

# Association between *Anaplasma marginale* and semen quality in bulls

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## ABSTRACT

**Objective:** to determine the association between the presence of *Anaplasma marginale* and sperm quality variables, including sperm concentration and percentage of individual motility.

**Design/methodology/approach:** A total of 92 animals from the Costa Chica region of Guerrero state, in Mexico, were evaluated. All animals were over 16 months of age, in good health and classified according to their origin and breed group. Semen samples were collected through an electroejaculation. Sperm concentration (SCo) and percentage of individual motility (%IM) were measured. Additionally, blood samples were collected via coccygeal vein puncture. The diagnosis of *A. marginale* was made by determination of antibodies. To analyze the association between *A. marginale* and other risk factors, a logistic model was used, with %IM and SCo as the dependent variables.

**Results:** More than 60% of the animals evaluated were from the municipality of Ometepec. Zebu-type cattle predominated, 75% of the sample was included in this category, followed by crossbred animals (Zebu×European). The incidence of *A. marginale* reached 50%. Logistic regression analysis indicated that origin and racial group are risk factors ( $p < 0.05$ ), whereas *A. marginale* is not a determinant ( $p > 0.05$ ) of seminal quality. However, its presence reduces the possibility of obtaining a sperm concentration and motility percentage suitable for reproductive function.

**Limitations on study/implications:** With Zebu×European crossbred animals, the possibility of males presenting better seminal characteristics increases.

**Findings/conclusions:** *A. marginale* is not a determining factor that modifies seminal characteristics in bulls.

**Keywords:** anaplasmosis, motility, sperm quality, ticks, tropics.

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## INTRODUCTION

In the selection of breeding males, it is essential to evaluate semen characteristics alongside phenotypic traits such as live weight or breed type (Butler *et al.*, 2020). According



to Torrado *et al.* (2016), the reproductive fitness of sires should be assessed when they are acquired, before the mating season begins, or during the breeding period if there is suspicion of reproductive failure attributable to the sire. However, this practice is not widely adopted and is mainly applied to sires intended for semen collection and cryopreservation for straw production, as well as to bulls selected for sale as breeders (Páez-Barón & Corredor-Camargo, 2014). As a result, at least 20% of bulls in non-selected populations are classified as subfertile and are unable to provide acceptable service or exhibit poor semen quality (Torrado *et al.*, 2016).

Regardless of whether the sire is used for semen collection for cryopreservation or under a natural mating system, the evaluation must include three aspects: a clinical examination, libido assessment, and semen quality evaluation (Páez-Barón & Corredor-Camargo, 2014). Specifically, in the case of semen quality, the assessment should consider factors such as color, volume, motility, morphology, viability, and sperm concentration. However, according to Boggio (2007), it is also important to conduct blood sampling to rule out the presence of infectious diseases that may affect reproduction, such as bovine viral diarrhea, brucellosis, infectious bovine rhinotracheitis, leptospirosis, neosporosis, among others.

The tropical region, which includes the Costa Chica region of the state of Guerrero, is known for its livestock activity under dual-purpose systems (meat and milk production). However, this can be affected by nutritional factors, viral agents, parasitic, and infectious agents (Avilés-Ruíz, 2022), which negatively impact reproductive factors, leading to economic losses for producers. This region is representative of tropical and subtropical areas where the presence of *Anaplasma marginale* causes economic losses (Olvera, 2021). *A. marginale* is a type of Rickettsia that parasitizes mature erythrocytes in cattle, causing hemolytic anemia, weight loss, reduced milk production, abortions, and even death (Corona *et al.*, 2014; Cora-Ibarra *et al.*, 2021). Although its effect on semen is not clear, it is believed to reduce semen quality by decreasing motility and sperm concentration. The objective was to determine the association between the presence of *A. marginale* and the percentage of individual motility and sperm concentration in male cattle from the Costa Chica region, under the hypothesis that animals that test positive for *Anaplasma* spp. will have lower sperm concentration and motility compared to negative animals.

## MATERIALS AND METHODS

### Description of the Study Area

This study was conducted in the municipalities of Cuajinicuilapa (16° 27' 60" N, 98° 24' 60" E) and Ometepec (16° 40' 57" N, 98° 24' 43" E), both located in the Costa Chica region of the state of Guerrero, Mexico. Both municipalities are situated between 0 and 50 meters above sea level, with a predominantly warm subhumid climate and summer rainfall (1,200 mm of annual average precipitation). Average temperatures range from 22 to 28 °C.

### Animals

A total of 92 male cattle, either bulls or potential bulls, all older than 16 months, without defects or pathologies in the reproductive organs, and in good general health, were evaluated. All animals were maintained under a semi-intensive grazing system with access

to mineral salts and water *ad libitum*. At night, the animals were housed in individual pens with access to clean, fresh water. Prior to the start of the study, the animals were dewormed and vaccinated according to regional recommendations.

The animals were classified according to their genetic group as zebu, european, and crossbred animals (Zebu×European) based on their phenotype and information provided by the bull owner. For their evaluation, all animals were placed in a handling chute, without sedation, minimizing any source of stress (Grandin, 2019). The animal management practices and the study design were approved by the Institutional Bioethics Committee of the Universidad Autónoma de Guerrero.

### **Semen Evaluation**

Semen samples were collected using an electroejaculator (Standar Precision, USA). Spermatozoa were observed under a microscope (i4 Infinity, LW Scientific, USA). The percentage of individual motility (%IM) was evaluated using the technique and parameters established by Koziol & Armstrong (2018), and classified as suitable (>60% IM) or unsuitable (<60% IM). Sperm concentration (SCo, millions of spermatozoa mL<sup>-1</sup>) was evaluated by counting in a Neubauer Bright line chamber following the methodology of the World Health Organization (2021), and categorized as suitable (>250 million spermatozoa mL<sup>-1</sup>) or unsuitable (<250 million spermatozoa mL<sup>-1</sup>, Koziol & Armstrong, 2018).

### **Determination of *Anaplasma marginale***

In the evaluated cattle, a blood sample was taken by puncturing the coccygeal vein using a polypropylene SARSTEDT tube with ethylenediaminetetraacetic acid (EDTA) as an anticoagulant (Monovette 4.9). The blood sample was homogenized with slow movements and allowed to rest for 15 minutes at room temperature. It was then stored at 5 °C until processing in the laboratory.

The diagnosis of *A. marginale* was performed through antibody determination. The plate was sensitized with Ag-2012 at 1.07 µg/200 µl of protein/well, and incubated overnight at 4 °C. Afterward, the plates were washed three times with PBS pH 7.2-Tween-20 at 0.05% (PBS-T20) and blocked with 5% skim milk in PBS-T20 pH 7.2 for 30 minutes. After three washes with PBS-T20, the control serum samples, diluted 1:100 in PBS-T20, were run in duplicate or triplicate. The plates were incubated for 1 hour at 37 °C and washed three times with PBS-T20. Rabbit anti-bovine IgG conjugated (Salinas *et al.*, 2022) with alkaline phosphatase (Jackson ImmunoResearch Laboratories, Lot: 112108), diluted 1:10,000 in PBS-T20, was added to each well. The plates were incubated for 60 minutes at 37 °C and washed as described. Finally, p-nitrophenylphosphate at 0.075% in Tris buffer pH 9.5 was added and incubated for 30 minutes at 37 °C.

The plates were read at 405 nm using a microplate absorbance reader (BioRad, iMark™), and optical density (OD) values were recorded. Two wells containing all components except the serum were used as blanks. The average absorbance of these blank wells was subtracted from the absorbance values of all other wells on the plate. The mean and standard deviation of the replicas and the positive and negative serum controls were also calculated.

### Statistical analysis

To study the association between *A. marginale* and other risk factors (origin and racial group), the data were analyzed using logistic regression (Hosmer *et al.*, 2013). The dependent variables (SCo and %IM) were analyzed using a binary logistic model, as the responses were considered dichotomous (Y=0, unsuitable; 1, suitable), using the SPSS statistical software (IBM<sup>®</sup>, 2023).

The model used to analyze the data can be represented as:

$$Y_{ijkl} \sim \text{binary dichotomus outcome } (P_{ijkl})$$

$$\text{Logit}(P_{ijkl}) = \beta_0 + \beta_1 \text{origin}_j + \beta_2 \text{group}_k + \beta_3 \text{presence of anaplasma}_l + e_{ijkl}$$

Where:  $\text{Logit}(P_{ijkl})$  = Probability that the *i*-th animal will present a suitable SCo or %IM, given that it is from the *j*-th municipality of origin, belongs to the *k*-th racial group, and shows the *l*-th presence of *A. marginale*;  $\beta_0$  = Independent term;  $\beta_1 \text{origin}_j$  = Effect of the *j*-th municipality of origin (1=Cuajinicuilapa, 2=Ometepec);  $\beta_2 \text{group}_k$  = Effect of the *k*-th racial group of the individual (1=zebu, 2=european, 3=crossbreed);  $\beta_3 \text{presence of anaplasma}_l$  = Effect of the *l*-th presence of *A. marginale* (0=healthy individual, 1=individual with *A. marginale*);  $e_{ijkl}$  = Residual random effect.

### RESULTS AND DISCUSSION

Table 1 shows the relative frequency of the observed values for the evaluated variables. Most of the sampled bulls originated from the municipality of Ometepec. Regarding the racial group, 75% of the sampled animals were zebu, classified by their phenotypic appearance and the information provided by the owners. Due to the tropical-subhumid and monsoonal climatic conditions (Köppen classification Aw) (INEGI, 2020), zebu animals are preferred by producers because of their adaptive characteristics to the adverse conditions of temperature, humidity, and presence of parasites (Rubio *et al.*, 2021).

The incidence of *A. marginale* was 50%, lower than the 67.1% reported by Mattos-Ferreira *et al.* (2022) in Mexico; however, it remains one of the highest in Latin America, comparable to Ecuador, where Muñoz-Guarniz *et al.* (2017) reported an incidence

**Table 1.** Frequency of independent variables related to seminal characteristics in the Costa Chica region of the state of Guerrero.

Variable	Nivel	(n) %
Origin	Cuajinicuilapa	31 (0.34)
	Ometepec	61 (0.66)
Racial Group	Cebuino	69 (0.75)
	Europeo	6 (0.07)
	Cruzas	17 (0.18)
<i>Anaplasma marginale</i>	Negativo	46 (0.50)
	Positivo	46 (0.50)

of *A. marginale* of 49.5%. The high incidence in both countries may be related to the abundance and poor control of its transmitting agent (Mattos-Ferreira *et al.*, 2022).

The results of the logistic model for SCo and %IM are presented in Tables 2 and 3. The risk factors that might influence the studied variables are more closely related to the municipality of origin and the racial group of the individuals ( $p < 0.05$ ), whereas the presence of *A. marginale* was not a significant risk factor for SCo ( $p = 0.324$ ) or %IM ( $p = 0.384$ ). The seminal quality of the bulls can be affected by multiple causes, both infectious and non-infectious, as well as various environmental factors, breed, age, nutritional factors, stress, and collection system, among others (Bhave *et al.*, 2020, Butler *et al.*, 2020).

There is a negative association between the municipality of origin of the animals and the values of SCo and %IM considering the values of the parameters  $\beta_i$  ( $-1.855$  to  $-1.940$ ) (Tables 2 and 3). The variable *municipality of origin* had OR values  $< 1$ , suggesting that the environmental differences between municipalities do not result in a decrease in seminal characteristics, indicating that the municipalities of origin share similar climatological factors (humidity, rainfall, and temperature), as well as management within the production units. On the other hand, a greater availability of sampled animals in Ometepec may be related to the sampling period, which may have coincided with the time of greatest reproductive activity of the bulls, reflecting in the quality of semen samples from recently worked bulls or those with little rest.

In the case of the racial group, the crossbreed between zebu and european cattle is positively associated with SCo and %IM ( $\beta_i = 1.511$  to  $1.903$ ), showing 4.53 to 6.70 (OR) times higher chances of achieving better SCo and %IM characteristics compared to European-type animals (OR  $< 1$  and  $p > 0.183$ ). While the productive traits of european-type animals are desirable in tropical regions, the use of crossbred animals (Zebu  $\times$  European) can be an option to increase reproductive efficiency. This alternative reduces the negative effect that high temperatures have on european animals, as developing above their thermoneutral zone can lead to alterations in metabolic activity, general health status, and hormonal concentration, ultimately resulting in reproductive deterioration (Façanha *et al.* 2019). Under tropical conditions, bulls of european origin suffer from heat stress, which may be related to thermoregulation failures and cause damage to testicular tissue (Sabés-Alsina *et al.* 2019). This can contribute to the production of heat shock proteins (Rahman *et al.* 2018), affecting spermatogenesis and leading to lower semen quality. The association between heat stress and reduced sperm viability is explained by the production of reactive oxygen species (ROS) in spermatozoa, which can lead to oxidative stress and DNA damage, affecting viability and fertilization capacity. According to studies involving metabolomics and metagenomics, alterations in taurine metabolism reduce the expression of genes linked to oxidative phosphorylation, decreasing the ability to generate adenosine triphosphate (ATP) and provide energy for sperm motility and functionality (Rao *et al.*, 2022). However, in the present study, crossbred animals (Zebu  $\times$  European) show a higher likelihood of achieving better semen characteristics due to the advantages conferred by crossbreeding—the adaptability of zebu cattle to high temperatures and the greater semen production and quality inherited from european breeds.

Although the presence of *A. marginale* had a negative relationship with SC ( $\beta_i = -0.476$ , OR=0.621, Table 2) and %IM ( $\beta_i = -1.561$ , OR=0.348, Table 3), its effect on these variables was not significant ( $p > 0.324$ ). While there is evidence that *A. marginale* reduces the percentage of normal spermatozoa, sperm motility, and even scrotal circumference in bulls due to the fever and anemia caused by the infection —affecting spermatogenesis both at the cellular and tissue levels (Lovett *et al.*, 2022)— some studies have found no association between the high prevalence of *A. marginale* and the results of breeding soundness examinations (BSE) (Johnson *et al.*, 2018; Jablinski *et al.*, 2023).

### Perspectives

Just as the febrile process affects semen viability (Rao *et al.*, 2022), anemia can cause hypoxia in the reproductive tissue, leading to oxidative stress, which may interfere with spermatogenesis and directly with mature spermatozoa (Esmailnejad *et al.*, 2018). Therefore, studies considering animals of different breeds in different climatic conditions will help clarify the relationship between the prevalence of *A. marginale* and negative results in BSE examinations, taking into account the underlying environmental mechanisms. It should be considered that bulls that survive *A. marginale* infection may return to their reproductive potential (Lovett *et al.*, 2022; Jablinski *et al.*, 2023). It is recommended to perform a new evaluation at least 61 days after the infection has been controlled, as this is the necessary time to complete the spermatogenesis process (Staub & Johnson, 2018). However, depending on the severity of the disease, a longer period may be needed before

**Table 2.** Risk factors associated with sperm concentration in bulls from the Costa Chica, Guerrero, Mexico.

Variable	$\beta$	$SE_{\beta}$	OR	CI (95%)	P
Constant	1.406	0.531	-	-	0.008
Origin	-1.855	0.549	0.157	0.053 - 0.459	<0.001
Racial Group	-	-	-	-	0.024
European	-0.433	0.985	0.649	0.094 - 4.474	0.660
Crossbreed	1.903	0.712	6.703	1.660 - 27.067	0.008
<i>A. marginale</i>	-0.476	0.483	0.621	0.241 - 1.602	0.324

$\beta$ =Estimator coefficient;  $SE_{\beta}$ =Standard error of the estimator; OR=Odds ratio; CI=Confidence interval.

**Table 3.** Risk factors associated with the % of individual motility in bulls from the Costa Chica, the state of Guerrero, Mexico.

Variable	$\beta$	$SE_{\beta}$	OR	CI (95%)	P
Constant	1.561	0.583	-	-	0.007
Origin	-1.940	0.616	0.144	0.043 - 0.481	0.002
Racial Group	-	-	-	-	0.043
European	-1.331	1.001	0.264	0.037 - 1.879	0.183
Crossbreed	1.511	0.721	4.532	1.104 - 18.614	0.036
<i>A. marginale</i>	-1.561	0.491	0.348	0.606 - 4.150	0.384

$\beta$ =Estimator coefficient;  $SE_{\beta}$ =Standard error of the estimator; OR=Odds ratio; CI=Confidence interval.

performing the semen quality test. Additionally, it should be assessed whether there is damage to the testes and if sperm production is economically viable to decide whether the bull should be slaughtered or retained as a breeder in the production unit. Finally, bulls that survive remain asymptomatic carriers, so it must be considered that they may be a source of infection for other animals in the herd.

## CONCLUSIONS

Under the conditions of the present study, the presence of *A. marginale* is not a risk factor that could modify the seminal characteristics of the cattle; however, it does decrease the likelihood of obtaining semen samples with adequate sperm concentration and individual motility percentage for bulls to develop their reproductive function properly.

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