

# Determination of body water compartments in neonatal foals by use of indicator dilution techniques and multifrequency bioelectrical impedance analysis

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**Objective**—To determine values for total body water (TBW), extracellular fluid volume (ECFV), intracellular fluid volume (ICFV), and plasma volume (PV) in healthy neonatal (< 24 hours old) foals and to create a multifrequency bioelectrical impedance analysis (MF-BIA) model for use in neonatal foals.

**Animals**—7 healthy neonatal foals.

**Procedures**—Deuterium oxide (0.4 g/kg, IV), sodium bromide (30 mg/kg, IV), and Evans blue dye (1 mg/kg, IV) were administered to each foal. Plasma samples were obtained following an equilibration period, and the TBW, ECFV, ICFV, and PV were calculated for each foal. An MF-BIA model was created by use of morphometric measurements from each foal.

**Results**—Mean  $\pm$  SD values were obtained for TBW ( $0.744 \pm 0.024$  L/kg), ICFV ( $0.381 \pm 0.018$  L/kg), ECFV ( $0.363 \pm 0.014$  L/kg), and PV ( $0.096 \pm 0.015$  L/kg). The 95% limits of agreement between the MF-BIA and indicator dilution techniques were within  $\pm 2$  L for TBW and ECFV.

**Conclusions and Clinical Relevance**—Fluid volumes in neonatal foals were found to be substantially larger than fluid volumes in adult horses. Multifrequency bioelectrical impedance analysis may be a useful technique for predicting TBW, ICFV, and ECFV in neonatal foals. (*Am J Vet Res* 2011;72:1390–1396)

Neonatal foals admitted to emergency veterinary facilities are often hemodynamically compromised and require rapid IV administration of fluids. The distribution of these fluids is affected by the physiologic status of each foal, disease process, and intrinsic properties of the fluid.<sup>1</sup> Investigators have described the fluid physiology of adult horses in a number of studies,<sup>2–6</sup> but there is little published information about fluid physiology and distribution in foals in the peer-reviewed literature. Knowledge of neonatal fluid volumes would help to guide fluid treatment and allow clinicians to assess whether critically ill foals have fluid deficits or excesses in comparison with values for clinically normal foals.

The TBW ranges from 0.623 to 0.677 L/kg in adult horses,<sup>3,5,6</sup> but it has not been determined in newborn foals. The ECFV of 2-day-old foals is 0.394 L/kg, as de-

## ABBREVIATIONS

ECFV	Extracellular fluid volume
ICFV	Intracellular fluid volume
MF-BIA	Multifrequency bioelectrical impedance analysis
$R_E$	Resistance of extracellular water
$R_I$	Resistance of intracellular water
TBW	Total body water

termined by use of thiocyanate as a dilution indicator, and this value was significantly larger than values obtained for adult horses (0.214 to 0.253 L/kg).<sup>2,4,5,7</sup> The ICFV has not been evaluated in foals, but it ranges between 0.356 and 0.458 L/kg in adult horses.<sup>3,5</sup> Plasma volume and blood volume have also been estimated in 2-day-old foals and reported to be 0.095 and 0.151 L/kg, respectively.<sup>7</sup> It is not known whether neonatal (< 24 hours old) foals have fluid volumes similar to those of 2-day-old foals. This information could impact drug dosage recommendations for medications that distribute primarily within a specific fluid space (eg, the ECFV).

Indicator dilution is 1 method used to determine fluid volumes in animals. Specifically, deuterium oxide has been used to estimate TBW in adult horses in a number of studies.<sup>3,5,6</sup> Bromide and sodium thiocyanate have been used to determine ECFV.<sup>2,4,5</sup> Plasma and blood volume have been estimated by use of Evans

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blue dye in both foals and adult horses.<sup>5,7,8</sup> In addition, MF-BIA has been used in adult horses and offers the advantage that it can provide immediate results and is a noninvasive technique.<sup>3,5,9</sup> Foal-specific coefficients and ideal morphometric measurements have not been determined for MF-BIA and are required before it can be applied in this age group of horses.

The purpose of the study reported here was to describe the fluid spaces in healthy neonatal (< 24 hours old) foals. In addition, the information obtained by use of indicator dilution was used to create an MF-BIA model that could be used to rapidly monitor fluid balance in neonatal foals during treatment. The hypothesis was that MF-BIA would provide a clinically acceptable method for use in estimation of TBW, ECFV, and ICFV.

## Materials and Methods

**Animals**—Seven clinically normal foals were included in the study, which was conducted at the Center for Equine Health at the University of California-Davis. Body weight ranged from 36.0 to 53.1 kg (median, 48.1 kg); body weight was measured with a digital walk-on scale. All foals were male Quarter Horses and were 8 to 19 hours old (median, 16.5 hours). All foals stood and suckled within 2 hours after birth. Foals were deemed clinically healthy on the basis of results of a physical examination, CBC, and serum biochemical analysis. In addition, all foals had a serum IgG concentration > 800 g/dL prior to the start of the study, as determined by use of a commercially available serum IgG test kit.<sup>a</sup> The study was approved by the Animal Use and Care Administrative Advisory Committee of the University of California-Davis.

**Experimental procedures**—A 14-gauge catheter<sup>b</sup> was placed in each jugular vein of all foals. Morphometric measurements were obtained; these measurements included the distance from the most cranial point of the shoulder joint to the point of the ipsilateral tuber ischii (length) and the distance from the ground to the top of the highest (dorsal) point of the spinous processes of the thoracic vertebrae (height). Two consecutive MF-BIA measurements were also obtained.

Sodium bromide<sup>c</sup> was diluted in deuterium oxide<sup>d</sup> to create a 7.9% solution of sodium bromide; sterile water then was added to achieve an osmolarity of 300 mOsm/L. This solution was administered IV during a 3-minute period through the catheter in the right jugular vein. The solution contained 30 mg of sodium bromide/kg, which was equivalent to a bromide dose of 25 mg/kg, and 0.4 g of deuterium oxide/kg. Immediately after infusion of the sodium bromide solution, 5% Evans blue dye<sup>e</sup> (1 mg/kg, IV) was administered via the same catheter.

**Collection of blood samples**—Venous blood samples (8 mL) were collected via the catheter in the left jugular vein before and 15 minutes and 2, 3, 4, and 5 hours after infusion of the sodium bromide solution and Evans blue dye. Samples were placed into 2 tubes for plasma harvest (1 contained heparin, and the other contained EDTA) and into a tube for serum harvest. The PCV and plasma total protein concentration were measured by use of the microhematocrit method and

refractometry, respectively, within 4 hours after sample collection. All other samples were centrifuged, and serum and plasma were harvested and stored at -80°C until analyzed to determine concentrations of deuterium, bromide, or Evans blue dye.

**Measurement of deuterium concentration and calculation of TBW**—Total body water was estimated by use of serum deuterium concentrations obtained before and 5 hours after infusion. The samples obtained 3, 4, and 5 hours after infusion were analyzed to ensure that deuterium concentrations had stabilized by the time of collection of the 5-hour sample. These time points were chosen on the basis of results of a prior study<sup>3</sup> conducted in adult horses. Analysis of serum deuterium oxide concentrations was performed as described elsewhere.<sup>5,6</sup> Briefly, the deuterium oxide in serum and water samples was reduced at 490°C to produce deuterium gas that was measured with an isotope-ratio mass spectrometer.<sup>f</sup> The data were expressed as the  $\delta$  deuterium per milliliter relative to Vienna standard mean ocean water. The value for TBW was calculated by use of the following equation:

$$\text{TBW (number of moles)} = (W \times A/18.02a) \times \frac{[\delta_{\text{dose}} - \delta_{\text{tap}}]/[\delta_{\text{after}} - \delta_{\text{before}}]}$$

where W is the number of grams of water used to dilute the deuterium, A is the dose of deuterium (number of grams) infused, a is the number of grams of deuterium diluted for analysis,  $\delta_{\text{dose}}$  is the measured deuterium concentration of the diluted dose,  $\delta_{\text{tap}}$  is the measured deuterium concentration of local tap water,  $\delta_{\text{before}}$  is the deuterium concentration determined for the sample obtained before infusion, and  $\delta_{\text{after}}$  is the deuterium concentration determined for the sample obtained 5 hours after infusion.

The TBW can be converted from moles to kilograms by use of the following equation: TBW (number of kg) = TBW (number of moles)  $\times$  18.02/1,000 g/kg. The TBW calculated by use of deuterium dilution is typically an overestimate (by 4%) because of the binding of deuterium to acidic amino acids and other nonexchangeable sites.<sup>3,5,6</sup> Thus, the corrected TBW (TBW<sub>corr</sub>) was obtained by use of the following equation:

$$\text{TBW}_{\text{corr}} = \text{TBW (number of kg)}/1.04$$

**Measurement of bromide concentration and calculation of ECFV**—Serum bromide concentration was measured by use of blood samples obtained before and 5 hours after infusion of the sodium bromide solution. A sample obtained at 2 hours was also analyzed to ensure that the serum bromide concentration had stabilized by the time of collection of the 5-hour sample. These time points were chosen on the basis of results of another study<sup>2</sup> conducted in adult horses. Analysis of serum bromide concentrations was performed as described elsewhere.<sup>10</sup> The serum bromide concentration was measured with a high-pressure liquid chromatographic method on an anion-exchange column.<sup>11,g</sup> The ECFV was calculated by use of the following equation<sup>2</sup>:

$$\text{ECFV (number of L)} = (\text{bromide dose} / [\text{bromide}_{\text{after}} - \text{bromide}_{\text{before}}]) \times 0.9 \times 0.95 \times 0.94$$

where bromide dose is the dose of bromide (number of  $\mu\text{mol}$ ) infused, bromide<sub>after</sub> is the serum bromide concentration (number of  $\mu\text{mol}$ ) in the sample obtained 5 hours after infusion, bromide<sub>before</sub> is the bromide concentration (number of  $\mu\text{mol}$ ) in the sample obtained before the infusion, 0.9 is the correction factor for non-extracellular distribution, 0.95 is the Donnan equilibrium factor, and 0.94 is the proportion of water in plasma.<sup>11</sup> Bromide loss in urine is typically 1.6% and has a negligible effect on the measurement of ECFV.<sup>12</sup>

**Calculation of ICFV**—The ICFV was calculated as the difference between TBW and ECFV by use of the following equation:  $\text{ICFV} = \text{TBW} - \text{ECFV}$ .

**Measurement of Evans blue dye concentration and determination of plasma volume**—The concentration of Evans blue dye was determined in plasma samples obtained 15 minutes after infusion. The measurement techniques have been described for samples obtained from foals at 2 days of age.<sup>7</sup> Lipemic plasma samples were cleared by use of polyethylene glycol as described in other studies.<sup>7,13</sup> All samples were measured in duplicate on a spectrophotometer<sup>h</sup> at 620 nm, and the mean of the 2 values was used for determination of the Evans blue dye concentration. Dye concentration was estimated on the basis of a standard curve for each foal that was created by use of predetermined dye concentrations in the range of all samples. Plasma volume was calculated for the sample obtained at 15 minutes after infusion on the basis of data from a study<sup>8</sup> in foals that revealed a good approximation of plasma volume by use of this time point (15 minutes). For determination of plasma volume, data from only 6 of the foals were used. Plasma volume was calculated by use of the following equation<sup>14</sup>: plasma volume (number of mL) = dose of Evans blue dye (number of  $\mu\text{g}$ )/dye concentration (number of  $\mu\text{g}/\text{mL}$ ). Blood volume was calculated by use of the following equation<sup>14</sup>: blood volume (number of L) = plasma volume (number of L)/(1 - PCV).

**Bioimpedance measurements**—Bioimpedance measurements were made via a head-tail configuration described for adult horses.<sup>3,9</sup> The hair was clipped from a 4 × 4-cm area over the right cranial border of the first cervical vertebra and another 4 × 4-cm area over the caudal aspect of the right tuber ischii. Skin surface of the areas was cleaned with alcohol and allowed to dry. Subdermal platinum electrodes<sup>i</sup> were then placed 2.5 cm apart in a configuration parallel to the ground within each area. A bioimpedance analyzer<sup>j</sup> was at-

Table 1—Mean ± SD estimations of fluid spaces in neonatal (< 24 hours old) foals determined by the use of indicator dilution techniques.

Fluid space	Volume (L)	Volume per body weight (L/kg)
TBW	33.8 ± 5.3	0.744 ± 0.024
ICFV	17.4 ± 3.0	0.381 ± 0.018
ECFV	16.5 ± 2.4	0.363 ± 0.014
Plasma volume	4.3 ± 1.0	0.096 ± 0.015
Blood volume	7.1 ± 1.6	0.159 ± 0.024

tached to the subdermal electrodes and recorded measurements of resistance and reactance at each of 50 frequencies within the range of 5 to 1,000 kHz. Impedance and phase angle were then computed from the measured values for resistance and reactance. Data were transmitted from the analyzer to a personal computer and stored until subsequent analysis.

The  $R_E$  and  $R_I$  were computed from the generated impedance and phase-angle spectral data for each electrode configuration. The impedance and phase-angle data were fitted to an enhanced version of the Cole-Cole model of current conduction through heterogeneous biological tissues by use of iterative nonlinear curve-fitting algorithms derived for use with the bioimpedance analyzer. The enhanced modeling program extended the original Cole-Cole model to allow for frequency-invariant time delays caused by the speed at which electrical information was transferred through a conductor.

Predicted ECFVs and ICFVs were estimated from the modeled  $R_E$  and  $R_I$  values; this was accomplished by use of equations formulated from the Hanai mixture theory, which describes the influence of nonconductive material on the apparent resistivity of surrounding conductive fluid. The ECFV was estimated by use of the following equation<sup>3,9</sup>:

$$V_{\text{ECW}} = k_{\text{ECW}} \times ([\text{MD}^2 \times \sqrt{\text{BW}}] / R_E)$$

where  $V_{\text{ECW}}$  is the predicted total extracellular water volume;  $k_{\text{ECW}}$  is a scaling factor that accounts for the geometry of measurements between a defined electrode array, resistivity of the extracellular fluid, and body density; MD is the morphometric distance (either height or length); BW is the body weight; and  $R_E$  is determined from the model. Values for  $k_{\text{ECW}}$  (a constant) were derived from regression of the ECFV predicted by use of the MF-BIA against the ECFV estimated by use of the corrected dilution space for sodium bromide.

The volume of intracellular water was predicted from further extrapolation of the Hanai theory by use of the following equation:

$$(1 + [V_{\text{ICW}}/V_{\text{ECW}}])^{5/2} = ([R_E + R_I]/R_I) \times (1 + [K_D \times V_{\text{ICW}}]/V_{\text{ECW}})$$

Table 2—Mean ± SD fluid spaces of neonatal (< 24 hours old) foals determined by use of MF-BIA with length or height of the foal as the morphometric measurement.

Fluid space	Length	Height
TBW (L)	33.9 ± 5.0	33.9 ± 4.1
(L/kg)	0.746 ± 0.044	0.749 ± 0.053
ICFV (L)	17.4 ± 2.7	17.4 ± 2.4
(L/kg)	0.385 ± 0.047	0.386 ± 0.053
ECFV (L)	16.5 ± 3.2	16.5 ± 2.7
(L/kg)	0.361 ± 0.028	0.363 ± 0.025

Length of a foal was determined as the distance from the most cranial point of the shoulder joint to the point of the ipsilateral tuber ischii and the height of the foal as the distance from the ground to the top of the highest (dorsal) point of the spinous processes of the thoracic vertebrae.

Table 3—Mean error and mean absolute error of 2 morphometric models for use of MF-BIA in the prediction of ECFV, TBW, and ICFV in neonatal foals.

Morphometric measurement	Mean $\pm$ SD error			Mean absolute error		
	ECFV (L)	TBW (L)	ICFV (L)	ECFV (L)	TBW (L)	ICFV (L)
Length	0.01 $\pm$ 0.9	0 $\pm$ 1.8	-0.01 $\pm$ 2.4	0.7	1.5	1.8
Height	0.01 $\pm$ 0.9	-0.03 $\pm$ 2.6	-0.04 $\pm$ 2.7	0.6	2	2.2

Mean error refers to the mean of the error between the predicted values obtained by use of MF-BIA and estimated values obtained by use of dilution techniques. Mean absolute error refers to the mean of the absolute value of the error between the predicted values obtained by use of MF-BIA and estimated values obtained by use of dilution techniques.  
See Table 2 for remainder of key.

where  $V_{ICW}$  is the volume of the intracellular water, and  $K_D$  is the ratio of the apparent resistivity of intracellular water to extracellular water. The value for  $K_D$  was derived from the iterative prediction of  $V_{ICW}/V_{ECW}$  and by adjusting  $K_D$  until a minimum mean error between the predicted and measured values was obtained.

The value for predicted TBW was calculated as follows:

$$TBW = V_{ICW} + V_{ECW}$$

**Statistical analysis**—Data were reported as mean  $\pm$  SD for all descriptive variables. Data were tested by use of the method of Kolmogorov-Smirnov and were found to be normally distributed. Predicted MF-BIA values and indicator dilution values were compared by use of linear regression, calculation of the mean value of the error between the 2 measurements, Student *t* tests, and the 95% limits of agreement. Bland-Altman plots were used to illustrate differences between mean values obtained by use of the 2 techniques. For all analyses, values of  $P < 0.05$  were considered significant. A standard statistical software program was used for analysis.<sup>k</sup>

## Results

**TBW, ECFV, ICFV, and blood and plasma volume**—We did not detect a significant difference in deuterium concentrations between the samples obtained at 4 and 5 hours ( $P = 0.298$ ) or in bromide concentrations between the samples obtained at 2 and 5 hours ( $P = 0.796$ ; Table 1). These time points were considered appropriate for use in determining volumes of the fluid compartments.

**MF-BIA**—Results of the MF-BIA calculated for the 2 morphometric measurements (ie, length and height; Table 2) were compared. The mean error and mean absolute error of the 2 equations were determined (Table 3). The mean absolute error is the mean of the absolute value of the error between the MF-BIA-predicted value and the value estimated from the dilutional data. The mean error and mean absolute error provided a comparison between the model developed by use of the data for height and the model developed by use of the data for length. There was not a significant difference between the 2 models for any of the fluid spaces.

The MF-BIA model determined by use of height yielded the smallest error when estimating ECFV as compared with results for dilutional calculations (Figure 1). There was not a statistical difference between

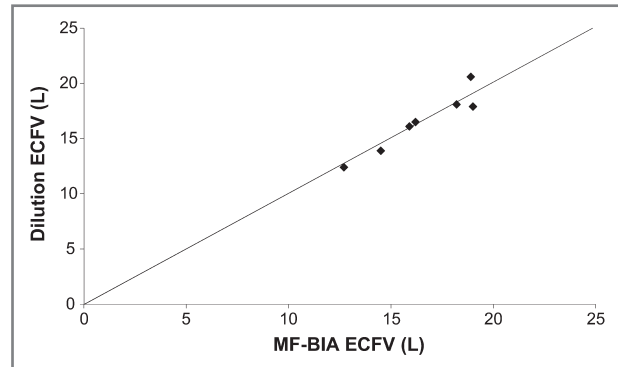


Figure 1—Graph illustrating the comparison of ECFV in 7 foals calculated by use of a dilution technique with sodium bromide and predicted by use of MF-BIA with height of the foal as the morphometric measurement. Each symbol represents results for 1 foal, and the line of best fit is indicated. Height of a foal was determined as the distance from the ground to the top of the highest (dorsal) point of the spinous processes of the thoracic vertebrae.

the MF-BIA models for ECFV estimated by use of height and length. Regression analysis of ECFV measured by use of MF-BIA and ECFV estimated by use of dilutional techniques yielded a high correlation ( $R^2$ , 0.90;  $P = 0.001$ ). The mean  $\pm$  SD difference between the 2 techniques was  $0.01 \pm 0.88$  L, and the 95% limits of agreement were  $-1.75$  to  $1.78$  L (Figure 2).

The MF-BIA model determined by use of length yielded the smallest error when estimating TBW, compared with results for dilutional calculations (Figure 3). There was not a statistical difference between the MF-BIA models for TBW estimated by use of height and length. Regression analysis of TBW measured by use of MF-BIA and TBW estimated by use of dilutional techniques yielded a high correlation ( $R^2$ , 0.88;  $P = 0.002$ ). The mean  $\pm$  SD difference between the 2 techniques was  $0 \pm 1.85$  L, and the 95% limits of agreement were  $-1.85$  to  $1.85$  L (Figure 4).

The MF-BIA model for length yielded the smallest error when estimating ICFV, compared with results for dilutional calculations (Figure 5). There was not a statistical difference between the MF-BIA models for ICFV determined by use of height and length. Results of regression analysis of ICFV measured by use of MF-BIA and ICFV estimated by use of dilutional techniques were not significant ( $R^2$ , 0.427;  $P = 0.111$ ). The mean  $\pm$  SD difference between the 2 techniques was  $-0.01 \pm 2.36$  L, and the 95% limits of agreement were  $-4.73$  to  $4.71$  L (Figure 6).

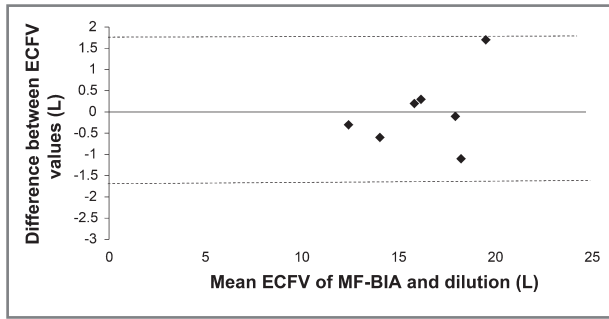


Figure 2—Bland-Altman plot illustrating the difference between ECFV estimated by use of MF-BIA with height of the foal as the morphometric measurement and ECFV estimated by use of a dilution technique plotted against the mean of the 2 methods for 7 foals. The solid line is the mean difference (bias), and dashed lines are the limits of agreement (mean difference  $\pm$  [1.96 X SD]). See Figure 1 for remainder of key.

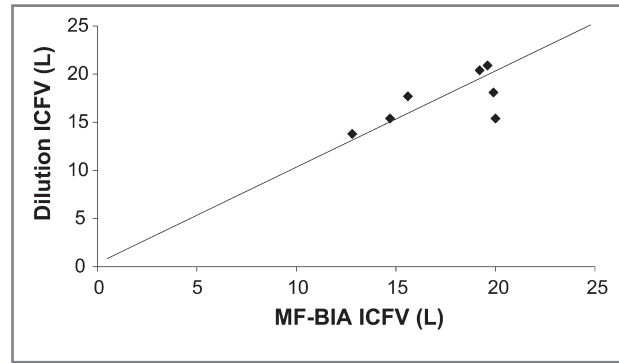


Figure 5—Graph illustrating the comparison of ICFV in 7 foals calculated by use of a dilution technique with deuterium oxide and predicted by use of MF-BIA with length of the foal as the morphometric measurement. See Figure 3 for key.

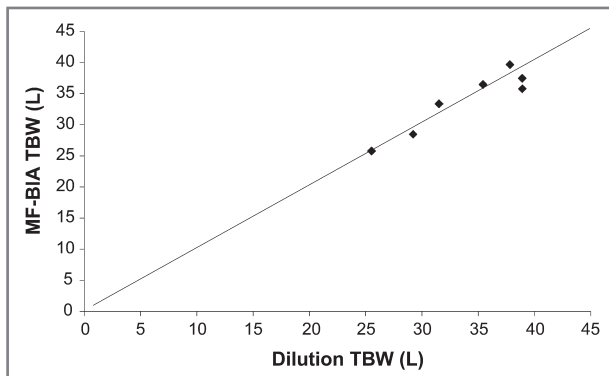


Figure 3—Graph illustrating the comparison of TBW in 7 foals calculated by use of a dilution technique with deuterium oxide and predicted by use of MF-BIA with length of the foal as the morphometric measurement. Each symbol represents results for 1 foal, and the line of best fit is indicated. Length of a foal was determined as the distance from the most cranial point of the shoulder joint to the point of the ipsilateral tuber ischii.

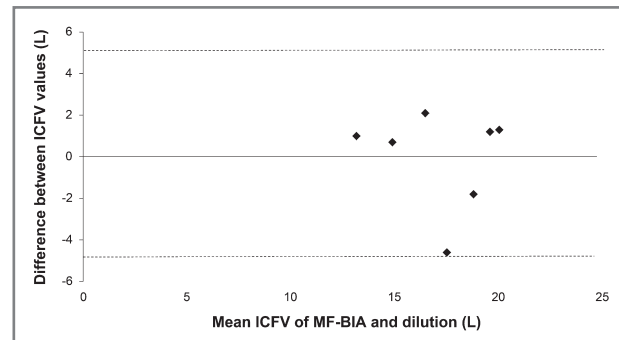


Figure 6—Bland-Altman plot illustrating the difference between ICFV estimated by use of MF-BIA with length of the foal as the morphometric measurement and ICFV estimated by use of a dilution technique plotted against the mean of the 2 methods for 7 foals. See Figures 2 and 3 for key.

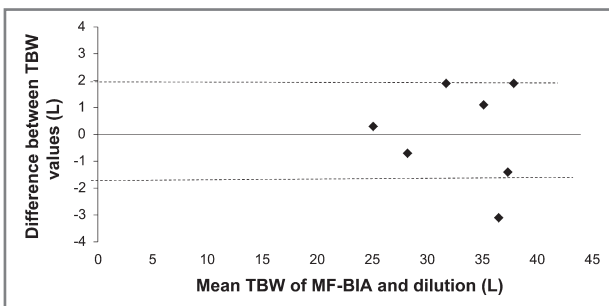


Figure 4—Bland-Altman plot illustrating the difference between TBW estimated by use of MF-BIA with length of the foal as the morphometric measurement and TBW estimated by use of a dilution technique plotted against the mean of the 2 methods for 7 foals. See Figure 2 for remainder of key.

## Discussion

The mean  $\pm$  SD ECFV ( $0.381 \pm 0.018$  L/kg) for the neonatal foals of the study reported here was similar to, but slightly less than, that reported in 2-day-old foals ( $0.394 \pm 0.029$  L/kg) in another study<sup>7</sup> The indicator dilution technique for ECFV used in the present study (bromide) was different than that used in the

study conducted to evaluate the 2-day-old foals (thiocyanate); this difference in technique could have been responsible for the small discrepancy in ECFV between the 2 studies. In other studies,<sup>2,4</sup> use of thiocyanate dilution resulted in larger ECFV estimates in adult horses than were obtained by use of bromide dilution. Further research is required to compare newborn foals of different ages by use of the same indicator technique. Foals in the present study were  $< 24$  hours old, whereas those of the other study<sup>7</sup> were 2 days old. For comparison, the ECFV decreases significantly in human infants after birth.<sup>15</sup>

The mean  $\pm$  SD plasma volume ( $96.2 \pm 15.4$  mL/kg) in the present study was also similar to that reported for 2-day-old foals ( $94.5 \pm 8.9$  mL/kg) in the aforementioned study.<sup>7</sup> However, both of these values are larger than that reported for adult horses ( $52.5 \pm 5.1$  mL/kg to  $63.3 \pm 8.9$  mL/kg).<sup>16</sup> Mean calculated blood volume ( $159.7 \pm 24.6$  mL/kg) in the foals of the present study was also similar to that for previously reported 2-day-old foals<sup>7</sup> ( $151.2 \pm 32.8$  mL/kg) but larger than that for adult horses<sup>16</sup> ( $77.5 \pm 3.8$  mL/kg to  $103.1 \pm 13.5$  mL/kg).

The mean  $\pm$  SD TBW ( $0.744 \pm 0.024$  L/kg) for the foals of the present study was larger than that for human infants at birth ( $0.696$  L/kg).<sup>17</sup> Values for the foals were also larger than those reported for adult horses ( $0.67 \pm 0.06$  L/kg).<sup>3,5</sup> In humans, TBW decreases with age, and on the basis of results of the present study,

there appear to be similar decreases in horses.<sup>17</sup> The mean ICFV ( $0.381 \pm 0.018$  L/kg) determined for foals in the present study was also larger than that for human infants ( $0.270$  L/kg).<sup>17</sup> It has been suggested that the relative ICFV of foals is the same as that of adult horses.<sup>18</sup> However, results of the present study, in conjunction with those of a similar study<sup>3</sup> in adult horses, suggest that the ICFV may decrease in horses as they mature. The foals in the present study had an ECFV and ICFV of similar size (ratio of 1:1), which contrasts to the ratio of ICFV to ECFV of almost 2:1 in adult horses.<sup>3</sup> Interestingly, the ratio of ICFV to ECFV is even more extreme in human infants (approx 0.6:1).<sup>17</sup>

The MF-BIA model created in the present study had similarities with the model developed for adult horses in another study.<sup>3</sup> As with other models, additional validation in another group of foals will provide better information about accuracy for various conditions.<sup>9</sup> Limitations of MF-BIA have been described for adult horses during acute fluid shifts, such as blood loss, and these same limitations may apply to foals.<sup>9</sup> Additionally, all foals in the present study were male, and a previous study<sup>19</sup> in humans suggests that it may be necessary to use sex-specific coefficients.

The MF-BIA models are created through estimates of fluid spaces made by use of indicator dilution. There is no criterion-referenced standard to determine ECFV, ICFV, TBW, and plasma volume in live animals. These fluid spaces are physiologic and do not necessarily coincide with a static volume; it is likely that they represent a dynamic process. Each indicator is prone to specific problems. Sodium thiocyanate was used in a prior study<sup>7</sup> in foals, but it has been found that sodium thiocyanate can penetrate cells and also bind to plasma proteins.<sup>20,21</sup> Bromide has a number of correction factors, which were indicated in the equations used in the present study, but has been used with high accuracy in horses.<sup>2,3</sup>

In the study reported here, as determined on the basis of regression analysis, MF-BIA did not appear to be as good for use in prediction of ICFV as was ECFV or TBW. This is similar to findings in adult horses, but the reason for this observation on the use of MF-BIA in horses is unclear.<sup>9</sup> It would appear to represent a species difference because this same discrepancy does not appear to be a consistent observation in studies of humans conducted by use of the same MF-BIA techniques and models. Results further illustrated the small discrepancy between the error in predicting the various fluid spaces in foals of the present study (Table 3).

Results of the present study apply to healthy neonatal foals; further research is needed to determine the fluid spaces in premature or septic foals. In humans, premature infants have a significantly larger TBW and ECFV than do term infants,<sup>16,22</sup> and this phenomenon may be the same in foals. Results of experiments on fetal vascular compliance and permeability in sheep indicate that fetuses may have a high filtration coefficient, compared with the filtration coefficient in adults, and therefore may differ in their response to fluid loading.<sup>23</sup>

For the study reported here, we concluded that neonatal foals < 24 hours old have ECFV, ICFV, and

TBW volumes that are larger than those in adult horses. Further research is needed to evaluate foals as they age up to 48 hours to determine whether there is an initial small expansion of the ECFV during the immediate postnatal period. Multifrequency bioimpedance analysis appears to be a useful technique for determination of physiologic fluid spaces (particularly ECFV and TBW) in healthy neonatal foals.

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- a. SNAP foal IgG, Idexx Laboratories Inc, Westbrook, Me.
  - b. BD Angiocath, BD, Sandy, Utah.
  - c. Sodium bromide, Sigma-Aldrich Inc, St Louis, Mo.
  - d. Deuterium oxide 99.9%, Sigma-Aldrich Inc, St Louis, Mo.
  - e. Evans blue dye, Sigma-Aldrich Inc, St Louis, Mo.
  - f. Hydra 20/20 isotope ratio mass spectrometer, Europa Scientific, Crewe, Cheshire, England.
  - g. 2690 high-performance liquid chromatography bromide, Waters, Milford, Mass.
  - h. Biowave diode array spectrophotometer, model S2100, Biochrom Ltd, Cambridge, England.
  - i. Grass platinum tetrapolar subdermal 30-gauge needle electrodes, 122 cm, Astro-Med Inc, West Warwick, RI.
  - j. Hydra ECF/ICF bioimpedance analyzer, model 4200, Xitron Technologies Inc, San Diego, Calif.
  - k. GraphPad InStat, GraphPad Software Inc, San Diego, Calif.
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