Published in final edited form as:

Ultrasound Med Biol. 2019 June ; 45(6): 1466–1474. doi:10.1016/j.ultrasmedbio.2018.12.004.

Quantitative ultrasound parameters based on the backscattered echo power signal as biomarkers of cervical remodeling: a longitudinal study in the pregnant Rhesus macaque

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Abstract

Clinical prediction and especially prevention of abnormal birth timing, particularly preterm, is poor. The cervix plays a key role in birth timing; it first serves as a rigid barrier to protect the developing fetus, then becomes the pathway to delivery of that fetus. Imaging biomarkers to define this remodeling process could provide insights to improve prediction of birth timing and elucidate novel targets for preventive therapies. Quantitative ultrasound (QUS) approaches that appear promising for this purpose include shear wave speed (SWS) estimation to quantify softness as well as parameters based on backscattered power, such as the mean backscattered power difference (mBSPD) and specific attenuation coefficient (SAC), to quantify organization of tissue microstructure. Invasive studies in rodents demonstrated that, as pregnancy advances, cervical microstructure disorganizes as tissue softness and compliance increase. Our noninvasive studies in pregnant women and Rhesus macaques suggested that QUS can detect these microstructural changes *in vivo*. Our previous study in the same cohort showed a progressive decline in SWS during pregnancy, consistent with increasing tissue softness, and we hypothesized that backscatter parameters would also decrease, consistent with increasing microstructural disorganization. In this study, we analyzed mBSPD and the SAC in the cervixes of Rhesus macaques (n=18). We found that both mBSPD and SAC decrease throughout pregnancy ($p < 0.001$ for both parameters), and that the former appears to be a more reliable biomarker. In summary, biomarkers that can characterize tissue microstructural organization are promising for comprehensive characterization of cervical remodeling in pregnancy.

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Keywords

Backscatter; Anisotropy; Cervix; Preterm birth; Cervical remodeling

Introduction

The cervix is the final gatekeeper to delivery. In pregnancy, through a process called cervical remodeling, it transforms from a barrier to protect the growing fetus into the pathway through which the fetus delivers (Myers et al., 2015a; Nott et al., 2016). This process is poorly understood and therefore, despite dedicated research effort, the preterm birth rate is rising (Martin et al., 2015) and it is estimated that even if all pregnant women were screened and offered appropriate available clinical intervention, 95% of preterm births would still occur (Chang et al., 2013). Postterm birth is also a significant problem because of its associated increased stillbirth risk and resource utilization (Feltovich, 2017). Imaging biomarkers to quantify remodeling are being explored because they could facilitate comprehensive understanding of the remodeling process, which could in turn improve prediction of birth timing and elucidate novel targets for therapies to prevent abnormal timing.

To date, quantitative ultrasound (QUS) biomarker exploration has focused on tissue softness and/or underlying microstructural organization. In general, parameters which measure softness are based on propagation of shear waves (Carlson et al., 2014a,b, 2015, 2018; Huang et al., 2016; Hernandez-Andrade et al., 2013; Molina et al., 2012; Muller et al., 2015; Rosado-Mendez et al., 2017, 2018) and those which quantify microstructural organization are based on backscattered echo signals from the propagation of compressional waves (McFarlin et al., 2006, 2010, 2015a,b; Labyed et al., 2011; Feltovich et al., 2010, 2012). Shear wave speed (SWS) estimation can quantify softness of the pregnant cervix in both women and Rhesus (Carlson et al., 2014a,b, 2015, 2018; Muller et al., 2015; Huang et al., 2016; Rosado-Mendez et al., 2017, 2018). Importantly, however, while SWS is a valuable biomarker of cervical remodeling, it provides no insight into *why* or *how* the tissue is becoming softer or more compliant (Feltovich and Hall, 2013). Rodent studies demonstrated that cervical tissue compliance is associated with changes in the composition and structure of the extracellular matrix (primarily collagen) throughout pregnancy (Read et al., 2007; Word et al., 2007; Akins et al., 2010; Timmons et al., 2010; Yoshida et al., 2014; Yao et al., 2016). QUS parameters based on the backscattered echo signal, such as the mean backscattered power difference (*mBSPD*) and the specific attenuation coefficient (*SAC*) provide information about these microstructural properties. For example, in both *ex vivo* and *in vivo* studies, *mBSPD* has been shown to detect changes in the organization of a primary acoustic scatterer (presumably collagen) in human tissue (Guerrero et al., 2018a,b) and it has been suggested that changes in the *SAC* correlate with changes in collagen content of the cervix in women and rodents (McFarlin et al., 2006, 2010; Bigelow et al., 2008; McFarlin et al., 2015b). Since obtaining tissues from pregnant women is nearly impossible for logistical reasons, and rodent models have limited applicability to humans, we have developed a Rhesus model for our studies of cervical remodeling (Hafez and Jaszczak, 1972; Shively and Clarkson, 2009; Ratajczak et al., 2010; Bezold et al., 2013).

In this longitudinal study in pregnant Rhesus, we explored QUS backscatter parameters (*mBSPD* and the *SAC*) in the cohort of macaques in which we previously studied *SWS*. We hypothesized that, like *SWS*, both parameters would decrease as pregnancy advanced. We found that both parameters did decrease, *mBSPD* more reliably than the *SAC*, suggesting increasing tissue microstructural disorganization. We conclude that *mBSPD*, and possibly the *SAC*, may be a useful biomarker of cervical microstructural disorganization in pregnancy. These biomarkers, especially in tandem with a biomarker that quantifies another property related to tissue remodeling (*SWS* to measure softness), could facilitate a more comprehensive understanding of the cervical remodeling process. This could lead to improved prediction of birth timing and, ultimately, novel strategies to prevent preterm and postterm birth.

Methods

This investigation was part of a larger study of QUS approaches to assess cervical remodeling. Details about the cohort and data acquisition can be found in Rosado-Mendez et al. (2018). The University of Wisconsin Institutional Animal Care and Use Committee approved the protocol and we acted in accordance with federal guidelines for proper animal care.

Cohort

The menstrual cycle of the Rhesus macaque is the same as that of women (averaging 28 days), but pregnancy is shorter (full term gestation is 24, as opposed to 40 weeks) (Rowell, 1963), making the Rhesus an excellent model for human pregnancy. Twenty macaques were selected for this study, 18 of which were successfully bred and thus the cohort consisted of 18 pregnant Rhesus primates (10 nulliparous and 8 multiparous). Ultrasound data were acquired at 7 timepoints: 0 (pre-pregnancy), 4 weeks, 10 weeks, 16 weeks, 20 weeks, and 23 weeks, and 2 weeks postpartum. For this study, we analyzed the timepoints during pregnancy (i.e. weeks 4, 10, 16, 20 and 23).

Data Acquisition

Scanning was conducted under supervision of Wisconsin National Primate Research Center (WNPRC) personnel in procedure rooms adjacent to primate housing at the WNPRC. Each macaque was sedated with 10-20 mg of ketamine per kilogram of body weight 5 minutes before scanning. Ultrasound exams (transrectal, the typical approach for studying the cervix of macaques) was performed with a prototype phased array transducer (128 element, 14 mm aperture, 3.3 mm diameter) operated as a linear array on a Siemens Acuson S2000 ultrasound system (Siemens Healthcare, Ultrasound Business Unit, Mountain View, CA, USA). Linear array operation (steering all acoustic beams at the same angle) enabled insonifying tissue at (roughly) the same angle to study anisotropy in QUS parameters estimates (see Guerrero et al. (2017)). The transducer was affixed to the examiner's index finger with Tegaderm™ dressing (to minimize motion) and inserted into a gel-filled glove as previously described (Carlson et al., 2015). The transducer was placed in the rectal cavity and the cervix identified via palpation and identification (from the B-mode image) of

anatomical landmarks (uterus, bladder, internal os, external os, and cervical canal). Figure 1(a) and (b) show the experimental setup and (c) a representative B-mode image.

Data were acquired from the “transition zone”, an area in the macaque cervix between the internal os and the tortuous distal canal, (Rosado-Mendez et al., 2017) where the cervix appears nearly identical to that of the human (primarily, the canal is relatively straight) (Rosado-Mendez et al., 2017, 2018). Raw radiofrequency (RF) echo signal data were captured using the Axius Direct Ultrasound Research Interface (Brunke et al., 2007) with 40 MHz sampling of the RF echo signals. Each set of RF data were captured from an independent plane of tissue through elevational translation of the transducer in the rectal cavity. Three independent sets of 15 frames of RF data were acquired by electronically steering the ultrasound beam from -28° to $+28^\circ$ in steps of 4° . Ten independent sets of beam steered RF echo signal data were acquired from a reference phantom after *in vivo* scanning. The homogeneous reference phantom was composed of glass beads (6 g/L; 3000E; Potter's Industries, Malvern, PA, USA; 5–20 μm diameter) suspended in a background mixture of agar and graphite (50 g/L; Superior Graphite, Chicago, IL, USA). This phantom had a sound speed of 1580 m/s and a linear attenuation of $0.67 \text{ dB}\cdot\text{cm}^{-1}\text{MHz}^{-1}$ in the 4–9 MHz bandwidth.

Selection of Region of Interest (ROI)

All RF echo signal data were converted into B-mode images, so that regions of interest (ROIs) could be selected by visual inspection of appropriate parenchymal tissue (i.e., excluding the cervical canal and grossly heterogeneous areas; data analysis beginning at least one aperture away). Figure 1(c) shows an example B-mode image with the ROIs demarcated.

QUS Parameter Estimation

The angle-dependence of backscattered power and specific attenuation coefficient were computed via the Reference Phantom Method (Yao et al., 1990). The Reference Phantom Method calculates a tissue's acoustic properties from echo signal power spectra relative to the reference phantom power spectra at equivalent depth. This step compensates for the system-dependence of power spectra providing system-independent QUS parameter estimates (Nam et al., 2011, 2012). Power spectra were estimated from RF echo signal data using Thomson's multitaper method (Thomson, 1982). The multitaper method has been shown to be the optimal method in terms of trade-off between spatial resolution and spectral variance when reducing the size of the power spectral estimation region (PSER) for attenuation coefficient estimation (Rosado-Mendez et al., 2013). The size of the PSER is typically 10–20 pulse-echo correlation lengths. Pulse-echo correlation lengths were estimated using the methods of Thijssen (2003) and found to be 230 μm (axial) and 320 μm (lateral). A $4\times 4 \text{ mm}^2$ PSER was determined to be the optimal size for spectral estimation.

Mean Backscattered Power Difference (mBSPD) Estimation

Backscattered power can be used to estimate the angle-dependence of acoustic properties of tissue via parameters based on the backscattered power difference (BSPD) (Guerrero et al., 2017). The BSPD is defined as the average of the logarithm of the ratio of sample to

reference phantom power spectral density in a chosen bandwidth. Computing the ratio of backscattered power for the sample to reference phantom allows comparison of a tissue's acoustic properties to that of spherical scatterers (Yao et al., 1990; Guerrero et al., 2017). The BSPD is computed at each beam steering angle in a chosen angular range. The normalized BSPD is computed by subtracting the magnitude of the highest BSPD among all beam steering angles (the angle of highest BSPD is referred to as the 'normalization angle') from the BSPD at all angles. The mean BSPD (*m*BSPD) is the average normalized BSPD among all beam steering angles. The larger the magnitude of the *m*BSPD, the greater the evidence of anisotropic scattering. Conversely, low *m*BSPD values suggest isotropic scattering. In this study, a 4×4 mm² parameter estimation region was used for each BSPD estimate.

Parameters which quantify the angle-dependence of backscattered power depend upon the angular range used in the analysis (Guerrero et al., 2017). In this study, as in our previous studies of the human cervix, (Guerrero et al., 2018b) we used a ±28° beam steering range for computing BSPD parameters. With the prototype linear array transducer used for these studies, this angular range allows estimation of *m*BSPD to adequate depths (1.4 cm) for these studies.

Specific Attenuation Coefficient (SAC) Estimation

The specific attenuation coefficient (SAC) (IEC 61391-2: 2010, 2010) is defined as the slope of the attenuation coefficient versus frequency (at a specific frequency). We calculated the attenuation coefficient, via the Reference Phantom Method with a linear least-squares fit of the logarithm of the ratio of sample to reference phantom power spectra versus axial depth at each frequency channel in the chosen bandwidth (Yao et al., 1991). The SAC was estimated as the linear least squares fit of the attenuation coefficient versus frequency in the chosen bandwidth. Based on our previous optimization protocol (Rosado-Mendez et al., 2013), we chose a 10 mm×4 mm attenuation coefficient parameter estimation region and a 4–9 MHz bandwidth for all SAC estimates.

Statistical Analysis

A multivariate mixed effects linear regression model was used to determine statistical significance of QUS parameters (statistical significance determined by $p < 0.05$). A maximum-likelihood model-building approach was used to obtain final linear models for (separately) *m*BSPD and SAC versus gestational age. Fixed effects included gestational age (GA, a continuous variable) and parity (a categorical variable) as follows:

$$mBSPD = a + b \times \text{Parity} + c \times \text{GA} \quad (1)$$

$$\text{SAC} = d + e \times \text{Parity} + f \times \text{GA} \quad (2)$$

Gestational age-dependence was computed using a linear least-squares fit to gestational ages (4–23 wk). Parity was modeled as multiparous = 0 and nulliparous = 1. Our analysis did not include interactions between parity and gestational age because this was unnecessary; the

models clearly and adequately demonstrated a significant decline in *mBSPD* with increasing gestational age. Multivariate mixed effects analysis was performed using R (R Core Team, 2013) and the lme4 package (Bates et al., 2015).

Results

Analysis of QUS Parameters in the Pregnant Cervix

mBSPD—Figure 2 shows the *mBSPD* as a function of gestational age for multiparous and nulliparous macaques. Median and interquartile range (IQR) values of the *mBSPD* are summarized in Table 1. The *mBSPD* was significantly dependent on both gestational age ($p < 0.001$) and parity ($p < 0.01$). The slope of the *mBSPD* versus gestational age was $c = -0.08 \text{ dB}\cdot\text{wk}^{-1}$. *mBSPD* was higher in nulliparous, as compared to multiparous, subjects ($b = 0.83 \text{ dB}$).

SAC—Figure 3 shows the SAC as a function of gestational age for multiparous and nulliparous macaques. Median and IQR values of the SAC are summarized in Table 1. The SAC demonstrated a significant dependence on gestational age ($p < 0.001$). The slope of the SAC versus gestational age was $f = -0.03 \text{ dB}\cdot\text{cm}^{-1}\text{MHz}^{-1}\text{wk}^{-1}$. SAC was not significantly different in nulliparous compared to multiparous subjects (i.e. not significantly different from 0; $p = 0.414$).

Discussion

Our goal is to identify complementary imaging biomarkers of cervical remodeling which can combine to provide comprehensive information about the process. Invasive, *ex vivo* studies in rodents demonstrate that cervical remodeling during pregnancy involves gradual disorganization of tissue microstructure, and this is associated with an increasingly soft and compliant cervix (Word et al., 2007; Akins et al., 2010, 2011; Mahendroo, 2012; Myers et al., 2015a). Invasive investigation in pregnant women is impossible due to logistical considerations, and therefore we developed a Rhesus macaque model because nonhuman primates are hormonally, anatomically and mechanically the closest we can get to human biology (Owiti et al., 1989; Golos, 2004; Shively and Clarkson, 2009; Ratajczak et al., 2010; Adams Waldorf et al., 2011; Micks et al., 2012; Bezold et al., 2013; Adams Waldorf et al., 2015). Our initial studies involved using (nonpregnant) hysterectomy specimens to verify shear wave speed estimates (SWS) and backscatter parameters (e.g. mean backscattered power difference, *mBSPD*) with actual tissue microstructure examined via nonlinear optical microscopy imaging in both Rhesus (Guerrero, 2018) and women (Guerrero et al., 2018b). Subsequently, we demonstrated *in vivo* with SWS that the cervix progressively softens as pregnancy advances in the Rhesus (Rosado-Mendez et al., 2017, 2018) and that the human cervix is significantly softer in late, as opposed to early, pregnancy (Carlson et al., 2015, 2018). The latter has been demonstrated by others as well (Muller et al., 2015). Further, biomechanical studies on cervical tissue from hysterectomy specimens in a group of women who were not pregnant and another group that underwent cesarean hysterectomy indicate that the tensile tangent modulus is softer by at least 3 orders of magnitude in the latter (Myers et al., 2008; Yao et al., 2016), and fiber-based material modeling suggest that this softening is accommodated by changes to cervical microstructural organization (specifically,

increased spreading of collagen fibers and intrinsic remodeling of fiber crosslinking) (Myers et al., 2015b). Consistent with all of these studies is our finding that *mBSPD* is significantly reduced in late, as compared to early, pregnancy in women, which suggests that softening is associated with changes in scatterers (presumably collagen) in cervical tissue microstructure (Guerrero et al., 2018a).

In this study, we hypothesized that the Rhesus cervix would demonstrate microstructural disorganization in late pregnancy as compared to early pregnancy. We further hypothesized that this disorganization would be steadily progressive, as in rodents. As expected, both the *mBSPD* and *SAC* declined progressively throughout pregnancy (Figures 2 and 3). The linear mixed effects model (Table 2) confirmed that both the *mBSPD* and *SAC* were significantly dependent on gestational age. These trends are consistent with studies of *SAC* (McFarlin et al., 2010) and *SWS* (Muller et al., 2015) in pregnant women, and also with invasive studies in rodents (Akins et al., 2010; Yoshida et al., 2014).

Similar to our findings in women, it appears that the *SAC* has larger estimate variance (compared to changes with gestational age) than the *mBSPD* and therefore may be less useful as an imaging biomarker of cervical remodeling. Table 3 compares women (McFarlin et al., 2010, 2015a,b; Guerrero et al., 2018a) to Rhesus in the first trimester (1T) and third trimester (3T). *QUS* parameter variability in the Rhesus decreases throughout pregnancy, mirroring reductions in *SWS* variability found throughout pregnancy in both Rhesus and women (Carlson et al., 2018; Rosado-Mendez et al., 2018), which suggests that the tissue becomes not only softer but also more homogeneous with remodeling. However, larger reductions in the *IQR* of the *mBSPD*, as compared to the *SAC*, are evident by the observation that the *IQR* of the *SAC* overlaps between P4 and P23 while the *IQR* of the *mBSPD* does not, which suggests that the latter may be a more precise indicator of remodeling. This high inter-subject variability has also been observed in studies of the *SAC* in the human cervix (McFarlin et al., 2010, 2015b). In addition, the *mBSPD* indicates that the Rhesus cervix in early pregnancy is more highly organized than the human cervix, but, by late pregnancy, the measurements are similar, while the *SAC* is less consistent with findings in women.

Although we are unsure of the mechanisms leading to softening of the cervix, the hypothesis that the collagen microstructure is reorganizing, as demonstrated in mice by Akins et al. (2010), is supported by the concurrent reduction in shear wave speed (Rosado-Mendez et al., 2018) and *mBSPD* shown in Figure 4. However, the limitations of this study, described below, deters drawing any strong conclusions at this time.

In summary, imaging biomarkers which objectively quantify cervical remodeling should help elucidate the mechanisms of remodeling, leading to improved prediction of birth timing, and, critically, strategies to prevent abnormal timing (preterm or postterm birth). The *mBSPD* and to a lesser extent the *SAC* are promising biomarkers of cervical microstructural organization.

A few limitations, all related to the prototype transducer we used, hinder the generalizability of this study. This transducer has a small aperture (14 mm) which limits interrogation depth

to about 1 cm or less for angle-dependent parameter estimates. Also, it is unfocused in the elevational direction, which results in suboptimal B-mode imaging and subsequent decreased ability for the operator to determine sampling location. In addition, the fact that it had to be affixed to an examiner's finger created logistical difficulties with respect to reaching the cervix in some subjects. To overcome these limitations, we have now an optimized transducer. This transducer has a larger aperture, elevational focusing, and a handle, all of which should facilitate more precise data acquisition, greater repeatability and reproducibility, and accessibility for other investigators. We are using this transducer in an ongoing longitudinal study in pregnant women, and we intend to use it for our next studies in macaques.

Conclusion

This longitudinal study suggests that quantitative ultrasound parameters based on the backscattered echo signal power may be useful biomarkers of cervical remodeling. We found progressive declines in the mean backscattered power difference (*mBSPD*) and the specific attenuation coefficient (*SAC*) throughout pregnancy, suggesting progressive disorganization of tissue microstructure, which is consistent with findings from invasive studies in other animal models as well as noninvasive studies in women. Further, trends in both parameters in Rhesus were consistent with those in women, making the Rhesus macaque an ideal animal model for studying cervical remodeling in pregnancy.

Acknowledgements

The authors would like to thank Ernest Madsen and Gary Frank for constructing the phantoms used in this study. We are also grateful for the technical support from Siemens Ultrasound. Research reported in this publication was supported by National Institutes of Health Grants T32CA009206 from the National Cancer Institute, R21HD061896, R21HD063031, and R01HD072077 from the Eunice Kennedy Shriver National Institute of Child Health and Human Development, UL1TR000427 the Clinical and Translational Science Award program, through the NIH National Center for Advancing Translational Sciences, and P51RR000167 to the Wisconsin National Primate Research Center from the National Center for Research Resources. This research was conducted in part at a facility constructed with support from Research Facilities Improvement Program grant numbers RR15459 and RR020141. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

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Figure 1:

(a) Experimental setup. The dashed-line box represents the approximate area of data acquisition (shown in Fig. 1c). (b) Beam steering angles. By convention, negative angles (red dash-dot line) are defined as moving toward the left, and positive angles (green dash-double-dot line) toward the right, side of the imaging plane. (c) B-mode image from a Rhesus macaque cervix. Regions of power spectral estimation are demarcated by solid boxes. The hyperechoic region between the boxes is the cervical canal.

Figure 2:

The mean BSPD as a function of gestational age for (a) multiparous and (b) nulliparous subjects. P# corresponds to the 'pregnancy week number' (gestational age). The horizontal line near the middle of each box is the median value for that group, the boxes represent the interquartile range (IQR) among parameter estimates, the whiskers represent the maxima and minima within $1.5 \times \text{IQR}$, and the dots are outliers outside of $1.5 \times \text{IQR}$. The solid line represents the linear mixed effects regression model fit to $m\text{BSPD}$ versus gestational age from P4 to P23.

Figure 3:

The SAC as a function of gestational age for (a) multiparous and (b) nulliparous subjects. P# corresponds to the week of pregnancy (gestational age). The solid line represents the linear mixed effects regression model fit to SAC versus gestational age (P4 to P23).

Figure 4:

A box plot of the shear wave speeds reported by Rosado-Mendez et al. (2018) and the mean backscatter power difference estimates for data acquired concurrently in the same animals.

Table 1:

Median (IQR lower – IQR upper) of the mean backscattered power difference (*m*BSPD in dB) and specific attenuation coefficient (SAC in dB·cm⁻¹MHz⁻¹) versus gestational age for multiparous and nulliparous macaques. P# stands for week of pregnancy (gestational age).

	<i>m</i> BSPD		SAC	
	Multiparous	Nulliparous	Multiparous	Nulliparous
P4	3.74 (3.21–4.49)	5.28 (3.70–5.94)	1.49 (1.29–1.89)	1.74 (1.32–2.31)
P10	3.68 (3.15–4.60)	3.98 (2.96–5.48)	1.17 (1.01–1.36)	1.23 (0.92–1.57)
P16	3.00 (2.09–3.76)	4.26 (2.99–5.56)	1.10 (0.94–1.33)	0.87 (0.26–1.17)
P20	3.02 (2.16–3.83)	3.58 (2.55–4.60)	1.16 (1.01–1.81)	0.75 (0.33–1.33)
P23	2.23 (2.02–3.45)	3.10 (2.20–3.41)	1.02 (0.98–1.11)	1.16 (0.57–1.43)

Table 2:

Mixed effect model coefficients, 95% confidence intervals (CI), and *p*-values resulting from linear regression of mBSPD and SAC (separate models) versus gestational age (4–23wk).

mBSPD				
Variable	Coefficient	Value	CI	<i>p</i>-value
Intercept	<i>a</i>	4.507	(3.703 – 5.310)	—
Parity	<i>b</i>	0.826	(0.213 – 1.439)	0.008
GA	<i>c</i>	−0.078	(−0.123 – −0.033)	<0.001
SAC				
Variable	Coefficient	Value	CI	<i>p</i>-value
Intercept	<i>d</i>	1.757	(1.418 – 2.096)	—
Parity	<i>e</i>	−0.144	(−0.489 – 0.201)	0.414
GA	<i>f</i>	−0.031	(−0.047 – −0.015)	<0.001

Table 3:

Comparison of median (IQR lower – IQR upper) or mean $\pm$ STD *m*BSPD and SAC estimates from this study and those found in women. Rhesus macaque data presented in this table includes nulliparous and multiparous specimen. 1T and 3T stand for first and third trimester, respectively.

		<i>m</i> BSPD	SAC
Rhesus Macaque	P4	4.30 (3.45 – 5.91)	1.55 (1.30 – 2.23)
	P23	2.50 (2.08 – 3.43)	1.04 (0.82 – 1.39)
Humans [*]	1T	2.79 (2.30 – 3.54)	1.56 (1.31 – 2.26)
	3T	1.96 (1.62 – 2.82)	1.45 (1.09 – 2.38)
Humans	1T [†]	—	1.4 $\pm$ 0.36
	3T [‡]	—	1.1 $\pm$ 0.4

Human data comes from

^{*} Guerrero et al. (2018a),

[†] McFarlin et al. (2010) and

[‡] (McFarlin et al., 2015a).