# Cardiac, ophthalmic and electrolytic parameters in pregnant american bully bitches

## Parâmetros cardíacos, oftalmológicos e eletrolíticos em cadelas american bully gestantes

## Parámetros cardíacos, oftalmológicos y electrolíticos en perras american bully preñadas

Received: 05/18/2022 | Reviewed: 05/28/2022 | Accept: 05/29/2022 | Published: 06/05/2022

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**Abstract**
The study aimed to assess the cardiovascular, ophthalmic, and electrolytic parameters of pregnant bitches. Ten American Bully bitches, with an average age of 22 ± 3.4 months, were included in this study. The evaluated parameters including the systolic blood pressure, electrocardiogram, Schirmer test 1, corneal sensitivity, intraocular pressure, serum potassium, sodium, ionized calcium, $Ca^{2+}$, and phosphorus. The evaluations were conducted ~3 days after the artificial insemination (day 0: D0) and during the gestational period (D30, D40, and D60). Despite the systolic blood pressure increased ($P < 0.01$), values were within the normal range in D40 to D60. The electrocardiogram indicated an increase in heart rate at D60 ($P < 0.001$; within normal values); and elevation of the P-wave and QRS complex durations in all evaluation periods, in comparison to the expected values for the species. Regarding electrolyte analysis, lower $Na^+$ was identified at D30 ($P < 0.009$) and D40 ($P < 0.01$). At the same time, the ophthalmic evaluation showed that tear production decreased in D60 compared to the other periods ($P < 0.01$) but remained within the normal range for the species. In addition, important electrolyte and electrocardiographic correlations were observed at D30 and D60. Our study demonstrated that, despite changes in electrocardiographic, blood pressure, electrolytic, and ophthalmic evaluation, they did not have any impact on physiological state, indicating only an adaptive condition to gestation. However, it is noteworthy that even with such results, the possibility of changes during this period is not ruled out, justifying the need for monitoring pregnant bitches throughout the whole gestational period.
**Keywords:** Pregnant bitches; Electrolytes; Physiological parameters; Heart; American Bully.

**Resumo**
O objetivo deste estudo foi avaliar os parâmetros cardiovasculares, oftálmicos e eletrolíticos de cadelas prenhes. Dez cadelas American Bully, com idade média de 22 ± 3,4 meses, foram utillizadas. Os parâmetros avaliados incluíram pressão arterial sistólica, eletrocardiograma, teste de Schirmer 1, sensibilidade corneana, pressão intraocular, potássio sérico, sódio, cálcio ionizado, Ca2+ e fósforo. As avaliações foram realizadas ~3 dias após a inseminação artificial (dia 0: D0) e durante o período gestacional (D30, D40 e D60). Apesar do aumento da pressão arterial sistólica (P < 0,01), os valores estavam dentro da normalidade no D40 ao D60. O eletrocardiograma indicou aumento da frequência cardíaca no D60 (P < 0,001; dentro dos valores normais); e elevação das durações da onda P e do complexo QRS em todos os períodos de avaliação, em comparação com os valores esperados para a espécie. Em relação à análise eletrolítica, menor Na+ foi identificado em D30 (P < 0,009) e D40 (P < 0,01). Ao mesmo tempo, a avaliação oftalmológica mostrou que a produção lacrimal diminuiu no D60 em relação aos demais períodos (P < 0,01), mas permaneceu dentro da normalidade para a espécie. Além disso, importantes correlações eletrolíticas e eletrocardiográficas foram observadas em D30 e D60. Nosso estudo demonstrou que, apesar das alterações na avaliação eletrocardiográfica, pressão arterial, eletrolítica e oftalmológica, elas não tiveram impacto no estado fisiológico, indicando apenas uma condição adaptativa à gestação. No entanto, vale ressaltar que mesmo com tais resultados, não se descarta a possibilidade de alterações neste período, justificando a necessidade de acompanhamento das cadelas gestantes durante todo o período gestacional.
**Palavras-chave:** Cadelas prenhez; Eletrólitos; Parâmetros fisiológicos; Coração; American Bully.

**Resumen**
El objetivo de este estudio fue evaluar los parámetros cardiovasculares, oftálmicos y electrolíticos de perras gestantes. Se utilizaron diez perras American Bully, con una edad media de 22 ± 3,4 meses. Los parámetros evaluados incluyeron presión arterial sistólica, electrocardiograma, prueba de Schirmer 1, sensibilidad corneal, presión intraocular, potasio sérico, sodio, calcio ionizado, Ca2+ y fósforo. Las evaluaciones se realizaron ~3 días después de la inseminación artificial (día 0: D0) y durante el período gestacional (D30, D40 y D60). A pesar del aumento de la presión arterial sistólica (P < 0,01), los valores estuvieron dentro del rango normal de D40 a D60. El electrocardiograma indicó un aumento de la frecuencia cardíaca en D60 (P < 0,001; dentro de los valores normales); y elevación de la duración de la onda P y del complejo QRS en todos los períodos de evaluación, en comparación con los valores esperados para la especie. En cuanto al análisis de electrolitos, se identificó menor Na+ en D30 (P < 0,009) y D40 (P < 0,01). Al mismo tiempo, la evaluación oftalmológica mostró que la producción de lágrimas disminuyó en D60 en relación a los demás períodos (P < 0.01), pero se mantuvo dentro del rango normal para la especie. Además, se observaron importantes correlaciones de electrolitos y electrocardiográficas en D30 y D60. Nuestro estudio mostró que, a pesar de los cambios en la evaluación electrocardiográfica, de presión arterial, electrolítica y oftalmológica, no tuvieron impacto en el estado fisiológico, indicando solo una condición adaptativa durante el embarazo. Sin embargo, cabe mencionar que aún con estos resultados, no se descarta la posibilidad de cambios en este período, justificando la necesidad de monitorear a las perras gestantes durante todo el período de gestación
**Palabras clave:** Perras preñadas; Electrolitos; Parámetros fisiológicos; Corazones; American Bully.

## 1. Introduction

Hemodynamic changes during pregnancy are complex in all mammals once is required increased blood supply by the uterus, mammary glands, and fetus, associated with the circulatory demand to other organs (Souza et al., 2017). The anatomical and physiological changes in the pregnancy result from hormonal secretion and body and behavioral alterations. Thus, it is essential to recognize and monitor these changes to preserve maternal and fetal health and the survival of the offspring (Simões et al., 2016).

Cardiovascular changes such as increases in heart rate (HR), cardiac output (CO), circulating blood flow, and ventricular remodeling, decreased peripheral vascular resistance (PVR) and variation in systemic blood pressure (SBP) are changes described during pregnancy in bitches and women (Abbott, 2010, Baumert, et al., 2010, Blanco, et al., 2012a). The changes play an essential role in maintaining uterine perfusion and fetal development (Almeida, et al., 2017). Other alterations in circulating hormones, autonomic tone, and electrolyte levels (sodium and potassium) may also contribute to cardiovascular changes (Blanco, et al., 2011).

There is only scarce data about cardiac electrophysiology during pregnancy in bitches. However, the pregnancy causes or exacerbates pre-existing arrhythmias, such as atrioventricular blocks, extrasystoles, and ventricular tachyarrhythmias. Several factors may be involved in arrhythmogenesis, including increased hemodynamic stress on the cardiovascular system

(Baumert, et al., 2010, Blanco, et al., 2012b, Batista, et al., 2017).

In pregnant women, eye changes are related to hormonal influence since there are androgen, estrogen, and progesterone receptors in the eye tissues, which interfere with visual quality. These changes could bring out pre-existing eye diseases or induce other eye diseases (Gupta, et al., 2005, Atas, et al., 2014). Decreased intraocular pressure, corneal sensitivity, increased corneal thickness and curvature, and variations in tear production are reported in women from the third gestational trimester (Gouveia, et al., 2009, Atas, et al., 2014, Naderan, 2018). In our knowledge, there are no reports on ophthalmologic changes in pregnant bitches.

Electrolyte concentrations change during pregnancy in women and bitches, especially serum levels of total (Ca2+) and ionized (iCa) calcium, which influence fetal skeletal development and delivery (Black, 2001, Rodrigues et al., 2017). In bitches, there is no description of the occurrence of interference from electrolytes, such as phosphorus (P+), sodium (Na+), and potassium (K+), on pregnancy, as described in women (Ivanova & Georgiev, 2018).

The American Bully breed was recognized in 2013 by the United Kennel Club and is considered a crossbreed of the American Pit Bull Terrier, American Bulldog, English Bulldog, and Old English Bulldog. Despite its powerful and robust appearance, its demeanor is gentle and friendly (UKC, 2013). Considering the interest in breeding, the American Bully has grown over the years, and the potential for diseases, especially related to pregnancy and parturition since this breed is associated with the high incidence of dystocia (Ackerman, 2021). Then, this study aimed to evaluate and correlate cardiovascular parameters such as electrocardiography and systemic blood pressure, ophthalmic parameters such as tear production, intraocular pressure, and corneal sensitivity, and electrolyte profile (Ca2+, iCa, Na+, K+, and P+), before and during pregnancy in American Bully bitches.

## 2. Methodology

***Ethical aspects***

The study was entirely conducted following the guidelines established by the National Council for the Control of Animal Experiments, approved by the Institutional Ethics Committee on Animal Use protocol number 7858060818.

***Animals, handling, and data collected***

Ten (n = 10) healthy bitches with 22 ± 3.4 months of age, American Bully breed, classified into the pocket (n = 7) and exotic (n = 3), were included in this study. Inclusion criteria were set as animals that had vital parameters (heart rate, respiratory rate, rectal body temperature, and mucous membrane color) and hematological, cardiovascular, and ophthalmological evaluations within the normal range for the species (Feitosa, 2014). All dogs were housed in ventilated environments, with an average 2 x 3 meters, were fed with super-premium dry food (500 g daily), and received water ad libitum. The samples and data were collected ~3 days (D0) after the last artificial insemination, D30, D40, and D60 of gestation confirmed by abdominal ultrasonography. At each period, cardiovascular, ophthalmic, and electrolyte parameters were evaluated. The same technician performed ultrasonography examinations, and the bitches were not subjected to sedation and/or general anesthesia during data collection.

***Cardiovascular data***

The systolic blood pressure was assessed by the non-invasive device (Veterinary Vascular Doppler, DV-610VD, Medmega) connected to an aneroid sphygmomanometer (Premium, NacionalVet). The bitches were kept in left lateral recumbency, with the cuff positioned in the middle third of the radius and right ulna. The average of three measurements was used (Brown & Henik, 2002).

The electrocardiogram was performed using the computerized method (ECGPC– TEB), directly connected to a microcomputer and TEB software, simultaneously recording tracings in leads I, II, III, aVR, aVL, aVF, at a speed of 50mm/second with vertical calibration of 1 cm/mV (Tilley, 1992, Wolf et al., 2000), for a predetermined time of 2 minutes. The dogs were positioned in the right lateral recumbency, followed by the placement of electrodes, according to Tilley (1992). After the electrocardiographic recordings, the interpretation of the results was performed in the lead II, obtaining the following parameters: cardiac rhythm, heart rate (beats per minute, bpm), the electrical axis in the frontal plane (degrees), measurement of waves and intervals (P wave: duration [seconds] and amplitude [mV]; R wave: amplitude [mV]; QRS complex duration [seconds]; QT interval [seconds]; T wave amplitude [mV] and ST-segment).

***Ophthalmic parameters***

The ophthalmic parameters were measured bilaterally, including tear production by Schirmer test 1 (Ophthalmos®, Rohto Pharmaceutical Group) without topical anesthesia, and evaluated for one minute.

Corneal sensitivity was assessed with an esthesiometer (Cochet-Bonnet®, Luneau Technology) positioned in the center of the cornea, making enough pressure up to the nylon filament suffered a slight arching and manifested closing of the eyelids. The length of the nylon thread was sequentially reduced every 0.5 cm, considering the length observed in the last blink reflex. According to the device-specific table, the value obtained in cm was converted to g/mm2.

Intraocular pressure was obtained by applanation tonometry (Tono-Pen Avia®, Reichert Inc.). The desensitization of the corneal surface by instillation of 0.5% proxymetacaine-based eye drops (Anestalcon®, Alcon) was performed, and intraocular pressure was measured by gently touching the center of the cornea with the tonometer probe, which was previously calibrated, until obtaining the record by the device. The average of three reading records provided by the device was considered.

***Electrolytic analysis***

The jugular vein was punctured with a 1 mL syringe and 25x6 needle containing 0.1 mL of heparin (Hemofol®, Cristália) and the sample was used for blood gas analysis (Cobas B 121®, Roche) within a maximum period of 1 hour. The potassium ($K^+$ - mmol/L), sodium ($Na^+$ - mmol/L) values, and ionized calcium (ICa – mmol/L) were measured.

A second blood sample was collected from the cephalic vein using a 5 mL syringe and 25 x 7 needle. The sample was centrifuged for 15 minutes at 1500 g and the plasma was used for serum calcium analysis $Ca^{2+}$ (mg/dL), (Ca - Labtest®), and phosphorus ($P^+$ - mg/dL), (P- Labtest®).

***Statistical analysis***

The variables with Gaussian distribution were analyzed by analysis of variance (two-way ANOVA), for fully randomized experiments, with the calculation of F and P-value. The obtained means were compared by Tukey's test, calculating the least significant difference. The ophthalmic conditions were evaluated using item analysis, a multivariate method that measures simultaneous correlations between variables involved in an event. The consistency of the correlations was measured by Cronbach's alpha, with values between 0.60 and 0.90 being considered significant. To assess the correlation between the cardiovascular, ophthalmic and electrolyte variables was used the Pearson test (-1 and +1), which considered the correlation weak ($r = 0.1 - 0.3$), moderate ($r = 0.4$ a $0.6$) strong ($r = 0.7$ to $1.0$) (Dancey & Reidy, 2005). Associations between the quantitative variables and the number of fetuses were established. Results were presented as mean and standard error. For analyses, differences were considered significant when $P < 0.05$ and GraphPad Prism 6 software was used.

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## 3. Results

*Cardiovascular parameters*

Table 1 represents the results obtained for the cardiovascular parameters at different times.

The bitches showed higher systemic blood pressure at D40 (P < 0.0007) and D60 (P < 0.007) compared to D0 but within the normal range for the species. An increase in heart rate within the normal range was observed at D60 (P < 0.001) (Table 1).

The P wave and QRS complex durations at evaluated periods were longer than those expected for the species (Table 1). The electrocardiogram showed that 52% of the bitches had sinus arrhythmia, 42% sinus rhythm, 2.6% sinus block, and 2.6% sinus tachycardia.

**Table 1.** Mean ± standard error of cardiovascular parameters obtained from American Bully bitches at pregnancy D0, D30, D40, and D60.

| Parameter | Period/day | | | |
|---|---|---|---|---|
| | **D0** | **D30** | **D40** | **D60** |
| Systemic blood pressure | 123.0 ± 4.1[a] | 126.0 ± 4.7[ab] | 135.0 ± 5.2[b] | 132.0 ± 6.2[b] |
| Heart rate (bpm) | 110.0 ± 6.9[a] | 110.0 ± 6.2[a] | 120.0 ± 6.2[a] | 130.0 ± 6.5[b] |
| P wave duration (s) | 0.05 ± 2.4 | 0.05 ± 2.6 | 0.06 ± 3.9 | 0.06 ± 2.5 |
| PR interval (s) | 0.10 ± 6.3 | 0.12 ± 4.6 | 0.11 ± 6.1 | 0.12 ± 4.9 |
| QRS duration (s) | 0.08 ± 5.8 | 0.07 ± 4.4 | 0.08 ± 3.2 | 0.09 ± 5.0 |
| QT interval (s) | 0.22 ± 5.8 | 0.21 ± 5.8 | 0.21 ± 7.5 | 0.22 ± 4.5 |
| P wave amplitude (mV) | 0.15 ± 0.01 | 0.13 ± 0.01 | 0.14 ± 0.01 | 0.16 ± 0.01 |
| R amplitude (mV) | 0.86 ± 0.14 | 0.79 ± 0.14 | 0.76 ± 0.12 | 0.78 ± 0.13 |
| T wave amplitude (mV) | 0.26 ±0.04 | 0.30 ± 0.07 | 0.23 ± 0.03 | 0.22 ± 0.03 |
| Segment S-T | 0.08 ± 0.01 | 0.07 ± 0.01 | 0.10 ± 0.01 | 0.20 ± 0.09 |
| Axe (degree) | 44.0 ± 2.9 | 44.0 ± 2.8 | 47.0 ± 4.6 | 47.0 ± 3.5 |

[ab]Different letters in same row indicate significant difference (P < 0.05). Source: Authors.

***Ophthalmic parameters***

There was no statistical difference in the parameters evaluated between right and left eyes, so the sample size of 20 eyes was used. The mean tear production value in D60 was lower than those obtained in the other periods (Table 2; P < 0.01). The corneal sensitivity and intraocular pressure did not change during pregnancy (P > 0.05); however, the intraocular pressure at D60 was 9.53% lower than that observed at D0.

The multivariate analysis (Cronbach's α = 0.62) showed an association between Schirmer test values, intraocular pressure, and corneal sensitivity at D60.

**Table 1.** Mean ± standard errors of Schirmer test, corneal sensitivity test, and intraocular pressure obtained from American bully bitches at D0, D30, D40, and D60 of pregnancy.

| Parameter | Period/day | | | |
|---|---|---|---|---|
| | **D0** | **D30** | **D40** | **D60** |
| Schirmer (mm/min) | 25 ± 0.9[a] | 25 ± 0.9[a] | 25 ± 0.5[a] | 22 ± 0.6[b] |
| Corneal sensitivity (cm) | 3.6 ± 0.6 | 3.8 ± 1.0 | 3.9 ± 0.4 | 3.8 ± 0.5 |
| Intraocular pressure (mm/Hg) | 21 ± 0.4 | 20 ± 0.7 | 20 ± 0.7 | 19 ± 0.5 |

[ab]Different letters in same row indicate significant difference ($P < 0.05$). Source: Authors.

***Electrolyte analysis***

At D30 and D40, the mean for serum Na+ concentration was reduced ($P < 0.009$ and $P < 0.01$, respectively). The other electrolytes were not changed during the evaluation period (Table 3).

**Table 2.** Mean ± standard errors of sodium ($Na^+$), potassium ($K^+$), phosphorus ($P^+$), total calcium ($Ca^{2+}$), and ionized calcium (iCa) serum concentrations from American bully bitches at D0, D30, D40, and D60 of pregnancy.

| Parameter | Period/day | | | |
|---|---|---|---|---|
| | **D0** | **D30** | **D40** | **D60** |
| $Na^+$ | 145.20 ± 0.55[a] | 143.70 ± 0.69[b] | 143.60 ± 0.61[b] | 145.00 ± 0.70[a] |
| $K^+$ | 4.24 ± 0.10 | 4.15 ± 0.01 | 4.19 ± 0.05 | 4.20 ± 0.07 |
| $P^+$ | 5.96 ± 0.87 | 6.50 ± 0.90 | 5.86 ± 1.10 | 5.79 ± 0.85 |
| $Ca^{2+}$ | 10.10 ± 0.35 | 10.00 ± 0.34 | 10.00 ± 0.48 | 10.00 ± 0.7 |
| iCa | 1.27 ± 0.05 | 1.35 ± 0.07 | 1.33 ± 0.05 | 1.37 ± 0.02 |

[ab]Different letters in same row indicate significant difference ($P < 0.05$). Source: Authors.

***Correlations among cardiovascular, electrolyte and ophthalmic data***

Strong negative correlation between QT interval and K+ concentration was observed at D30 ($r = -0.77$, $P < 0.008$). At D60, T wave amplitude was negatively correlated with serum Na+ concentration ($r = -0.75$, $P < 0.03$) and positively with K+ ($r = 0.90$, $P < 0.002$).

Moreover, a negative correlation was observed between Na+ and K+ at D60 ($r = -0.79$, $P < 0.005$). Regarding ophthalmic parameters, there were no correlations. Furthermore, the variables were not influenced by the number of fetuses.

## 4. Discussion

Despite several studies on canine pregnancy, the mechanisms that induce tissue and organic changes resulting from the hormonal, immunological, hemodynamic, and electrolytic variations (Taradaj, et al., 2018) were not completely elucidated. Simões et al., (2016) and Blanco et al., (2012b) studied the cardiovascular system of pregnant bitches during the third phase of gestation and at the prepartum period (both eutocic and dystocic) and described electrocardiographic variations. However, ocular changes were not defined in the pregnant bitches, as reported in pregnant women (Naderan, 2018). Thus, this study is the pioneer in describing cardiovascular and ophthalmic parameters and their correlations with the values of Ca+2, iCa, P+, K+, and Na+ in all gestational stages in American Bully bitches.

In our study, the heart rate increase was evident at D60 of gestation but within the normal range. This finding was different from another study that reported an increased heart rate in bitches with 40 days of pregnancy, while bitches at gestational risk, heart rate was changed at 30 days of gestation (Blanco, et al., 2012b). Moreover, increased heart rate was observed at 5 days before delivery (Abbott, et al., 2010), similar to our results at D60. Despite that, Blanco et al. (2011) reported that increased heart rate between 40 and 60 days of gestation did not cause adverse effects in purebred bitches, unlike the decrease in heart rate results from uterine perfusion alterations, as described in women (Baumert, et al., 2010).

Contrary, other authors did not describe variation in heart rate in bitches studied in several breeds and ages (Almeida, et al., 2017; Souza, et al., 2017), different from our study in that the females were from the same breed and lower age range. The authors still suggested that the findings resulted from the activation of vagal tone, triggering a decrease and/or maintenance of heart rate in response to the increased demand for blood flow so that heart rate was not capable of causing injuries to the cardiovascular system.

The heart rate progressively decreases in the postpartum period and during lactation (Abbott, 2010; Batista, et al., 2017), highlighting the importance of monitoring cardiovascular parameters until the end of lactation, aiming to investigate hemodynamic and cardiovascular regression changes during pregnancy.

The reduction in the systemic blood pressure of pregnant bitches influences peripheral vascular resistance (Aguiar, et al., 2018). In women, there is a compensatory increase of 40% in cardiac output, and in bitches of 23% (Brooks & Keil, 1994, Prestes & Landim-Alvarenga, 2006); circulating hormones decrease the pressure of the vascular system, significantly decreasing vascular resistance and, therefore systemic blood pressure (Almeida, et al., 2017). Although within the normal limits the increase in systemic blood pressure at D40 and D60 observed in our study differs from those reported in women and bitches of other breeds (Almeida, et al., 2017, Aguiar, et al., 2018). In contrast, our findings corroborate with Rosa and Paulino-Junior (2018), who monitored the systemic blood pressure in 25 bitches of different breeds. The results can be explained by breed behavior once close to parturition, the bitches were restless and reluctant to remain in the left lateral recumbency for measurement, justifying the increase in systemic blood pressure at this pregnancy stage. In addition, other studies found a correlation between the mean systemic blood pressure and the number of fetuses; however, regarding these parameters, we did not find a correlation in our study.

The increase in QRS and P wave duration suggests an increase in left ventricular and atrial overload, respectively, already described in pregnant bitches (Kittleson, 1998), due to eccentric cardiac hypertrophy established as a compensatory mechanism for volume overload (Blanco, et al., 2011, Blanco et al., 2012b). In our study, there was an increase in QRS complex and P wave durations from D0 to D60, suggesting that the finding is related to the physical condition of the American bully since the breeds (UKC, 2013) involved in its origin are robust and demand a high energy load and muscle gain. Thus, myocardial adaptations occur to maintain this condition to allow adequate blood flow up to the heart (Levine & Poray-Wybranowska, 2016).

In the late gestation, we expected an increase in the cardiac axis due to the uterine distension that causes compression of adjacent organs and diaphragm, leading to repositioning the thoracic structures (Gowda, et al., 2003, Blanco, et al., 2012b). However, in our study, the frontal plane cardiac axis remained within typical values for the species, disagreeing from reported in previous studies (Blanco, et al., 2012b, Aguiar, et al., 2018).

In the early stages of pregnancy, renal plasma flow is lower to minimize glomerular Na+ loss (Aguiar, et al., 2018). However, the consumption of high nutritional quality diets, such as those offered to bitches in our study, maintained adequate energy balance and Na+ levels throughout pregnancy.

The dietary Na+ and K+ did not influence systemic blood pressure or induced hypertension in bitches, as observed in women (Lane-Cordova, et al., 2017) since the bitches were fed with a balanced diet. Serum blood K+ concentration depends on several homeostatic controls, mainly by the renal system, via aldosterone (Lane-Cordova, et al., 2017). Sex hormones are not considered to affect renal K+ homeostasis, although pregnancy results in ion retention due to the state of relative resistance to aldosterone, and the progesterone antagonizes the mineralocorticoid effects (Wingo & Greenlee, 2011).

A strong negative correlation, observed in the late gestation (D60), between Na+ (increased) and K+ (decreased), which can be explained by the progesterone drop around the D50 of gestation and lower expression of progesterone receptors in the renal collecting duct, reducing K+ levels. Progesterone plays an essential role in K+ control during pregnancy (Veiga, et al., 2009).

The negative correlation between K+ and QT interval corroborates with the findings of another study in pregnant mice, which identified variation in the QT interval throughout the gestation (Eghbali, et al., 2005). On the contrary, Blanco et al. (2012b) reported no change in the QT interval during canine pregnancy and related to maternal heart rate acceleration. According to the authors, this acceleration occurs in association with short QT intervals and can hide changes in the cardiac parameter. This study suggested to correct the heart rate concerning the QT interval since after the correction (corrected QT interval - QTc) the authors found higher value in pregnant bitches than in nonpregnant, indicating that pregnancy alters the time required for ventricular depolarization and repolarization. The increase in QTc was explained by the prolonged action potential, in which K+ has a significant influence (Baumert, et al., 2010)

Regarding electrocardiographic exam, the changes found in our study in pregnant bitches may be associated with electrolyte abnormalities such as hyperkalemia, hyponatremia, hypermagnesemia, and hypocalcemia described in another study (Blanco et al., 2012b). Hyperkalemia induces the myocardium to lose the suitable cardiac depolarization and repolarization (Simões, et al., 2016). Our study observed negative relation between serum K+ and T wave amplitude, although the parameters remained within the normal range for canine species in evaluated periods. Similarly, other reports mentioned increased K+, despite staying within the normal range for dogs, also did not compromise atrial and ventricular cardiac depolarization and repolarization (Aguiar, et al., 2018; Simões, et al., 2016).

Regarding ophthalmic changes during gestation, there is a reduction in tear production in women from the third trimester of pregnancy (Skare, et al., 2012, Duran & Güngör, 2019, Nwachukwu, et al., 2019) that returns to normality gradually in the postpartum period (Skare *et al*. 2012, Duran & Güngör 2019). Our findings are according to those in women since Schirmer values significantly decreased on D60. Furthermore, the meibomian gland function is related to the balance between estrogen and testosterone levels in women (Duarte, et al., 2007, Nwachukwu, et al., 2019). Then, the increase in estrogen and progesterone during pregnancy induces reduced lacrimal and meibomian gland secretion and changes the quality of lipid secretion, increasing the evaporation of tears and contributing to dry eye (Nwachukwu, et al., 2019).

Several studies reported a gradual decrease in intraocular pressure in women, especially in the final third of pregnancy (Saylık & Saylık, 2014), including values below the normal range (Tolunay, et al., 2016). The return to initial values occurs gradually in the puerperium (Tolunay, et al., 2016). This decrease was related to the increased uveoscleral drainage and decreased aqueous humor production, triggered by great estrogen (Atas et al., 2014) and progesterone (Gouveia, et al., 2009, Atas, et al., 2014, Naderan, 2018). Estrogen induces vasodilation, which decreases blood pressure in women (Omoti et al., 2008). Consequently, there is a decrease in peripheral vascular resistance and episcleral venous pressure during the final third of pregnancy in bitches, justifying the reduction of intraocular pressure (Chawla, et al., 2013, Tolunay, et al., 2016). Another hormone related to reducing the intraocular pressure in women is relaxin, which decreases corneal rigidity and aqueous humor production (Phillips & Gore, 1985, Tolunay, et al., 2016). In bitches, similar changes can be associated with relaxin that starts to be produced between the 3rd or 4th week of pregnancy, peaks 2 to 3 weeks before parturition, and then begins to fall until stable during lactation for 4 to 9 weeks (Steinetz, et al., 1987).

In our study, the American Bully bitches, unlike the reports in pregnant women (Saylık & Saylık, 2014). The intraocular pressure remained unchanged throughout the gestational period. The absence of similar studies makes it difficult to discuss. It is known that brachycephalic dogs can present high intraocular pressure values, as the shallow orbit keeps the eyeball more exposed. By keeping the eyelids open for measurement, the direct pressure on the bulb would increase intraocular pressure (Klein, et al., 2011).

Studies describe decreased corneal sensitivity in women with advanced gestation and an increase in corneal thickness, both returning to normal within six weeks after delivery (Chawla, et al., 2013, Taradaj, et al., 2018). The expression of mRNA of estrogen, progesterone, and androgen receptors was demonstrated in the cornea and other ocular tissues in animals and humans (Wickham, et al., 2000). These findings suggested that the increase in corneal thickness would be related to the peak of estrogen in the final third of pregnancy, implying greater water retention in stromal cells, increased ocular refractive index, culminating in decreased corneal sensitivity (Chawla, et al., 2013, Taradaj, et al., 2018). Contrary, there was no alteration in corneal sensitivity in American Bully pregnant bitches; the lack of similar studies in the same species restricts the establishment of comparisons. Moreover, brachycephalic dogs have decreased corneal sensitivity (Barrett, et al., 1991, Arnold, et al., 2014).

It is noteworthy that hormonal variations (concentrations and peaks) in pregnant women differ from the canine species; therefore, a comparison cannot be established, so additional studies in the canine species are needed to demonstrate and address the adaptations of the canine species canine pregnancy. Although this study is a pioneer in evaluating these parameters in the American Bully breed, there are some limitations, such as the number of bitches studied. Additional reports with a more significant number of bitches could bring new results and/or better standardization of the studied variables for the American Bully breed. Furthermore, monitoring hormone levels, especially estrogen and progesterone; proteomic, biochemical, lipid, and tear osmolarity analysis; corneal thickness and curvature; Doppler echocardiography would better understand the changes that follow pregnancy in bitches, and their repercussions.

## 5. Conclusion

Our study observed that the adaptations noticed during canine pregnancy in electrolyte levels and the cardiovascular system can be evidenced from the second third of pregnancy. Changes in the visual system occur in the final third of gestation in American Bully bitches. However, these changes had no impact on the physiological state of the bitches, probably indicating only an adaptive condition to pregnancy. We emphasize monitoring these variables throughout the gestational period, especially in bitches that already show abnormal values before pregnancy.

### Acknowledgments

To Dr. Maricy Appar\u00edcio for the help with the English language and to Alex Reis from the *Reis Kennel Bulls* kennel (@reiskennelbulls) for consenting to participate in this research.

### Ethics approval and consent to participate

The study was conducted after obtaining approval by the Ethical Commis-sion in the Use of Animals (CEUA) of the University of Franca (number 7858060818). This study was entirely conducted following the guidelines established by the National Council for the Control of Animal Experiments (CONCEA), Brazil.

### Funding

This study was financed in part by the Coordena\u00e7\u00e3o de Aperfei\u00e7oamento de Pessoal de N\u00edvel Superior – Brazil (CAPES) – Finance Code 001”.

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