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EFFECTS OF SEASON, MAXIMUM TEMPERATURE AND RELATIVE HUMIDITY ON THE GESTATION SUCCESS OF TROPICAL MILKING CRIOLLO COWS

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ABSTRACT

The season of the year (ES), maximum temperature (Tmax) and relative humidity (RH) affect gestation success of Tropical Milking criollo (TM) heifers, but their effects on the gestation of TM cows are not completely known. The study aimed to determine the effects of environmental factors along with artificial insemination (AI) service and calving on the gestation success of TM cows. Data were collected during 14 years, 959 records of AI of 155 cows in three seasons: hot-dry (HD), hot-humid (HH) and fresh-dry (FD). The response variables were gestation success of cows at first service after first calving (GF), first calving first to fifth service (GS) and global gestation success (GG). Model effects were ES, Tmax and RH seven days before the AI (PRAI), the day of the service (DAI) and seven days after (POAI); and days open (DO), cumulative milk production (MP), number of service (NS) and calving number (CN). GF was analyzed with logistic regression, and GS and GG with generalized linear models. Over 50 % of estrus occurred in the HH season. Tmax affected GF $\hat{\beta}_1$ = -0.13 ± 0.07 ($p \leq 0.05$) and GG $\hat{\beta}_1$ = -0.08 ± 0.04 ($p \leq 0.05$) in PRAI. There were no effects of ES, RH, NS or MP on GF, GS and GG for PRAI, DAI and POAI ($p > 0.05$). DO affected GG in PRAI, DAI and POAI $\hat{\beta}_2$ = -0.005 ± 0.002 ($p \leq 0.01$). GG were as close to 52.0 % for the three seasons. Although with no statistical differences in multiparous cows ($p > 0.05$), for NS maximum gestation were 60 % at first and third, and minimum 40 % at fourth services; for CN they were 60 % at fourth, and 40 % at sixth and seventh calving, respectively. TM cows are adapted to a hot tropical Climate and their seasonal changes, showing similar pregnancy frequencies among seasons.

Keywords: Adapted cattle, artificial insemination, climatic change, dairy, intertropical region.

INTRODUCTION

The hottest climates are found in the planet´s intertropical zone, where maximun temperature (Tmax) and relative humidity (RH) are high and the rainfall can be abundant. In addition, climate change has caused the temperature to rise and the rainy period to shorten, with higher duration and intensity of the dry season (Fuentes-Franco *et al*., 2014; Byrne *et al*., 2018;).

The main characteristics of hot tropical climates are the mean annual temperature greater than 22 °C, mean temperature of the coldest month greater than 18 °C and altitude less than 1200 m. In these climates, pastoral livestock systems predominate, where animals are exposed throughout the year to adverse climate factors and, parasites and diseases typical of these environments (Hernández-Castellanos *et al*., 2019).

Heat stress is one of the main reasons for infertility in cattle (Hansen, 2019). In females, high temperatures and RH before fertilization cause high concentrations of cortisol, which reduce the pulses of gonadotropin-releasing hormone (GnRH) and the release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH). This produces low concentrations of estradiol and alteration in the communication of the granulosa cells and the oocyte with irreparable damage to the DNA, which in turn is manifested in low percentages of estrus and ovulation (Schüler *et al*., 2017).

As high environmental temperatures correlate with rectal and body temperatures during the fertilization period, increases in rectal temperature and in the cervical canal are generated (López-Gatius *et al*., 2021). These changes reduce the viability and motility of sperm and its fertilizing capacity (Monterroso *et al*., 1995). After fertilization, embryos are sensitive to heat stress during the first stages of their development, with more embryonic reabsorptions and poor-quality blastocysts (Hansen, 2019). Furthermore, climate change is expected to affect cattle exposed to inter-annual climatic variability of grazing systems (Rust, 2019).

In Tropical Milking criollo (TM) heifers, it was verified that Tmax before and RH after artificial insemination (AI) affected their gestation success (%) at first service ($p \leq 0.05$; Rosales-Martínez *et al*., 2021). Importantly, TM cows have proved to be productive, more resistant to adverse climatic effects and to endo and ectoparasites, due -like adult animals- to their pigmented and thick skin, short and shiny hair and medium size, among other characteristics favorable to hot environments (De Alba, 2011). The breed has more adaptation genes at different frequencies than exotic European breeds (O'Neill *et al*., 2010). However, the changing weather conditions in the TM habitat, with Tmax and RH above 30 °C and 84 %, make it necessary to quantify their effect on cows to plan their reproduction programs; where the calving-conception interval and the level of milk production could also affect cows´ fertility. Therefore, the objective of this study was to quantify the effect of season of the year, maximum temperature and relative humidity, artificial insemination service, and calving number related factors on the gestation success of Tropical Milking criollo cows.

MATERIALS AND METHODS

Characteristics of the seasons of the year

The study was carried out in Veracruz, Mexico at 19° 11´N and 96° 20´W, at an altitude of 23 m. Three climatic seasons of the year were considered, as developed by cluster analysis by Rosales *et al*. (2021), with Tmax and RH as: hot-dry (HD, March-May, 31.6 ± 1.0 °C and 77.2 ± 1.1 %), hot-humid (HH, June-October, 31.4 ± 0.4 °C and 84.7 ± 1.0 %) and fresh-dry (FD, November-February, 26.8 ± 0.5 °C and 84.6 ± 0.3 %). Annual mean temperature and precipitation were 24.8 °C and 1,525.8 mm (INIFAP, 2018; SMN, 2018); the climate of the region is AW_0(w)(i')gw'' hot subhumid with rains in summer (García, 1988).

Cow management and data source

Cows were fed with para grass (*Brachiaria mutica* Forssk) and native grasses (*Paspalum* spp.). Milking was manual, once a day in the morning, with the calf presence. The cows in estrus were detected daily throughout the year, with the support of a watch bull. Artificial insemination (AI) was performed 12 h after estrus detection, with straws from TM bull stallion containing 25 million sperm (0.5 mL) and progressive individual motility greater than 60 %. The pregnancy diagnosis was done by transrectal palpation 45-55 d after the AI (Rosendo-Ponce and Becerril-Pérez 2015).

The TM cows´ datasets more complex and bigger than the TM heifers come from 14 years of cows of 82.12 ± 1.15 months mean age. A total of 959 AI records from 155 cows were used.

Response variables

Frequencies of estrus were recorded according to watch bulls´ detection and estimated by season in relation to year total (%). Regarding the number of AI services as cows´ gestation success by season was studied, for the first service after first calving (GF, %; n = 121) and from the first to the fifth service (GS, %; n = 167) and the global gestation success of multiparous cows from the second to the seventh calving and all services (GG, %; n = 792).

Statistical analysis

The effects of season of the year (SE), Tmax and RH on GF, GS and GG were analyzed for the following seven days before the AI service (PRAI), a period in which the preovulatory follicle dominance occurs (Wilson *et al*., 1998); the service day (DAI), when there is an increase in the uterine temperature and possible reduction in the sperm fertilizing capacity (Monterroso *et al*., 1995); and seven days after AI service (POAI), a period in which embryos are sensitive to heat stress (Hansen, 2019).

In addition, the effects of days open (DO) and cumulative milk production on the day of service (MP), number of service (NS) and calving number (CN) were also studied. To analyze GF, the following logistic regression model was used:

$$y_{ij} = \mu + ES_i + \beta_1 (x_{1ij} - \bar{x}_{1..}) + \beta_2 (x_{2ij} - \bar{x}_{2..}) + \beta_3 (x_{3ij} - \bar{x}_{3..}) + \beta_4 (x_{4ij} - \bar{x}_{4..}) + \in_{ij}$$

where y_{ij} = is the response variable measured the i-th season of the year, of the j-th cow, which can take values of 1 pregnant or 0 non-pregnant; μ = Constant that characterizes the population; ES_i = fixed effect of the i-th season of the year. i = 1 hot-dry, 2 hot-humid and 3 fresh-dry; β_1 = regression coefficient that relates the mean maximum temperatures with the response variable; x_{1ij} = mean of the maximum temperature observed in the i-th season of the year that occurred during the insemination of the j-th cow; $\bar{x}_{1..}$ = mean of mean maximum temperatures; β_2 = regression coefficient that relates the mean relative humidity with the response variable; x_{2ij} = mean of the relative humidity observed in the i-th season of the year that occurred during the insemination of the j-th cow; $\bar{x}_{2..}$ = mean of mean relative humidity; β_3 = regression coefficient that relates days open with the response variable; x_{3ij} = days open in the i-th season of the year during insemination of the j-th cow; $\bar{x}_{3..}$ = mean open days; β_4 = regression coefficient that relates cumulative milk production with the response variable; x_{4ij} = cumulative milk production the i-th season of the year during insemination of the j-th cow; $\bar{x}_{4..}$ = mean cumulative milk production; $\in_{ij}$ = random error. $\in_{ij} \sim B\ (n, p)$.

The data were processed with PROC LOGISTIC; opportunity ratios and 95 % confidence intervals were obtained for Tmax, RH, DO and MP. GS and GG were analyzed with generalized linear models using PROC GLIMMIX, with a binomial distribution and a logit link function; including NS and CN as independent factors (SAS Institute Inc., 2010).

RESULTS AND DISCUSSION

Gestation success in the first service of first calving cows (GF)

The frequency of the first estrus with AI per season was HD 19.8, HH 56.2, FD 24.0 %, favoring HH, where 88 % of the annual precipitation occurs and there is greater availability of fresh forage, although Tmax and RH were high**.**

In a previous study, TM heifers had a more homogeneous frequency per season of 22.7, 39.2 and 38.1 %, respectively (Rosales-Martínez *et al*., 2021). For cows served in HD, HH and FD, the GF was 58.3, 48.5, 44.8 % and 49.6 % overall mean. In heifers it was 35.0, 57.9, 58.2 % and 53.0 % overall mean (Rosales-Martínez *et al*., 2021). Therefore, the cows of this study had a better performance in HD than the heifers of the similar research, but decreasing in HH and FD.

For cows, the highest and lowest frequency of annual gestations success were in HH at 55.0 and FD 21.7 % compare to 43.0 and 41.9 % of heifers in a previous research. The heifers responded favorably to the HH and FD seasons and the cows stood heat stress better in HD with 23.3 %, which is 8 % higher than the heifers (Rosales-Martínez *et al*., 2021). The TM bulls showed better mass motility and a higher number of normal

sperm in HD (Villatoro *et al*., 2016). It is preferable to conceive the cows at the first AI service, since labor and semen straws costs are reduced and obtain a new lactation, and a calf every year (Kim and Jeong 2019).

Tmax affected GF only in PRAI ($p \leq 0.05$; Table 1). For TM heifers in the previous study, ES affected GF ($p \leq 0.05$) and a similar effect of Tmax in GF was estimated ($p \leq 0.02$; Rosales-Martínez *et al*., 2021). No effects of ES, RH, DO and MP were observed in GF for PRAI, DAI and POAI ($p > 0.05$). Heat dissipation by convection, conduction and evaporation are lower in heifers because they have a smaller body surface than cows; by increasing their size, heifers increase their ability to dissipate latent heat (Dahl *et al*., 2020).

Table 1. Effect of season, maximum temperature (Tmax), relative humidity (RH), days open (DO) and cumulative milk production (MP), seven days before the artificial insemination on gestation success at the first service after first calving in Tropical Milking criollo cows.

Variable	OR	CI	*p*
Hot-dry season	1.0		0.7031
Hot-humid season	0.69	0.26 – 1.81	
Fresh-dry season	0.64	0.20 – 1.96	
Tmax	0.87	0.76 – 0.99	0.0472
RH	1.03	0.96 – 1.11	0.4427
DO	0.99	0.99 – 1.01	0.7975
MP	1.00	0.98 – 1.00	0.8790

OR= Opportunity ratio; CI = Confidence interval (95%); *p*-value = for season effect and covariables.

MP was 535.1 ± 20.7 kg with DO of 118.7 ± 6.3 d, which did not influence GF ($p > 0.05$). As Tmax increased by 1 °C in PRAI, the probability of GF decreased, $\hat{\beta}_1 = -0.13 \pm 0.07$ ($p \leq 0.05$) in the three seasons (Figure 1).

This coefficient value in TM heifers of previous research shows that the adverse effect of Tmax was higher $\hat{\beta}_1 = -0.20 \pm 0.09$ ($p \leq 0.02$) (Rosales-Martínez *et al*., 2021). In Holstein and Jersey cows, for each increase of 1 point in the Temperature and Humidity Index (ITH), the follicular size decreased 0.1 mm (Schüler *et al*., 2017).

Holstein cows exposed to ITH under 31-35 had 1.08 more chances of becoming pregnant compared to those with ITH over 70 (García-Ispierto *et al*., 2007).

The decrease in GF in bovine females is related to increase cortisol under heat stress (Idris *et al*., 2021). High Tmax and RH reduce the GnRH pulses that disrupt the synthesis of steroid hormones in the follicle (Schüler *et al*., 2017). Exposure to long periods of high temperature and relative humidity can generate chronic stress that reduces estradiol concentrations in the follicles (Abdelnour *et al*., 2020) and frequently inhibits ovulation; in addition, poor communication between the granulosa cells and the oocyte and irreparable damage to the DNA (Paula-Lopes *et al*., 2012).

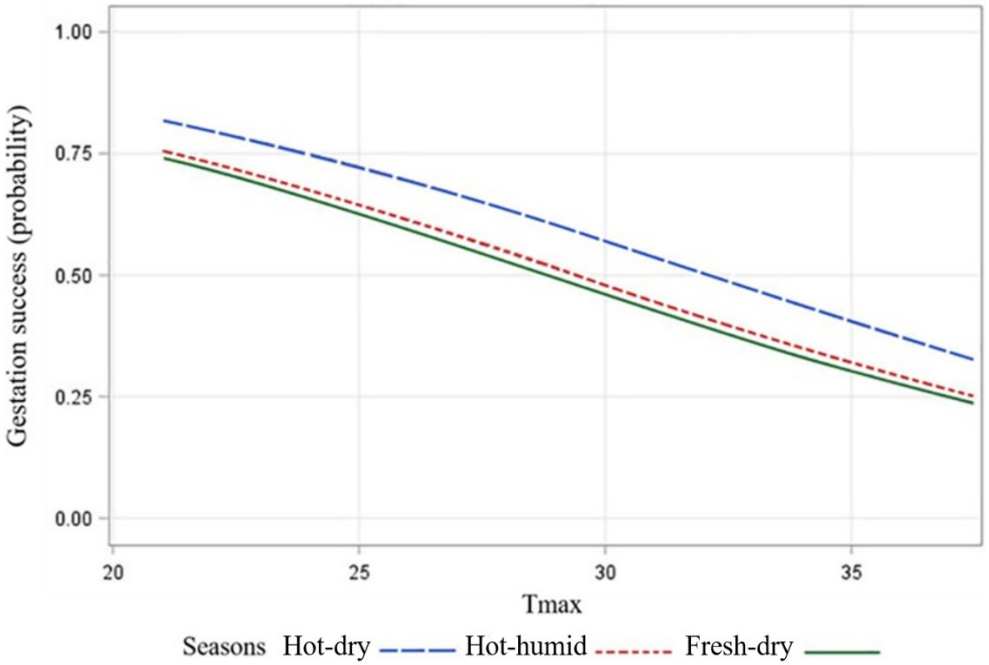

Figure 1. Relationship between the maximum temperatures (Tmax, °C) of seven days before artificial insemination with the probability of gestation success at the first service after first calving in Tropical Milking criollo cows.

Gestation success in all services of first calving cows (GS)

The frequency of estrus per season was HD 19.8, HH 47.9 and FD 32.3 % similar to the first estrus in heifers (Rosales-Martínez *et al.*, 2021). In HD, the temperature exceeded 30 °C, which affects the development of the dominant follicle, with low estradiol secretions that can generate prolonged follicular dominance, affect the manifestation of estrus and decrease ovulation (Schüler *et al.*, 2017).

No effects of ES, Tmax, RH, DO, MP and NS were observed in GS for PRAI, DAI and POAI ($p > 0.05$). The mean probabilities estimated by season were HD 0.48 ± 0.11, HH 0.67 ± 0.07 and FD 0.60 ± 0.08 ($p > 0.05$). In TM heifers, estimated probabilities lower than 0.50 in HD and higher than 0.70 in HH were different ($p \leq 0.05$; Rosales-Martínez *et al.*, 2021). Dairy cows from temperate climates show fertility problems when introduced to hot tropical regions; however, *Bos taurus* naturalized for centuries are adapted to these regions (Lima *et al.*, 2020).

In this study, TM cows showed similar gestation success probabilities at first pregnancy in the three seasons and in the three AI periods in the five AI services. Criollo breeds have adapted to the changing seasons of the different climates of the tropical regions (De Alba, 2011).

Global gestation success in multiparous cows (GG)

The estrus frequency of multiparous cows per season was HD 25.4, HH 50.6 and FD 24.0 %, similar to the first calving cows. There were no effects of ES, RH, MP, NS or CN on GG for PRAI, DAI and POAI; however, Tmax affected GG in PRAI ($\hat{\beta}_1$ = -0.08 ± 0.04; $p \leq 0.05$).

In cows under heat stress, theca and viability of granulosa cells of the follicles are affected (Khan *et al*., 2020), hormonal synthesis is interrupted and estradiol concentration is reduced, reducing follicular development with damage to the oocyte (Abdelnour *et al*., 2020), so it takes at least three estrous cycles to reestablish their competence.

Although no effect of Tmax was observed three days before AI in dairy cows, when the ITH increased from 70 to 80 points the gestation rate decreased from 30.6 to 23 % (García-Ispierto *et al*., 2007). Holstein cows exposed to ITH greater than 72 points in the 42 to 1 days before AI were 31 % less likely to become pregnant (Schüler *et al*., 2014).

MP was 634.2 ± 22.8 kg, almost 100 kg more than at first service after first calving cows, which did not affect GS in any of the three AI models ($p > 0.05$). Moderate amounts of high-solids milk production and the medium size of the multiparous cows generate little metabolic heat that can be dissipated in high temperatures and RH.

Animals under stress for several years generate metabolic and genetic adaptations to contribute to the performance of the physiological process in turn (Collier *et al*., 2019). High milk-producing cows by genetic selection in temperate regions require more AI services per conception (Walsh *et al*., 2011).

DO in multiparous cows was 120.7 ± 4.8, similar to the first service after first calving cows. DO affected GG in PRAI, DAI and POAI ($\hat{\beta}_3$ = -0.005 ± 0.002; $p \leq 0.01$). When the gestation rate increased 1 %, a decrease of four was observed in days open (VanRaden *et al*., 2004), for which it is convenient that the cows are gestated at the first AI service. Mean GG probabilities by season for PRAI, DAI and POAI were all close to 0.52 (Table 2).

Schüler *et al.* (2014) estimated GG of 31.0 % in Holstein cows under Tmax of 29.8 °C and maximum RH of 96.1 %, and when the ITH increased from 72 to more than 78

Table 2. Global gestation success probability of Tropical Milking criollo multiparous cows in three seasons ($p > 0.05$).

Period of Tmax and RH effect	Season		
	Hot-dry	Hot-humid	Fresh-dry
AI previous seven days	0.50 ± 0.06	0.54 ± 0.05	0.52 ± 0.06
AI day	0.50 ± 0.06	0.52 ± 0.05	0.55 ± 0.06
AI posterior seven days	0.49 ± 0.06	0.54 ± 0.05	0.53 ± 0.06

Tmax = Maximum temperature; RH = Relative humidity; AI = Artificial insemination.

points, the gestation decreased from 39.4 to 31.6 % (Lozano *et al.*, 2005). Although no statistical differences were observed ($p > 0.05$) of GG by period of service for NS and CN (Figure 2), probabilities greater than 0.50 were observed in the first three services and in the first four calvings.

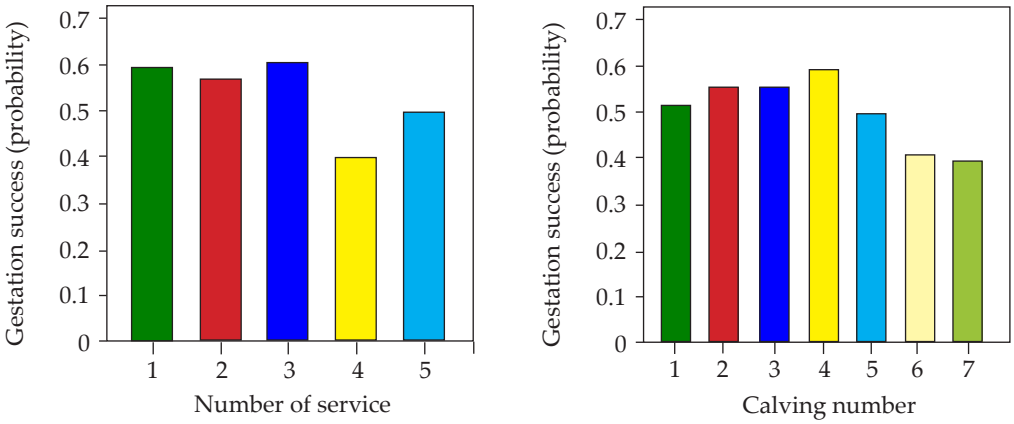

Figure 2. Global gestation success probability in multiparous Tropical Milking criollo cows by number of service and by calving number ($p > 0.05$).

Lozano *et al.* (2005) observed 38.2 % of gestation in Holstein cows of first calving and in cows of three calvings or more the gestation decreased to 32.7 %. *Bos taurus* introduced and maintained in unfavorable environments through centuries have adaptive characteristics such as short hair (Baena *et al.*, 2019), low concentrations of triiodothyronine and thyroxine to decrease the production of thyrotropin-releasing hormone, as a metabolic heat reduction mechanism, and increases in heat dissipation through sweat glands and respiratory rate (Sejian *et al.*, 2018). Romosinuano criollo embryos were more tolerant to heat stress than other non-adapted breeds (Hernández-Cerón *et al.*, 2004). TM cows are long-lived; some can even reach up to 20 years (De Alba, 2011), which shows their ability to adapt to a hot tropical climate, making it a useful genotype to face the challenges of animal production in adverse and changing scenarios.

CONCLUSIONS

Tropical Milking cows showed the highest frequency of estrus in the hot humid season, and no season effect on gestation success, evidence of their adaptation and ability to reproduce even under changing and adverse climatic conditions. Nevertheless, the maximum temperature of the seven days before artificial insemination affected the gestation success at the first service after first calving cows and the global gestation success of multiparous cows. Before artificial insemination, the detrimental effects of

maximum temperature were lower in multiparous cows than in the first service after first calving cows.

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