

Haematocrit changes in healthy periparturient bitches that underwent elective caesarean section

K.G.M. De Cramer ^{a,*}, K.E. Joubert ^b, J.O. Nöthling ^c

^a Rant en Dal Animal Hospital, Mogale City, Gauteng, South Africa

^b Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, Gauteng, South Africa

^c Department of Production Animals, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, Gauteng, South Africa

*Corresponding author. Tel.: þ27 01166031110; fax: þ27 8656351114. E-mail address: kdramer@mweb.co.za (K.G.M. De Cramer).

Abstract

Haematocrits were measured before each of 406 caesarean sections performed on 324 bitches at term and again following crystalloid fluid therapy administered at 35 ml/kg over 1½ to 2 h starting from induction. The mean haematocrit was 44.2% (95% CI 43.8 to 44.6%) before caesarean section and 37.8% (95% CI 37.3 to 38.2%) following caesarean section and fluid therapy, with a mean decrease of 6.4 percentage points (95% CI 6.1 to 6.7%) over all 406 caesarean sections. These results provide the clinician with clear guidelines of the normal expected ranges of haematocrits in bitches before and after caesarean section. Results of this study show that bitches have haematocrits at term that are at the lower end of the normal reference ranges for non-pregnant dogs and that there is no true anaemia of pregnancy. It is therefore suggested that if late term bitches present with anaemia, other causes besides pregnancy should be considered.

Keywords: Cesarean section, hematocrit, dog, blood loss, anemia, blood transfusion trigger

1. Introduction

Poor oxygen delivery may negatively affect bitches undergoing a caesarean section (CS) and their foetuses [1]. Various physiological changes during pregnancy affect oxygen delivery. Oxygen delivery depends on oxygen carrying capacity (haemoglobin), cardiovascular function and respiratory function [1]. As early as 1977, Concannon et al. [2] referred to a physiological normocytic, normochromic anaemia in pregnant bitches. They reported that the increase in body weight of the bitches they observed during pregnancy was accompanied by a decrease in haematocrit and proposed that this may have been due to a large increase in plasma volume.

The haematocrit of normal healthy non-pregnant dogs may lie between 42% and 62% (average 52%) [3] or between 37% and 55% (average 50%) [4]. Four studies have reported a lowering of haematocrit during gestation in bitches: In 1974, Hayashi [5] reported a steady decrease in the haematocrit of 15 bitches that became statistically significant on Day 50 of pregnancy, when it also reached a nadir of 33.7% (SEM 1.8%) from when onwards it increased to 38.7% (SEM 1.8%) on Day 60. Hayashi also reported the mean postpartum haematocrit as 37%. In 1977 Concannon et al. [2] reported that the haematocrit in 12 pregnant bitches was consistently lower than that of 12 non-pregnant bitches from Day 20 after the onset of oestrus onwards and continued to decline to reach a nadir of 30.6% (SD 0.8) by Day 60 to 62. In 1993 Kaneko et al. [6] showed that the haematocrit of 23 beagles decreased from between 40% and 52% before pregnancy to between 28% and 42% at term and that the litter size had an influence on haematocrit [6]. Finally, in 2013, Dimço et al. [7] reported a mean haematocrit of 45.4% (SD 3.6%) in 16 non-pregnant bitches and a mean of 41% (SD 4.9%) in 16 bitches of similar body weight and age in the

last trimester of pregnancy. Due to varying results, litter sizes and the small sample sizes of these studies, no definitive conclusions about haematocrits during pregnancy can be made.

The haematocrit of bitches that had been pregnant increased slowly after parturition but were still slightly lower by 145 days after the onset of oestrus than those of bitches that had had non-pregnant oestrous cycles [2]. There is no absolute decrease in erythrocyte mass, and the haematocrit returns to normal within 8 to 12 wk after parturition as the plasma volume returns to normal [1].

Fluid therapy is recommended as a standard for CS in bitches [8-14]. Fluids are given to correct any fluid and electrolyte deficits, acid-base balances, the hypotensive effects of anaesthesia and maintain cardiac output and uterine blood flow [8]. Fluid rates of 10 to 30 ml/kg/h with additional boluses have been suggested to maintain perfusion [8;10;14]. Fluid therapy has the potential to cause additional change to the haematocrit.

Caesarean sections are associated with additional blood loss from surgery. There is no literature describing the haematocrits of bitches in late pregnancy before and after caesarean sections. This is a stumbling block in the periparturient risk assessment of bitches that delivered by CS. Following CS, bitches and their puppies are generally not kept in veterinary hospitals for long due to the risk of disease exposure and better nursing environments at home. Assessing risk prior to discharge following CS in bitches is particularly important because this may take place as soon as 2 to 3 h after surgery. This study comprises a retrospective analysis of data on haematocrits before and after CSs in healthy bitches undergoing elective CS to assess what changes can be expected.

2. Materials and Methods

The protocol was approved by the Animal Ethics Committee of the Faculty of Veterinary Science, University of Pretoria, (Onderstepoort, South Africa) (protocol number V048-14). All experimental animals were housed and fed commercial dry pellets twice daily and with ad-lib water. This study included 406 CSs in 324 healthy, privately owned bitches presented to a private veterinary clinic for management of parturition during march 2012 to October 2015. Only healthy bitches destined for elective CS were included in the current study. No ovariohysterectomies were done and the placentas were removed with each puppy. A blood smear evaluation was performed before surgery. A clinical examination was performed before and after surgery which included assessment of: skin for turgor, mucous membranes for colour, moistness and capillary refill time, respiratory and heart rates, rectal temperature and habitus. The decision to perform a CS was based upon the first appearance of dilatation of the cervix on vaginoscopy performed every 6 th. The bitches were weighed immediately prior to surgery and anaesthetised using the standard anaesthetic protocol in the practice which included low dose alpha2-adrenergic agonist premedication (Medetomidine 7 µg/kg iv) (Zoetis Animal Health, Sandton, South Africa), propofol (1 to 2 mg/kg iv) (Fresenius Kabi, Midrand, South Africa) as induction agent and sevoflurane (1 to 2%) (Safeline Pharmaceuticals, Northcliff, South Africa) in oxygen for maintenance of anaesthesia. The CS was performed in standard fashion as described by Gilson [15]. The blood required for haematocrit assessment (approximately 1 ml) was collected by jugular venipuncture using a syringe and 23 G needle directly before anaesthetising the bitch for surgery and again 1½ to 2 h following induction for surgery and after the bitch had already received the set fluid volume (Ringer lactate, Fresenius Kabi, South Africa). No blood was collected from indwelling catheters used for fluid and drug administration as this would lead to potential errors in measuring the

haematocrit. The blood was immediately transferred to a heparinised (sodium heparin 80 iu/ml) microhaematocrit capillary tube (Marienfeld laboratory glassware, Germany) (74.5 to 75.5 mm in length and 1.1 to 1.2 mm internal diameter) and centrifuged at 12000 revolutions per min for 10 min producing a relative centrifugal force of 14800 g and the haematocrit expressed as a percentage. This calculation was performed by measuring the red blood cell column in mm and dividing this value by the total length in mm of the blood column (plasma and packed cell column) and multiplying by 100 to obtain a percentage. If the serum appeared with reddish discolouration after centrifugation it was assumed that haemolysis had taken place during the blood collection or centrifugation processes and blood collection was then repeated.

The total volume of fluids administered was 35 ml/kg body weight to each bitch. The fluid was administered over 1½ to 2 h including surgery time, starting at time of induction, using simple fluid administration sets with the fluid rate ranging from 17.5 to 26.25 ml/kg/h. In order to standardise the effect of haemodilution on haematocrit in all the bitches, it was ensured that the bitch got the set fluid volume and approximate fluid rates. This was achieved by calculating the required amount of fluids the bitch should receive and removing it from the fluid bag. For instance, if the dog weighed 20 kg, the required amount is 700 ml and thus 300 ml would be removed from the one litre fluid bag in a sterile fashion. Because infusion pumps were not used, care was taken to ensure that the calculated fluid volume did not infuse in a time shorter than 1½ hour and not longer than 2 hours following induction for anaesthesia. The haematocrit after CS was not collected until the required fluid volume had been infused. For each bitch the following data were recorded: Date of CS, name of owner, name of bitch, breed, haematocrit before CS (Htbefore), haematocrit after CS (Htafter), body weight before CS, total number of

puppies delivered (litter size)—irrespective of whether they were stillborn or delivered alive.

2.1.Data analysis

Linear regression was used to determine the effects of breed and litter size on Htbefore, and of Htbefore on Htafter. Each breed having 11 or more caesarean sections (CSs) in the data set was included in the regression models. These breeds were English bulldogs (n = 119 CSs, labelled “Bulld”), Boerboels (n = 203 CSs, labelled “Boerb”), bull terriers (n = 21 CSs, labelled “Bull t”), German shepherds (n = 11 CSs, labelled “G s d”) and Labradors (n = 11 CSs, labelled “Labr”). For analyses that include breed as independent variable, the English bulldog was used as the baseline category and each other breed was compared thereto. The English bulldog was chosen as the baseline group because the group was large and, globally, English bulldogs often requires CS. (Figure 1)

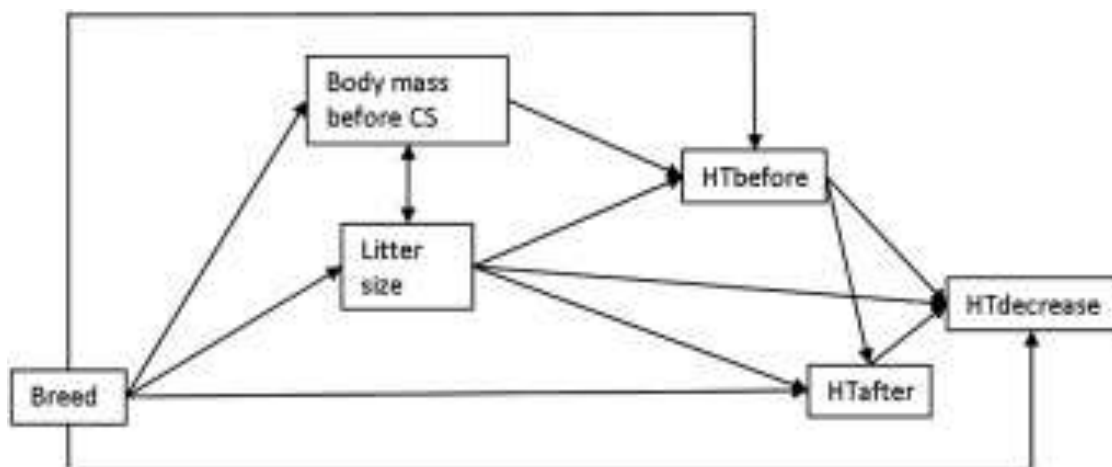


Fig. 1. Path diagram of possible effects of independent variables on factors affecting hematocrit before (Htbefore) and hematocrit after (Htafter) cesarean section in bitches.

Prior inspection revealed that litter size was significantly larger in Boerboels than in bulldogs, bull terriers, German shepherds and Labradors, whereas it was the same among

the latter four breeds. In all models where litter size was the independent variable of interest, breed was included to control its confounding effect on that of litter size [16].

Prior inspection of body weight revealed that it was distinctly bimodal because each Boerboel bitch was heavier than any other bitch included in the regression models. When the regression models were run with and without body weight, we found that the effect of body weight is spurious and it is perfectly included in the effect of breed. Bodyweight was then excluded from all the regression models.

Although not included in the regression analyses, Chows (seven CSs on seven bitches), Staffordshire terriers (seven CSs on five bitches), 16 CSs on a group of 14 bitches belonging to eight breeds varying in size from Toy Pom to Rottweiler that occurred in low frequency in the data set (labelled “rare”) and 11 CSs on a group of 11 bitches of which the breed was not recorded, varying in size from 3 kg to 72 kg (labelled “unknown”), were included in the summary statistics of the raw data.

Sixty three bitches each underwent two or more CSs. Data were considered clustered in these bitches and a mixed-effects linear regression was used to determine the effect of repeated CS on $H_{t\text{before}}$ and $H_{t\text{after}}$, respectively. For this analysis repeated CS was used as a categorical variable with values of 1, 2, 3, 4 or 5 for the first, second, third, fourth and fifth CSs, respectively. The haematocrits of the second to fifth CSs were each compared to those of the first.

For regressions of $H_{t\text{before}}$ and $H_{t\text{after}}$ on independent variables, each CS, including the second to fifth CSs done on 63 bitches, was used as an independent observation.

Huber/White sandwich estimators of the standard errors were used for all regression analyses. No interactions were included in the analyses. Effects were considered

significant if $P < 0.05$. Data analysis was done using Stata 14 (Stata Corp College Station, Texas, USA).

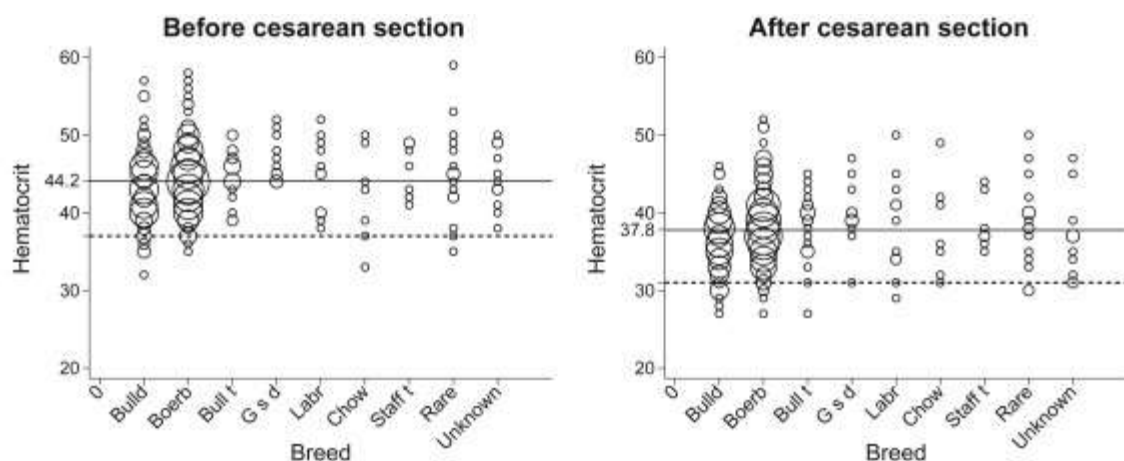
3. Results

Based on the clinical examinations, no bitch showed any illness or clinical evident dehydration before induction of anaesthesia or prior to discharge of the bitch and her puppies.

3.1. Summary of the data

Table 1 summarizes the haematocrits before and after CS, as well as the decrease in haematocrit during the CS in the various breeds.

Figure 2 shows the distribution of the haematocrits before and after CS according to breed and Figure 3 shows the distribution of haematocrits before and after CS according to litter size.



Smallest circles indicate single values, larger circles indicate more frequent occurrences of hematocrits. Solid lines indicate overall means. Dashed lines indicate 5th percentiles (37.0 before CS, 31.0 after CS).

Fig. 2. Distribution of hematocrits before and after 406 elective cesarean sections on healthy bitches according to breed. Boerb, Boerboel; Bulld, Bulldog; Bull t, Bull Terrier; Chow, Chow; G s d, German Shepherd dog; Labr, Labrador; rare, eight breeds occurring in low frequency in the data set; Staff t, Staffordshire Terrier; unknown, 16 bitches for which the breed is unknown.

Table 1. Haematocrit of healthy bitches undergoing elective caesarean section (CS) before and after CS, and the decrease during CS

	No. of CSs	No. of bitches	Before CS		After CS		Decrease during CS	
			Mean	SD	Mean	SD	Mean	SD
Bulldog	119	89	43.15	4.25	36.40	3.65	6.75	2.93
Boerboel	203	162	44.46	3.93	38.33	4.26	6.13	2.78
Bull terrier	21	16	44.90	3.13	38.14	4.41	6.76	3.37
German shepherd	11	11	46.91	2.95	39.82	4.24	7.09	3.56
Labrador	11	9	44.73	4.84	38.36	6.38	6.36	2.46
Chow	7	7	42.14	6.23	38.00	6.38	4.14	2.73
Staffordshire terrier	7	5	45.43	3.41	38.57	3.51	6.86	2.61
Rare ^a	16	14	45.06	6.04	38.63	5.63	6.65	2.40
Unknown ^b	11	11	44.45	3.96	37.69	5.35	7.64	4.11
Over all	406	324	44.17	4.18	37.77	4.35	6.41	2.92

^a This group consists of 8 breeds occurring in low frequency in the data set; they varied in size from Toy Pom to Rottweiler.

^b The breeds of these bitches were unknown or not recorded; they varied in body mass from 3 to 72 kg.

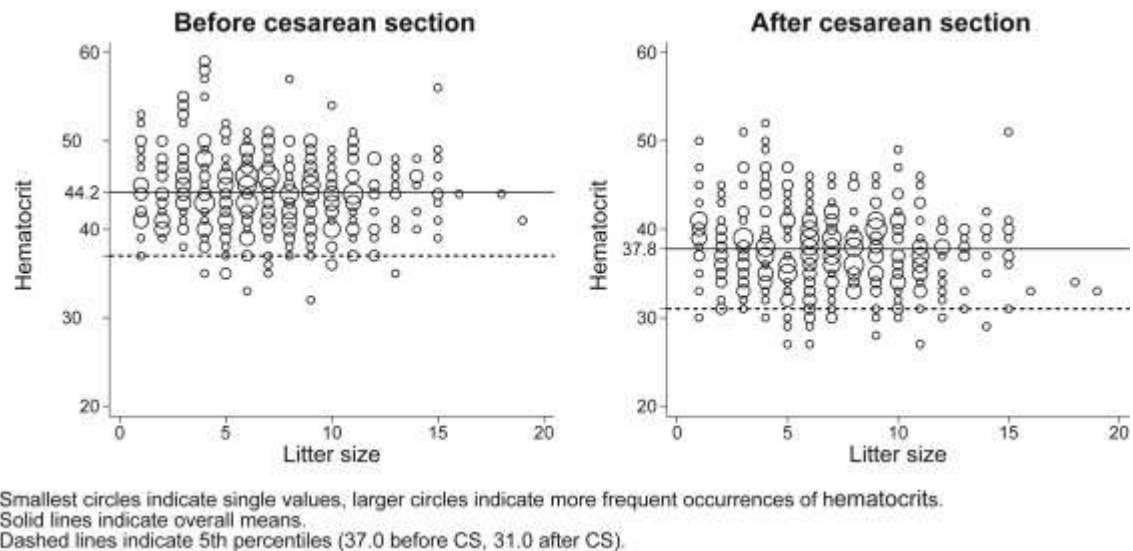


Fig. 3. Distribution of hematocrits before and after 406 elective cesarean sections on healthy bitches according to litter size.

The mean haematocrit before CS was 44.2% (95% CI 43.8 to 44.6%) and the 5th percentile of Htbefore over all 406 CSs 37.0% (95% CI 37 to 38.8%). Ten haematocrits were below 37% prior to CS (mean 34.8%, SD 1.32%, minimum 32%). The mean haematocrit after CS was 37.8% (95% CI 37.3 to 38.2%) and the 5th percentile over all 406 CSs was 31% (95% CI 30.0 to 31.8%). The mean decrease in haematocrit during CS and fluid therapy was 6.4 percentage points (95% CI 6.1 to 6.7 percentage points) over all 406 CSs.

The decrease in haematocrit was quite symmetrically distributed around the median of six percentage points (Figure 4), with 50% of caesarean sections associated with decreases between five and eight percentage points and 95% with decreases between one and 13 percentage points. Five percent of CSs were associated with a decrease in haematocrit of 12 percentage points or more (12 to 18 percentage points).

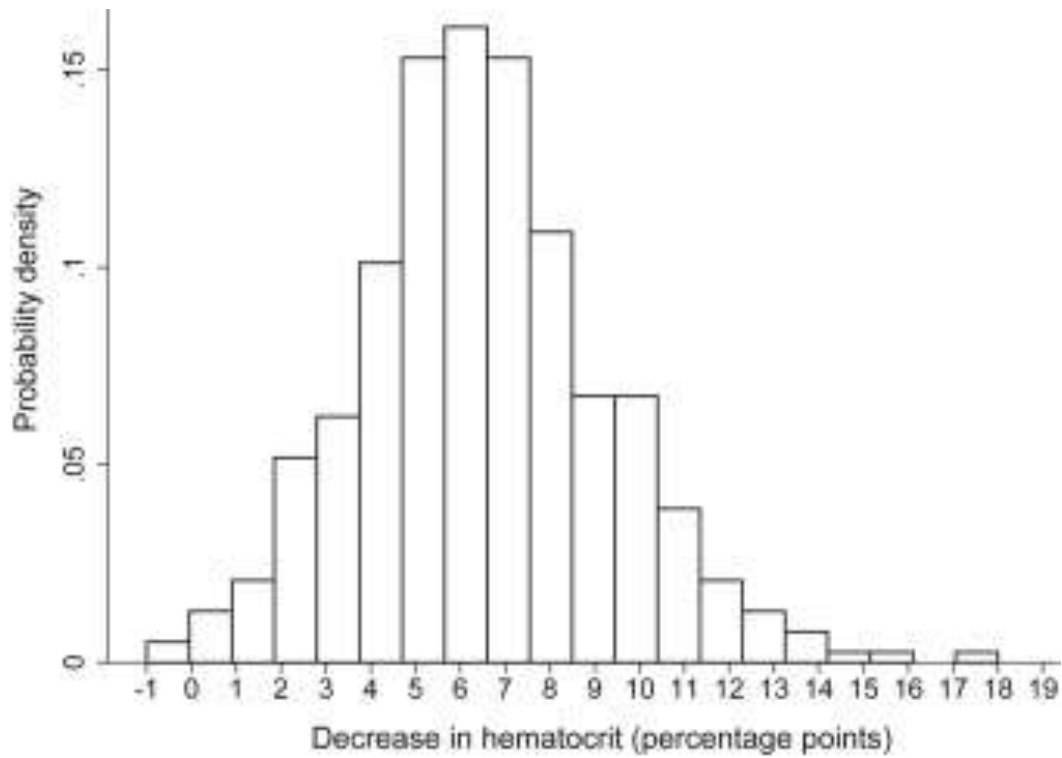


Fig. 4. Histogram of the decrease in hematocrit during 406 cesarean sections in bitches.

Sixty three bitches each had two to five CSs (Table 2). Htbefore and Htafter were independent of the number of CSs a bitch had ($P > 0.1$).

Table 2. For bitches that underwent more than one caesarean section (CS), haematocrit before and after CS was independent of the number of CSs a bitch had ($P > 0.1$)

Caesarean section	n ^a	Haematocrit			P ^d
		Mean	95% CI L ^b	95% CI U ^c	
Haematocrit before CS					
First of 2 or more	63	44.70	43.40	46.00	
Second of 2 or more	63	43.67	42.71	44.62	0.21
Third of 3 or more	13	44.85	42.75	46.94	0.91
Fourth of 4 or more	5	43.40	40.95	45.85	0.36
Fifth	1	44			0.29
Haematocrit after CS					
First of 2 or more	63	38.03	36.72	39.34	
Second of 2 or more	63	37.25	36.24	38.27	0.36
Third of 3 or more	13	39.85	37.77	41.92	0.15
Fourth of 4 or more	5	39.2	36.11	42.29	0.49
Fifth	1	37			0.12

4. ^a Number of caesarean sections in the category

5. ^{b,c} Lower and upper limit of the 95% confidence interval of the mean

6. ^d Within a time relative to CS (before or after), the mean haematocrits of the second to fifth CS were each compared to the mean of the first CS.

6.1. The effects of breed and litter size on Htbefore

Regressing Htbefore on breed shows that breed had a significant unconditional effect on Htbefore ($F(4,360) = 4.81, P < 0.001$). Apart from the G s d, which had a mean Htbefore

that was 3.76 percentage points higher than that of the breed with the lowest Htbefore (Bulld), the difference among breeds were below two percentage points (Table 3).

Table 3. Linear prediction of the effect of breed on haematocrit before caesarean section

Breed	Haematocrit (%)				P ^d
	Mean	95% CI L ^a	95% CI U ^b	Effect size ^c	
Bulldog	43.15	42.38	43.92		
Boerboel	44.46	43.92	45.01	1.31	0.006
Bull terrier	44.90	43.58	46.22	1.75	0.024
German shepherd dog	46.91	45.23	48.59	3.76	0.000
Labrador	44.72	41.97	47.48	1.58	0.279

^a Lower limits of the 95% confidence interval of the mean

^b Upper limits of the 95% confidence interval of the mean

^c Magnitude (percentage points) of the difference between the breed and the baseline breed (Bulldog)

^d Significance of the difference between the breed and the baseline breed (Bulldog)

Figure 5 graphically displays the effect of breed on Htbefore.

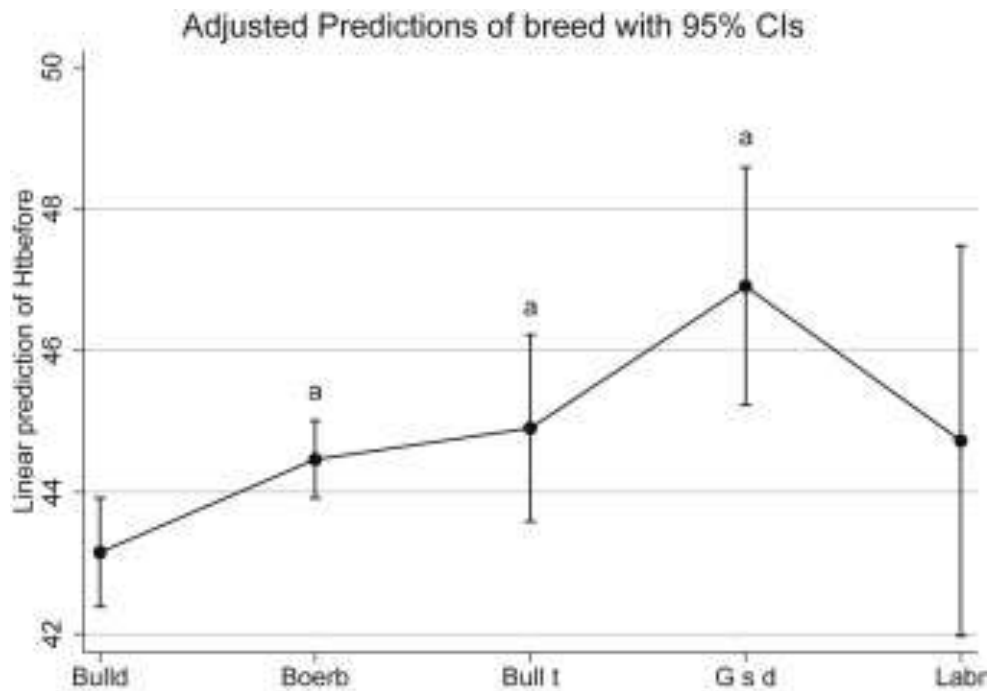


Fig. 5. Unconditional effect of breed on hematocrit before CS (Htbefore). Predicted means marked “a” are significantly higher than that of Bulld ($P < 0.05$). Boerb, Boerboel; Bulld, Bulldog; Bull t, Bull Terrier; G s d, German Shepherd dog; Labr, Labrador.

Figure 6 suggests that, in bulldog-, Boerboel-, German shepherd- and Labrador bitches, there exists a variable negative linear relationship between Htbefore and litter size.

Regression of Htbefore on litter size and breed showed that, controlling for breed, there was a significant ($P = 0.013$) but weak effect of litter size on breed. Htbefore is expected to decrease by 0.15 percentage points (95% CI 0.03 to 0.28 percentage points) for each unit increase in litter size.

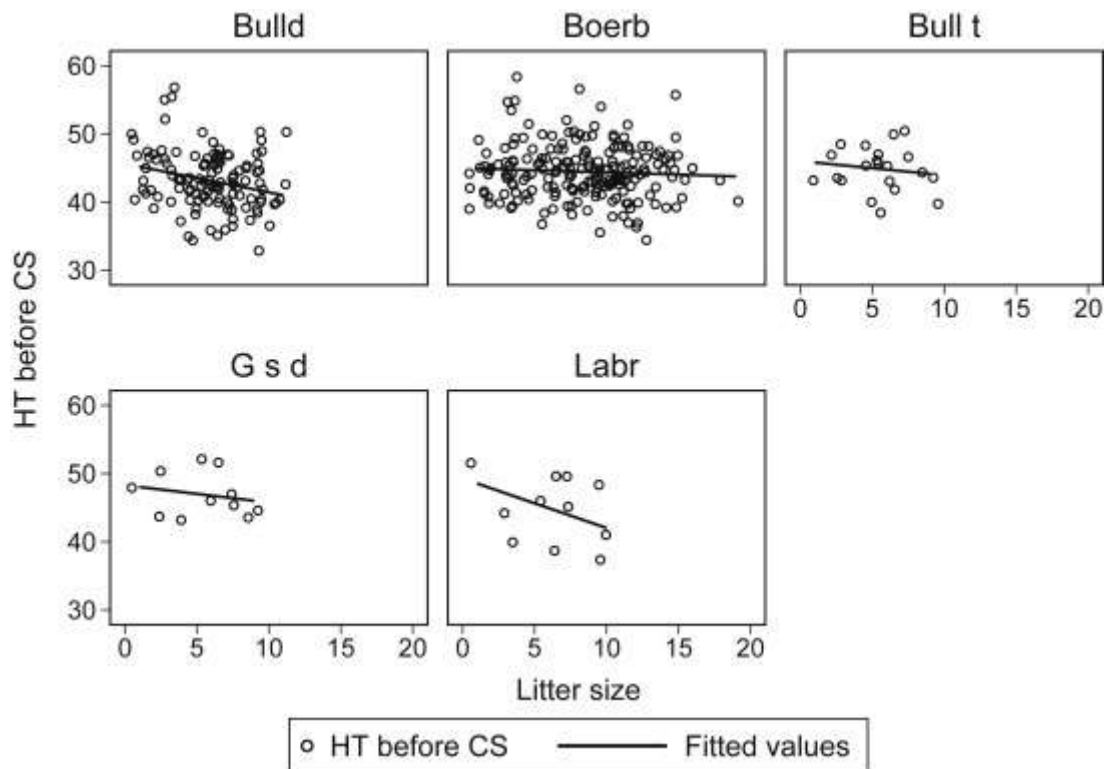


Fig. 6. Hematocrit before cesarean section against litter size, with the best linear prediction in five breeds. Boerb, Boerboel; Bulld, Bulldog; Bull t, Bull Terrier; G s d, German Shepherd dog; Labr, Labrador.

6.2. The effect of Htbefore on Htafter

Figure 7 suggests that Htbefore has a strong positive, linear effect on Htafter in bulldog-, Boerboel-, German shepherd and Labrador bitches. Regressing Htafter on Htbefore with litter size and breed revealed that, controlling for litter size and breed, Htafter increases by 0.77 percentage points (95% CI 0.70 to 0.84 percentage points) for each percentage point increase in Htbefore ($P < 0.001$).

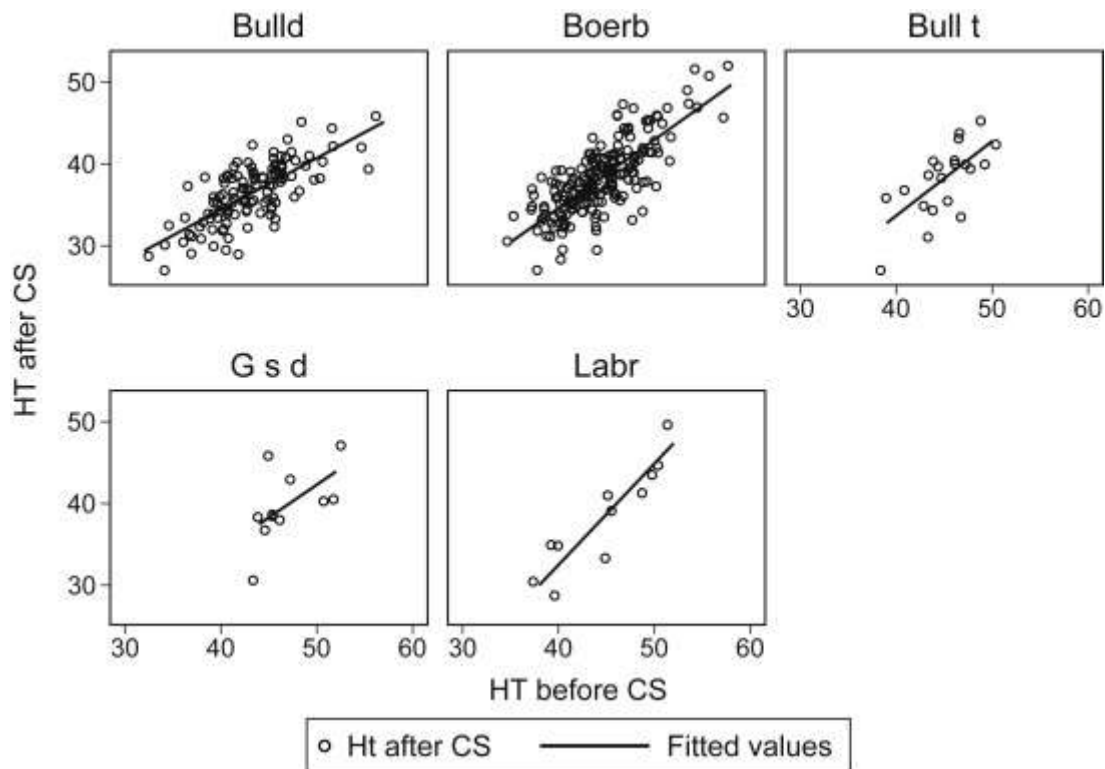


Fig. 7. Hematocrit after caesarean section against hematocrit before caesarean section, with the best linear prediction in five breeds. Boerb, Boerboel; Bulld, Bulldog; Bull t, Bull terrier; G s d, German shepherd dog; Labr, Labrador.

7. Discussion

Anaemia may be expressed as haematocrit but haemoglobin concentration in the blood is the preferred way [17]. Haematocrit was chosen in the current study as it requires no laboratory assistance, is easily available to all veterinary practitioners and is rapidly performed in any private practice. Furthermore in humans, haematocrit change was reported as an objective indicator of blood loss and anaemia [18;19] and is well correlated to blood haemoglobin content [20].

Concannon et al. [2] reported that pregnant bitches develop a physiologic anaemia presumably due to plasma expansion, with mean haematocrits in close-to-term bitches of 30.6 (SEM 0.8%, n = 12). This was later termed anaemia of pregnancy [21]. In contrast, the current study shows that the expected haematocrit of healthy bitches of various breeds

before CS is substantially higher at 44.2% (SD 4.2%), with a 95% confidence interval for the mean of 43.8 to 44.6%. Should the study be repeated, the mean haematocrit is expected with 95% certainty to fall within this narrow confidence interval. The Japanese have reported a preterm haematocrit of 38.7% (SEM 1.8%) in 15 dogs [5]. In contrast, Dimço et al. [7] reported haematocrits of 41% (SD 4.9%, n = 16) and are in closer agreement to the results in the current study. In another study, all haematological parameters and serum biochemical profiles in pregnant bitches remained within normal ranges without adjustment to account for haemodilution and concluded that these parameters did not differ significantly from those found in normal adult dogs [22]. Our results have an important clinical implication as pregnant bitches presenting with haematocrits below the normal reference range should be examined for other concurrent disease that may cause anaemia. This is particularly important if such bitches are close to term, giving natural birth or are to be subjected to CS and further potential blood loss. The reference intervals for normal healthy non pregnant dogs' haematocrits at a laboratory at similar altitude (1500 m above sea-level) to the experimental animals were 37 to 55% (Personal communication, Scheepers E, 2014). In the current study, only 10 of the 406 CSs were associated with a haematocrit before CS below 37%. This may in part be explained because all the bitches in the experimental group were from well-managed breeding colonies where the nutrition and veterinary care was of a high standard.

Kaneko et al. [6] studied the effects of litter size on the extent of anaemia of pregnancy in 23 beagle dogs and observed a correlation between litter size and a decrease in haematocrit, haemoglobin concentration and red blood cell count. They reported a 1.1% decrease in haematocrit for each pup present. The current study shows that, controlling for breed, litter size has a significant but weak effect on Ht_{before}, with Ht_{before} on average decreasing by 0.14 percentage points for each increase of litter size by one pup. Over the

range of litter sizes seen in the current study this means that the mean litter size is expected to decrease by 2.5 percentage points from the smallest litter size of one to the largest of 19. The expected decrease in haematocrit before CS with an increase in litter size found in the current study is seven to eight times smaller than that found by Kaneko et al. [6]. The small sample size in the study by Kaneko et al. [6] may have resulted in a less precise estimation of the relationship between haematocrit and litter size. Although the current study shows that increased litter size results in a slightly lower haematocrit, the difference is of minimal clinical significance.

The current study shows that breed affects the haematocrit of bitches before caesarean section. Controlling for litter size, Boerboel and bull terrier bitches are expected to have 1.7% higher and German shepherd bitches 3.7% higher mean haematocrits before caesarean section than the mean (43.2%) of English bulldog bitches. Breed specific changes in haematological variables have been reported and related to genetic phenotypes [23]. Our data support this contention.

It seems unlikely that the effects of breed and litter size would be sufficient to explain the distinctly lower haematocrits before CS reported in other studies [2;5;6] compared to those reported in the current study.

The current study shows that the haematocrit of a bitch prior to CS is independent of the number of previous CSs that she has had.

The effects of altitude on haematocrit is not expected to have influenced results in the current study. This assumption is based on the finding that although exposure to hypoxia in dogs occurring at moderate to marked high altitude results in measurable cardiovascular changes, including increased heart rate, increased systemic and pulmonary artery pressure,

and changes in systolic and diastolic cardiac function, it does not result in an increase in haematocrit [24].

The haematocrit after caesarean section in this study was decreased by both the surgery and the fluid therapy. Nevertheless, fluid therapy should be considered standard practice during surgery for CS section in the bitch [1]. In the current study, the hydration status of the bitches was assessed using assessment of skin turgor, mucous membranes and capillary refill time. Although imperfect, clinical assessment of hydration by looking for gross abnormalities during a clinical examination appears practical.

During normal parturition in the bitch a small amount of blood loss occurs and this has been shown in one study to reduce the mean haematocrit by 1.7% from 38.7% (SEM 1.8%) to 37.0% (SEM 0.8%) [5]. No study prior to the current one has reported the effects of CS on haematocrit in dogs.

Changes in haematocrit have been used to estimate blood loss during obstetric surgery in women [19] and allowed the prediction of bleeding even with ongoing fluid resuscitation [25]. Stafford et al. [19], used a formula to reliably identify excessive blood loss during caesarean section in women from the haematocrits before and after caesarean section.

Fluid redistribution following acute blood loss may take several hours but may be achieved in one hour when fluids are administered intravenously [25]. If not enough time is allowed for fluid redistribution following acute blood loss, haematocrit is likely to be inaccurate and give false low estimates of blood lost and or false high haematocrits [26]. In the current study 1½ to 2 h was allowed for equilibration but this may not have been long enough. Despite the shortcomings of haematocrit, the American College of Obstetricians and Gynaecologists has used haematocrit in their official definition of postpartum haemorrhage as a drop of more than 10% in haematocrit during delivery [27].

In the current study the average haematocrit after CS was 37.8 (SD 4.4) and was largely dependent on haematocrit before CS. Controlling for litter size and breed, H_t after is expected to decrease by 0.77 for each decrease of one in haematocrit before CS. The average decrease in haematocrit following CS and fluid therapy was 6.4% (SD 2.9). It can be speculated that the extent of blood loss and, hence, the haematocrits may be different following CS when the placentas are left in situ or removed and whether an ovariohysterectomy was performed or not. In this study no ovariohysterectomies were performed and the placentas were removed from the uterus with each puppy. The effect of blood loss associated with vaginal births and CS in humans is ameliorated for by autotransfusion of blood into the circulation from the contracting uterus if left in place [28]. It is fair to speculate that autotransfusion may also act as a compensatory mechanism to protect against effects of maternal blood loss during natural whelp and CS in dogs as has been reported in woman [28]. In the current study, the effect of autotransfusion on haematocrit was not studied.

In a report on CS in bitches it was suggested that blood loss during surgery should be compensated for at a level of 3 ml of crystalloid for every 1 ml of blood lost [14]. The author did not explain how the amount of blood lost was estimated or determined. Change in haematocrit before and after CS in the bitch seems an objective parameter to mitigate this shortcoming.

All our bitches made an uneventful recovery and survived beyond 7 d and nursed litters successfully, yet five percent of bitches showed a decrease in haematocrit of 12 to 18 percentage points. It should however not be assumed that a decrease in haematocrit by 12 to 18 percentage points is never a reason for concern. A study in woman suggested that a change in haematocrit of more than 10 percentage points may indicate a need for blood transfusion [19]. The significance of such a decrease should be based on basic veterinary

clinical principles. In humans both a minimum haematocrit as well as a maximum change in haematocrit before and after surgery are used as trigger for blood transfusion [29-31]. Further research is required to better define transfusion triggers in the periparturient bitch undergoing CS.

8. Conclusions

Results of this study show that bitches have haematocrits at term that are at the lower end of the normal reference ranges for non-pregnant dogs and that there is no true anaemia of pregnancy. In contrast to current recommendations [32], pregnant bitches near term with haematocrits below normal reference ranges for non-pregnant dogs should be evaluated for disease that may affect haematocrit. Also, our results show that there is a small yet significant negative correlation between litter size and haematocrit at term in bitches but that this has no clinical significance. On average the haematocrit decreased by 6.4% during CS, while bitches were receiving 35 ml/kg of crystalloids over 1½ to 2 h. Further research is required to better define transfusion triggers in the periparturient bitch undergoing CS.

Author contributions

J.O. Nöthling and K. Joubert were the supervisors of the scientific protocol and assisted in drafting the protocol and manuscript up to the final drafts. J.O. Nöthling performed the statistical analyses. K.G.M. De Cramer was the main person involved in experimental work and wrote the protocol and manuscript.

Conflicts of interest

Conflicts of interest: none.

References

- [1] Ryan SD, Wagner AE. Cesarean section in dogs: Physiology and perioperative considerations. *Compendium on Continuing Education for the Practicing Veterinarian* 2006;28:34-43.
- [2] Concannon PW, Powers ME, Holder W, Hansel W. Pregnancy and parturition in the bitch. *Biol Reprod* 1977;16:517-26.
- [3] Moritz A, Fickenscher Y, Meyer K, Failing K, Weiss DJ. Canine and feline hematology reference values for the ADVIA 120 hematology system. *Veterinary Clinical Pathology* 2004;33:32-8.
- [4] Duncan JR, Prasse KW. *Veterinary laboratory medicine: Clinical Pathology*, ed 5. Arnes (IA), Iowa State University Press, 2011.
- [5] Hayashi M. Composition of the blood of adult Beagle bitches during the estrous cycle, pregnancy and postpartum period. *Japanese Journal of Animal Reproduction* 1974;11:89-94.
- [6] Kaneko M, Nakayama H, Igarashi N, Hirose H. Relationship between the number of fetuses and the blood constituents of beagles in late pregnancy. *The Journal of veterinary medical science/the Japanese Society of Veterinary Science* 1993;55:681-2.
- [7] Dimço E, Abeshi J, Lika E, Dharmo G. Effect of pregnancy in hematological profile of dogs. *Albanian Journal of Agricultural Sciences* 2013;12:159-62.
- [8] Ryan SD, Wagner AE. Cesarean section in dogs: Anesthetic management. *Compendium on Continuing Education for the Practicing Veterinarian* 2006;28:44-5.
- [9] Moon PF, Erb HN, Ludders JW, Gleed RD, Pascoe PJ. Perioperative risk factors for puppies delivered by cesarean section in the United States and Canada. *J Am Anim Hosp Assoc* 2000;36:359-68.
- [10] Robertson SA, Moon PF. Anesthetic management for cesarean section in bitches. *Veterinary Medicine* 2003;98:675-96.
- [11] Traas AM. Surgical management of canine and feline dystocia. *Theriogenology* 2008;70:337-42.
- [12] Von Heimendahl A, Cariou M. Normal parturition and management of dystocia in dogs and cats. *In Practice* 2009;31:254-61.
- [13] Kushnir Y, Epstein A. Anesthesia for the pregnant cat and dog. *Israel Journal of Veterinary Medicine* 2012;67:19-23.
- [14] Smith FO. Guide to Emergency Interception During Parturition in the Dog and Cat. *Veterinary Clinics of North America - Small Animal Practice* 2012;42:489-99.
- [15] Gilson SD. Cesarean section; in Slatter DH (ed): *Textbook of Small Animal Surgery*. 2003, vol 1, p 1517-20.
- [16] Dohoo I, Martin W, Stryhn H. *Veterinary Epidemiologic Research*, ed 2nd. Manitoba Canada, VER Inc, 2009.
- [17] Goonewardene M, Shehata M, Hamad A. Anaemia in pregnancy. *Best Practice and Research: Clinical Obstetrics and Gynaecology* 2012;26:3-24.
- [18] Hidar S, Jennane TM, Bouguizane S, Lassoued L, Bibi M, Khaïri H. The effect of placental removal method at cesarean delivery on perioperative hemorrhage: a randomized clinical trial ISRCTN 49779257. *European Journal of Obstetrics & Gynecology and Reproductive Biology* 2004;117:179-82.
- [19] Stafford I, Belfort MA, Dildy III GA. Etiology and Management of Hemorrhage; in Saade GR, Foley MR, Phelan JP, Dildy III GA (eds): *Critical Care Obstetrics*. Chicester (UK), Wiley, 2010, p 308-25.

- [20] World Health Organization. Iron deficiency anaemia assessment prevention and control: a guide for programme managers. Report . 2001. Geneva, (WHO/NHD/01.3).
Ref Type: Report
- [21] Concannon PW, McCann JP, Temple M. Biology and endocrinology of ovulation, pregnancy and parturition in the dog. *J Reprod Fertil Suppl* 1989;39:3-25.
- [22] Kimberely ET, Casal ML, O'Donnell PA, Haskins ME. Effects of pregnancy on complete cell counts and serum biochemistry profiles in dogs. *Theriogenology* 2006;66:670.
- [23] Lawrence J, Chang YMR, Szladovits B, Davison LJ, Garden OA. Breed-specific hematological phenotypes in the dog: a natural resource for the genetic dissection of hematological parameters in a mammalian species. 2013.
- [24] Glaus TM, Hauser K, Hässig M, Lipp B, Reusch CE. Non-invasive measurement of the cardiovascular effects of chronic hypoxaemia on dogs living at moderately high altitude. *The Veterinary record* 2003;152:800-3.
- [25] Thorson CM, Ryan ML, Van Haren RM, Pereira R, Olloqui J, Otero CA et al. Change in hematocrit during trauma assessment predicts bleeding even with ongoing fluid resuscitation. *The American surgeon* 2013;79:398-406.
- [26] Nicol B, Croughan-Minihane M, Kilpatrick SJ. Lack of value of routine postpartum hematocrit determination after vaginal delivery. *Obstetrics & Gynecology* 1997;90:514-8.
- [27] ACOG. American College of Obstetricians and Gynecologists. Postpartum hemorrhage. Technical Bulletin No. 243. 1998. Washington, DC. Ref Type: Report
- [28] Ciliberto CF, Marx GF. Physiological changes associated with pregnancy. *Adv Anesth* 1998;9:1-3.
- [29] Van Woerkens ECSM, Trouwborst A, Van Lanschot JJB. Profound hemodilution: what is the critical level of hemodilution at which oxygen delivery-dependent oxygen consumption starts in an anesthetized human? *Anesthesia & Analgesia* 1992;75:818-21.
- [30] Freitag M, Standl T, Horn EP, Wilhelm S, Schulte Am Esch J. Acute normovolaemic haemodilution beyond a haematocrit of 25%: Ratio of skeletal muscle tissue oxygen tension and cardiac index is not maintained in the healthy dog. *European Journal of Anaesthesiology* 2002;19:487-94.
- [31] Mannucci PM, Levi M. Prevention and treatment of major blood loss. *N Engl J Med* 2007;356:2301-11.
- [32] Linde-Forsberg C, Eneroth A. Abnormalities in pregnancy, parturition, and the periparturient period; in Ettinger SJ, Feldman EC (eds): *Textbook of veterinary internal medicine*. St. Louis (MO), Saunders Elsevier, 2000, p 1527.