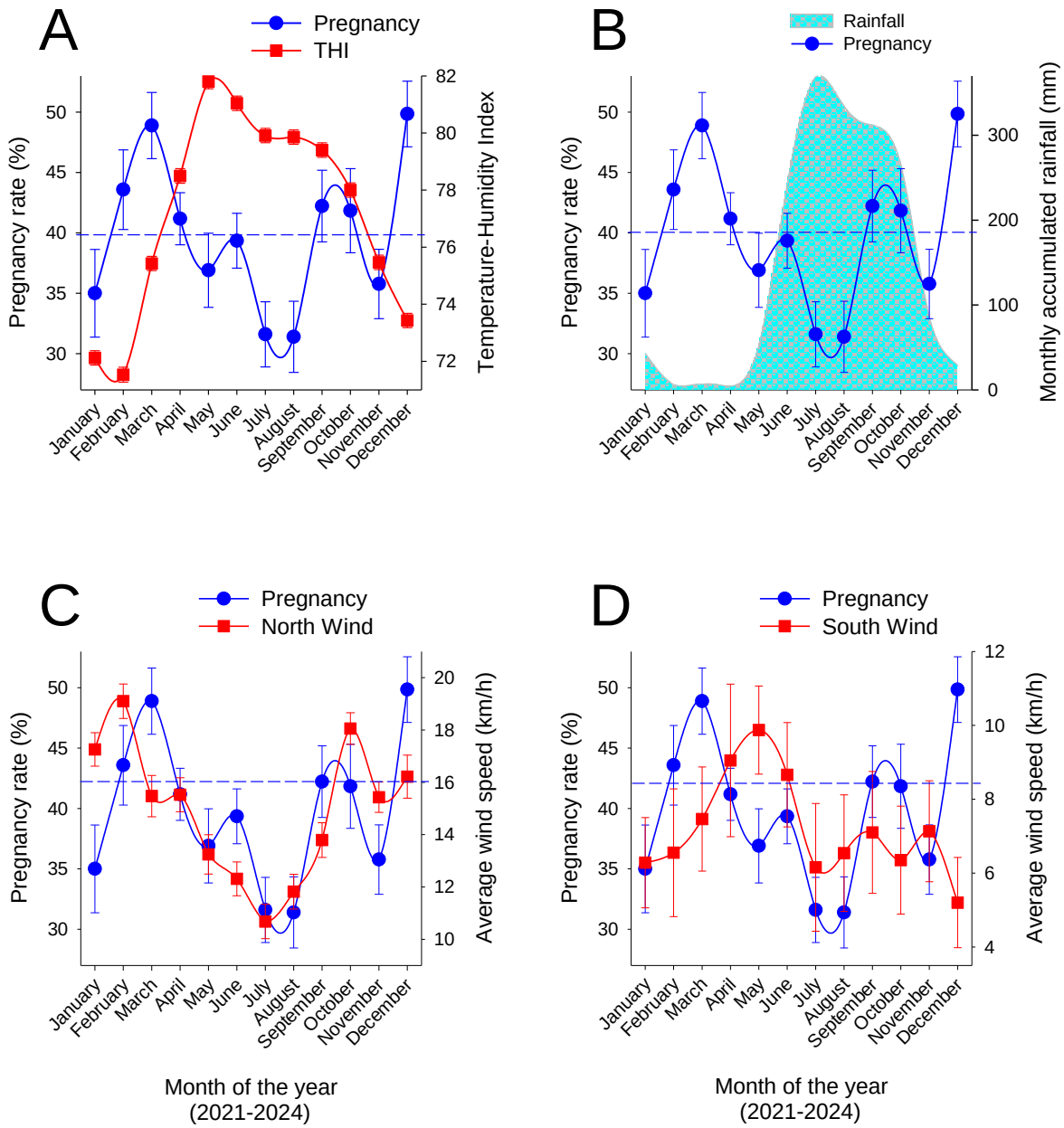


Figure 4. Impact of weather variability on the percentage of successful pregnancies in RC to which Bos *indicus* embryos were transferred in the humid tropics of Mexico. A. Effect of the Temperature-Humidity Index on pregnancy success percentage per year. B. Effect of monthly accumulated rainfall on PR per year. C. Effect of the prevailing north wind on PR per year. D. Effect of the prevailing south wind on PR per year.

As shown in Figure 5A, CL3 (>25 mm ∅) yield greater PR ($p<0.05$) in comparison with CL1 (<15 mm) and CL2 (15-25 mm). Environmental comfort (Figure 5B) affects the percentage of CL3 because, when RC are in an environment of emergency (THI > 84), their amount is reduced ($p< 0.05$). Regarding the seasons of the year (Figure 5C), no significant changes were observed in summer, autumn nor winter ($p>0.05$); changes were only perceived in spring ($p< 0.05$). The dry period favors the presentation of CL3 and a lower proportion of CL1 compared to the rainy period ($p < 0.05$).

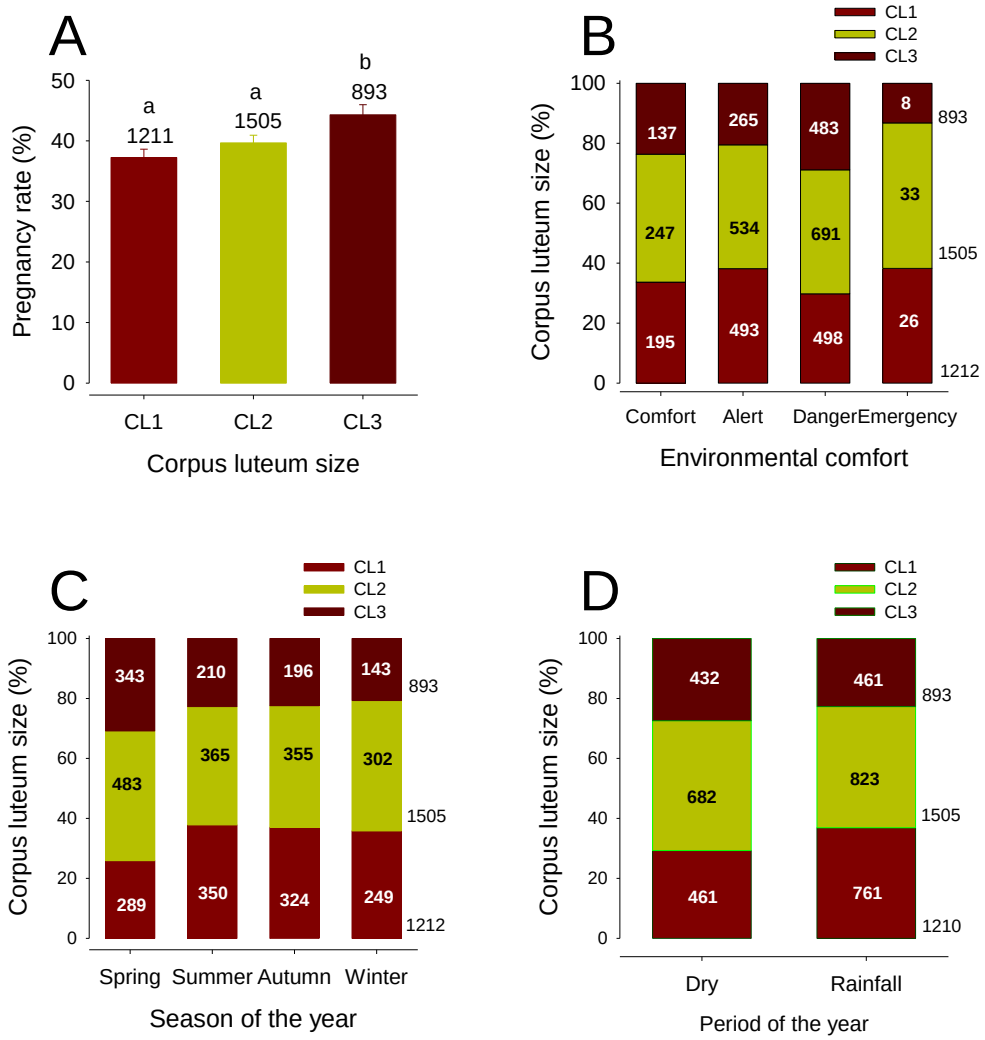


Figure 5. Effect of environmental comfort per season and period on corpus luteum size. A. Corpus luteum size and its' association with successful pregnancy percentage. B. Environmental comfort and its' effect on the frequency of every CL size. C. Effect of the seasons of the year on the frequency of every CL size. D. Effect of the different periods of the year on the frequency of every CL size. Notice. The numbers shown in the error bar represent the amount of CL analyzed; the difference between the letters in each column of A is significant ($p< 0.05$)

As illustrated in the previous results, weather variability impacts PR; in this regard and with the objective of obtaining the pregnancy success rate based on the heat stress provoked by the combination of temperature and humidity (THI), it was decided to use logistic regression, which allows to find the probability (Y) for each THI value (X), these results are shown in Figure 6.

The average values ± standard error of the THI found in this study in the analysis period (2021-2024), were of 77.23 ± 0.11, with a minimum of 59.10 and a maximum of 87.49 and the variation coefficient was 5.63%. Thus, with a minimum THI value (59.10), the probability of a successful pregnancy is 0.5685 (56.85%) and 0.3224 (32.24%) for the maximum THI value (87.49).

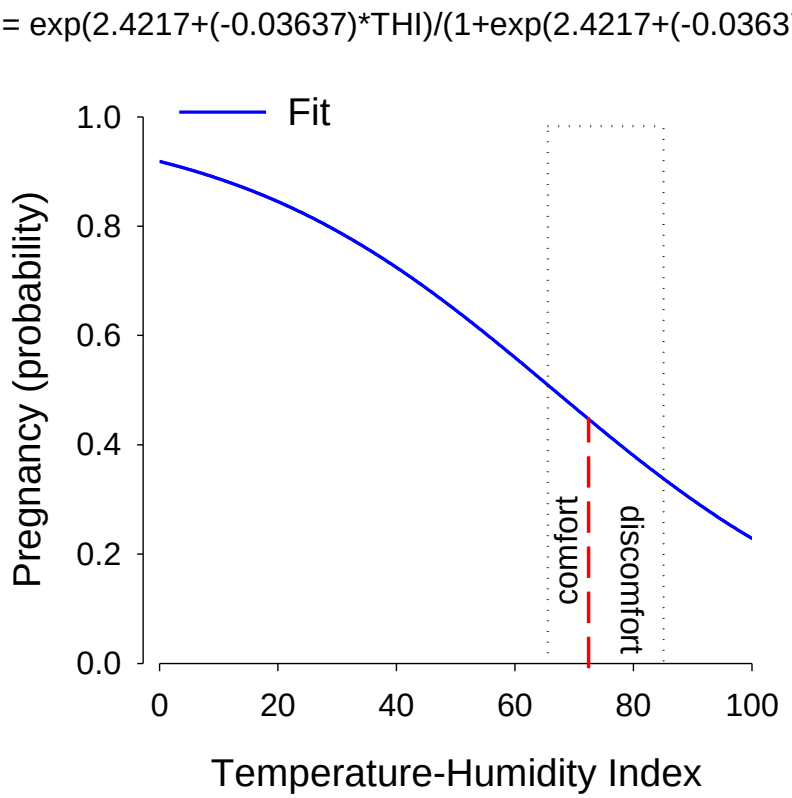


Figure 6. Effects of Temperature-Humidity Index on the successful pregnancy rate of the transfer of in vitro produced embryos *Bos indicus*. The equation is the logistic regression adjustment (solid blue line). The dotted square represents the window of biological analysis in the humid tropics, and the vertical dashed red line separates thermal comfort from discomfort (alert, danger and emergency).

Lastly, it was decided to elaborate a dendrogram to holistically demonstrate the correlations (unions) and report the explained variability of the studied phenomenon, the weather variability and the PR, which shows the variables that have a negative or positive effect on the latter. Moreover, with the use of PCA method, the contribution of each variable (eigenvectors) and the variance of two factors were quantified. These results are shown in Figure 7.

The results of the union dendrogram in Figure 7A show a clear association between environmental variables (THI category and wind) and the chronology (month); Additionally, another association can be appreciated between pregnancy and temperature but, as it has been reported before, it is negative. Figure 7B summarizes the results of the principal components analysis PCA of the biplot, where factor 1 = 43.40% (x axis) and factor 2 = 18.18 (y axis) and explains 61.57% of the variation of the phenomenon studied. Each arrow represents a variable (eigenvector over factor 1 - factor 2). It can be observed that the temperature, the THI and its' categories are in the opposite direction to the pregnancy and that the wind variable is on the same direction (they are correlated). Rainfall and humidity are in the opposite direction.

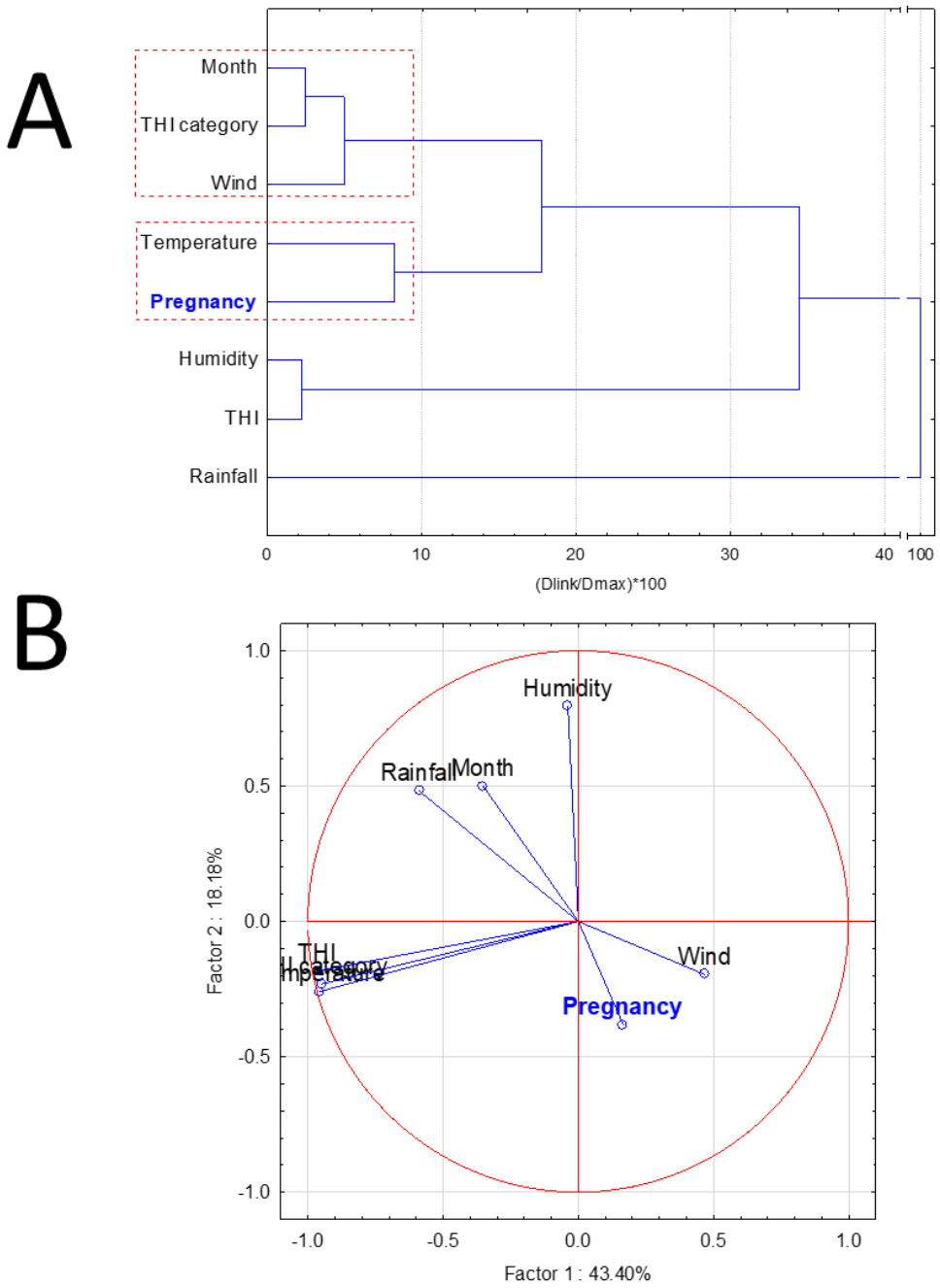


Figure 7. Multivariate analysis of the climate variation and its relationship with of pregnancy of the transfer of in vitro produced embryos Bos *indicus* in the Mexican humid tropics. A. Union dendrogram, non-weighted and normalized pair clustering with the Pearson 1-r correlation coefficient ([Correlation distance/maximum distance] * 100). B. Bidimensional PCA image (biplot), the sense and magnitude of the original variables are shown in the new plot.

DISCUSSION

The results of the study confirm that the weather variability of the Mexican humid tropics significantly affects PR in RC transferred of in vitro produced (IVP) embryos Bos *indicus.* The regional climograph, the annual distribution of the Temperature-Humidity index (THI), the analyses per season and period (rain and dry), as well as the analysis for obtention of the probability (logistic regression) and the multivariate analysis (dendrogram and PCA) agree in that environmental stress, especially that provoked by high temperatures (≥30°C) and relative humidity (≥75%), reduces the

possibility of successful pregnancies, while the success of embryo transfer programs increases in cooler seasons and periods (lower THI, cooler and drier wind and lesser accumulation of rainfall).

These results confirm the initial hypothesis that environmental variation, particularly the combined effect of high temperature and humidity, significantly reduces the probability of successful pregnancy following the transfer of Bos *indicus* embryos IVP under tropical conditions. By integrating the Temperature-Humidity Index (THI), rainfall, and wind variables, this study demonstrated that heat stress and relative humidity act as limiting environmental factors for reproductive efficiency, thereby fulfilling the main objective of associating these variables with pregnancy success in humid tropical environments.

From the results obtained and plotted it can be appreciated that weather variability has an influence on PR of RC, given the heat stress, since it causes a rise of body temperature, as well as physiological and behavioral disturbances due to the extreme environmental conditions (CORREA-CALDERON et al. 2022). Temperature is one of the most studied climatological factors (MUZZO et al. 2025); recent studies (PORRÚA et al. 2023), mention that the increase of the mean annual temperature in Mexico is 2.88 °C (2.77 - 3.33 °C), above the increase of the mean global temperature, which is 1.90 °C (1.78 - 1.97 °C), while at the beginning of the 20th century it was 1.69 °C (1.59 - 1.81 °C) for Mexico and 1.23 °C globally. In the northern states of the country, the increase reached 6 °C and 5 °C in the southern states, while it reached 2-4°C in the rest of the country in the period from 1975-2021 (PORRÚA et al. 2023).

On a yearly basis, 2023 had the highest PR, while 2021, 2022 and 2024 had the lowest rates. This variation coincides with the proportion of days per THI category, in which the years that had the most days in “danger” and “emergency” conditions presented an adverse environment for embryo implantation and thus for maternal recognition of pregnancy. These findings coincide with the physiology of heat stress in livestock, which compromises ovarian function and the quality of the uterine environment (BOVA et al. 2014, DÍAZ et al. 2020, KHAN et al. 2023). Particularly, the threshold value of the THI >74 is associated with compensatory tachypnea, endocrine alterations and inflammation (NIENABER & HAHN 2007, DÍAZ et al. 2018, SAIZI et al. 2019, KHAN et al. 2023).

The seasonal pattern in the Mexican humid tropics supports this interpretation. PR diminished in summer, which coincided with the high temperature period, high relative humidity and peak rainfall. On the other hand, the best PR obtained is in autumn and winter, with spring being moderate but variable. The better rates during the dry period, in comparison with the rainy period, shown in Table 1, may be explained by a relatively lower THI, given that there is less relative humidity and less periods of intense rainfall that may be associated with sanitary issues (BARUSELLI et al. 2010, MARTINEZ et al. 2021, GALINA & GEFFROY 2023).

Moreover, the prevailing north wind and northeast, driven by cold air masses (cold fronts) moving down from Canada and the United States toward the Gulf of Mexico. The collision with the warmer air in the region generates strong wind currents that cause a significant drop in temperature, an increase in humidity, and precipitation, the peak season occurs during autumn, winter, and early spring was positively related

to PR. While winds from the south and southeast, favored by intense anticyclone systems (high pressure) in the Gulf of Mexico, are strong, dry, and warm. They don't usually bring rain and create a hot and stable environment.

These winds are more frequent during the summer and in transition periods between cold front seasons, was negatively associated with PR. Which underlines the fact that it is not only temperature, but the combination of temperature, humidity and circulation of air currents that regulates the thermal balance of the RC (VIDAL-ZEPEDA 2005, COSTAMAGNA et al. 2024). This group of results overlaps with studies done in tropical conditions, which highlight the most successful seasonal windows for AIE and FTAI, as well as for embryo transfers when the environment is less stressful, both for the donor cows and RC (BARUSELLI et al. 2010, PÉREZ-MORA et al. 2020, MARTINEZ et al. 2021).

It is worth remarking that, when THI is categorized (comfort, alert, danger, emergency) with the purpose of interpreting physiological responses (respiratory rate, thermoregulation) of the RC, it is negatively associated with the observed reproductive yield. The longer the danger and emergency conditions last (heat waves) around the time of the transfer, the greater the possibility of implantation failure (ZAVALETA-MARTINEZ et al. 2024b) and with it PR diminish. This reinforces the prior recommendation of planning transfers around time windows of lesser daily THI and monitor short-term predictions to minimize the exposure of the embryos to heat waves (HANSEN 2004, BARUSELLIET al. 2010, MARTINEZ et al. 2021).

In this sense, the monthly-accumulated rainfall variable had a negative impact on PR when it reached peak values (July-August). Intense rainfall is associated with a greater parasitic load and sanitary problems that may affect body condition (BARUSELLI et al. 2010, GALINA & GEFFROY 2023). In contrast with this environmental variable, the prevailing north wind lessened heat stress, while the prevailing south wind increased it. These patterns respond to the physics concepts of convection and evaporation. With less humidity, the vapor gradient between the skin and the environment is more favorable and facilitates heat dissipation; in high humidity conditions, evaporation is inefficient, which promotes hyperthermia (COSTAMAGNA et al. 2024).

Regarding the analysis of corpus luteum, their size on transfer day has been considered an indirect physiological indicator of progesterone production. CL3 (>25 mm) were associated with a greater PR, in comparison with CL1 and CL2, thus confirming that luteal activity is a key element of early embryonic survival (ALKAN et al. 2020, CHOI et al. 2023, VALCHUK et al. 2023). Moreover, CL3 was less frequent in high temperatures and relative humidity conditions (THI ≥ 84), which supports the notion that heat stress compromises luteogenesis and steroidogenesis (KHAN et al. 2023, DÍAZ et al. 2018, DÍAZ et al. 2020, GÓMEZ-GUZMÁN et al. 2024).

This notion is consistent with evidence of a reduction of progesterone in a CL that is smaller, or less competent in hot environments, such as those observed in the tropical regions; this impacts endometrial receptivity and interferon-τ signaling (SAKATANI 2017, SARTORI et al. 2025). From a practical point of view, the results support the notion of classifying RC by corpus luteum of sizes ≥15 mm (CL2 and CL3),

before transfer, to maximize the probability of successful pregnancy (BARUSELLI et al. 2010, ALKAN et al. 2020, CHOI et al. 2023).

The use of logistic regression allowed the transformation of the environmental comfort indicator (THI) into an operational risk indicator. The probability of success fell from ~0.57 at the observed THI minimums (~59) to ~0.32 at THI maximums (~87). This ~0.40 decrease in probability demonstrates the non-linear sensitivity of pregnancies to thermal stress, which is consistent with the extant bibliography, that documents failure of maternal recognition of pregnancy, decrease in the secretion of progesterone and endometrial alterations when temperature and relative humidity have increased (KHAN et al. 2023, NARANJO-GOMEZ et al. 2021, SAKATANI 2017).

The discovery of a pronounced gradient within the “biological analysis window” of the humid tropical regions can be explained by threshold physiological responses. Once the value of THI has been exceeded by 74 units, further increases can intensify oxidative damage due to the presence of reactive oxygen species (ROS), a cell disfunction of the endometrium and the embryo, which hinders the implantation of the transferred embryos (BOVA et al. 2014, SAKATANI 2017, QU et al. 2024). ROS and antioxidant agents not only can affect embryo implantation, but also oocyte quality is reduced, causing “aged oocytes” (WANG et al. 2021).

Oxidative stress (OE) increases the presence of the superoxide anion and hydrogen peroxide, affecting the mitochondria of the embryo cells, the mitochondrial DNA and causing apoptosis (SLIMEN et al. 2014). In addition to this, it has been documented that an increase of THI has an effect on the size of the preovulatory follicle, as well as on the intensity of the estrus signs, which is attributed to hormone alterations, reduction of the estradiol concentration (in the liquid of the dominant follicle) and concentration of LH in plasma. Moreover, it changes the concentration of progesterone (<1.0 ng/m per each point of increase in THI), while follicle size is reduced by 0.1mm per each point of THI increase (SCHÜLLER et al. 2017).

In the multivariate analysis, the union dendrogram showed an association of the THI categories and prevailing wind with chronology (the season of the year), which reinforces the notion that the local climate calendar can have an impact of the probability of reproductive success. The PCA biplot explained 61.57% of the variance (43.40% + 18.18%); THI and temperature were in the opposite direction to the pregnancy variable, while wind was in the same direction.

The monthly-accumulated rainfall and relative humidity also were oriented to opposite directions with respect to PR. This vector geometry summarizes the conceptual model of the study; a successful pregnancy happens as a “balance” between the thermal load (T, RH), ventilation (wind), sanitary and handling stability associated with monthly-accumulated rainfall. This integration coincides with the results of previous evaluations of embryo transfer programs in the humid tropics, where seasonality and the microclimate on transfer day have been proposed as extrinsic factors for successful pregnancies (PÉREZ-MORA et al. 2020, HANSEN 2020, MARTINEZ et al. 2021).

Although intrinsic factors such as breed composition, age, body condition, and physiological state are known to influence fertility and embryonic survival, the present study aimed to isolate the environmental component affecting pregnancy outcomes.

All RC were crossbred Bos indicus × Bos taurus animals under homogeneous management, non-lactating condition, and similar body scores (3-4 on a 1 to 5 scale; AYRES et al. 2009).

Weather conditions were comparable among the livestock production units analyzed, as all were located within the humid tropical belt of Mexico under similar temperature-humidity profiles. Minor microclimatic differences (e.g., local rainfall intensity or wind direction) were normalized using standardized weather station data and portable hygrothermographs obtained during each transfer. These adjustments ensured that the environmental variables used in the analysis reflected regional climate behavior rather than isolated local effects.

Lastly, even if the *in vitro* embryos are cultivated under control conditions and following IETS quality criteria (IETS 1998, STRINGFELLOW & GIVENS 2010), their post-transfer destiny (a healthy calf) depends on a favorable uterine environment in the RC. Heat stress can: (i) affect the Fallopian tubes and endometrium, altering their secretions, (ii) reduce the amount of progesterone by compromising luteogenesis and, (iii) induce oxidative stress and damage to the embryonic DNA, lowering its restorative and developmental capacity (HANSEN 2004, DÍAZ et al. 2018, GÓMEZ-GUZMÁN et al. 2024).

In this sense, the results coincide: when THI increases, pregnancy success rate and CL3 frequency both decrease; when the prevailing wind is cool and dry and THI is low, the pregnancy success rate increases. It can be inferred that uterine receptivity and embryonic development are two interdependent factors in tropical humid environments (HANSEN 2020, SARTORI et al. 2025). The main limitation of this study was the inability to incorporate intrinsic reproductive variables of RC, such as parity or detailed hormonal pro-filing, which could further explain individual variation in pregnancy outcomes.

Additionally, despite the use of standardized environmental data, local management practices may have introduced minor uncontrolled variation across livestock units. Nevertheless, the consistency of the statistical results and the strong association between THI and PR suggest that climatic stress remains the predominant factor influencing reproductive efficiency under these tropical conditions

There is a high probability that the Mexican humid tropical regions will experience more frequent and intense heat waves and more erratic rain patterns in the future (GODDE et al. 2021, TOLEDO-ALVARADO & OROZCO-LUCERO 2025, MUZZO et al. 2025, DOMÍNGUEZ-MANCERA et al. 2017). In this context, embryo transfer programs must promote adaptation schedules, strengthen micro-environmental infrastructure and use genomic tools to raise thermal resilience without compromising productivity (SILVA-NETO et al. 2025, DE ALMEIDA et al. 2022, HANSEN 2020, CASANOVA-PEREZ et al. 2018). These results can guide timing of embryo transfer programs in humid tropical environments to improve reproductive outcomes.

CONCLUSION

Weather variability in the Mexican humid tropics significantly modulates the pregnancy success rate of *Bos indicus* embryos IVP. Seasonal adjustment and

environmental management are key to maintaining reproductive efficiency under heat stress conditions.

The findings of this study provide quantitative evidence that the Temperature-Humidity Index (THI) can serve as a reliable environmental indicator for predicting reproductive performance in tropical cattle systems. Understanding these climatic thresholds is crucial for optimizing the timing of embryo transfers, improving animal welfare, and increasing the profitability of dual-purpose herds under tropical conditions.

Future studies should integrate physiological and molecular indicators, such as progesterone profiling, gene expression of heat-shock proteins, and oxidative stress biomarkers in RC to elucidate the mechanisms underlying thermal stress on embryo implantation. Additionally, incorporating genetic resilience traits and evaluating microclimate modification strategies in livestock facilities could enhance adaptation to on-going climate change in tropical regions.

NOTES

AUTHOR CONTRIBUTIONS

Conceptualization, methodology, and formal analysis, Alondra Zavaleta-Martínez, Manuel Barrientos-Morales and Belisario Dominguez-Mancera; software and validation, José M. Martínez-Hernández, Yasser Kayser-Alarcon and Belisario Dominguez-Mancera; investigation, Alondra Zavaleta-Martínez, Manuel Barrientos-Morales and Araceli Rodríguez-Andrade; resources and data curation, Víctor Absalón-Medina, Yasser Kayser-Alarcon and Belisario Dominguez-Mancera; writing-original draft preparation, Manuel Barrientos-Morales, Manlio Alpírez- Mendoza and Belisario Dominguez Mancera; writing-review and editing, Araceli Rodríguez-Andrade and Belisario Dominguez Mancera; visualization, Víctor Absalón-Medina, Manuel Barrientos-Morales and Belisario Dominguez Mancera; supervision, Manuel Barrientos-Morales and Belisario Dominguez Mancera; project administration, Alondra Zavaleta-Martínez, Manuel Barrientos-Morales and Belisario Dominguez Mancera; funding acquisition, Alondra Zavaleta-Martínez and Belisario Dominguez Mancera. All authors have read and agreed to the published version of the manuscript.

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The handling and immobilization procedures used on the embryo recipient cows were reviewed and approved by the bioethics committee of the Universidad Veracruzana, School of Veterinary Medicine and Animal Sciences, folio n°: COBIBA011/2021.

INFORMED CONSENT STATEMENT

Not applicable because this study did not involve humans.

DATA AVAILABILITY STATEMENT

The data can be made available under request.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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