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RESEARCH ARTICLE

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The regional effect of serum hormone levels on cerebral blood flow in healthy nonpregnant women

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Abstract

Sex hormones estrogen (EST) and progesterone (PROG) have received increased attention for their important physiological action outside of reproduction. While studies have shown that EST and PROG have significant impacts on brain function, their impact on the cerebrovascular system in humans remains largely unknown. To address this, we used a multi-modal magnetic resonance imaging (MRI) approach to investigate the link between serum hormones in the follicular phase and luteal phase of the menstrual cycle (MC) with measures of cerebrovascular function (cerebral blood flow [CBF]) and structure (intracranial artery diameter). Fourteen naturally cycling women were recruited and assessed at two-time points of their MC. CBF was derived from pseudo-continuous arterial spin labeling while diameters of the internal carotid and basilar artery was assessed using time of flight magnetic resonance angiography, blood samples were performed after the MRI. Results show that PROG and EST had opposing and spatially distinct effects on CBF: PROG correlated negatively with CBF in anterior brain regions ($r = -.86$, $p < .01$), while EST correlations were positive, yet weak and most prominent in posterior areas ($r = .78$, $p < .01$). No significant correlations between either hormone or intracranial artery diameter were observed. These results show that EST and PROG have opposing and regionally distinct effects on CBF and that this relationship is likely not due to interactions with large intracranial arteries. Considering that CBF in healthy women appears tightly linked to their current hormonal state, future studies should consider assessing MC-related hormone fluctuations in the design of functional MRI studies in this population.

KEYWORDS
arterial spin Labelling, cerebral blood flow, estrogen, menstrual cycle, neurovascular coupling, progesterone, time-of-flight

1 | INTRODUCTION

Over the last decades, several lines of research have demonstrated that ovarian hormones are not only important for female reproduction, but they can also interact with the central nervous system and thereby influence cognitive function (Almey, Milner, & Brake, 2015; Le, Thomas, & Gurvich, 2020; Luine, 2014; Sellers, Raval, & Srivastava, 2015). The neurosteroids Estrogen (EST) and Progesterone (PROG) can be synthesized by neurons themselves or enter the parenchyma by crossing the blood–brain barrier (Kellogg & Frye, 1999; Pardridge & Mietus, 1979). EST's effects on behavior and brain function has been widely studied (Comasco & Sundström-Poromaa, 2015; Luine, 2014; Poromaa & Gingnell, 2014; Sacher, Okon-Singer, & Villringer, 2013; Sherwin & Grigorova, 2011) and results suggest that EST may boost neuronal excitability by interacting with glutaminergic neurotransmission (Barth, Villringer, & Sacher, 2015; Del Río et al., 2018; Finocchi & Ferrari, 2011; Foy et al., 1999; Pelligrino & Galea, 2001; Woolley, 2007). On the other hand, PROG, along with its derivatives pregnanolone and allopregnanolone, interacts with gamma amino butyric acid (GABA) receptors (Barth et al., 2015; Birzniece et al., 2006; Bitran, Purdy, & Kellog, 1993; Carver & Reddy, 2013; Del Río et al., 2018; Reddy, 2018; Smith, Shen, Gong, & Zhou, 2007) and inhibit neuronal activity (Belelli, Bolger, & Gee, 1989; Bitran et al., 1993; Gee, Bolger, Brinton, Coirini, & McEwen, 1988; Jeffrey et al., 2014; Puia, Gullo, Dossi, Lecchi, & Wanke, 2012; Taubøll & Lindström, 1993). For example, intravenous injection of 200 μg/kg PROG has been found to decrease the spontaneous spiking of cortical excitatory neurons by ∼50% in animals (Phillis, 1986). Behaviorally, PROG and its metabolites have been shown to reduce anxiousness in rodents in a dose-dependent manner (Bitran et al., 1993; Wieland, Lan, Mirasedeghi, & Gee, 1991), and having sedative properties in humans at high doses (Wu & Burnham, 2018). As changes in neuronal activity are typically associated with changes in regional cerebral blood flow (CBF; Dukart et al., 2018; Iadecola, 2017; Spanaki et al., 1999; Wolff, 1990), Magnetic Resonance Imaging (MRI) measures of whole-brain perfusion such as arterial spin labeling (ASL) could provide a noninvasive window into how hormones affect metabolism and/or neuronal activity in different cortical regions.

As it stands, relatively little is known regarding the role sex hormones play in regulating blood flow. There is indirect evidence in humans that EST is associated to enhanced endothelial function, and protects against hypertension (August & Suthanthiran, 2006), stroke, and cerebrovascular incidents (Lisabeth & Bushnell, 2012). Moreover, animal models have shown direct links between EST and increased production of vasodilators such as nitric oxide and prostaglandins by acting on receptors in the endothelial and smooth muscle cells of arteries (Krause, Duckles, & Pelligrino, 2006; Stanhewicz, Wenner, & Stachenfeld, 2018; Stirone, Duckles, & Krause, 2003). These vascular effects are linked to relaxation of the large cerebral arteries in animals (Duckles & Krause, 2007; Littleton-Kearney, Agnew, Traystman, & Hurn, 2000; Miller & Mulvagh, 2007; Pelligrino & Galea, 2001; Ramírez-Rosas, Cobos-Puc, Sánchez-López, Gutiérrez-Lara, & Centurión, 2014). Considering the excitatory and vasodilatory effects of EST, increasing serum levels could lead to widespread increases in CBF. Interestingly, PROG may have opposing effects on CBF since GABAergic agonists are generally associated with decreases in CBF (Dukart et al., 2018; Wolff, 1990) and metabolism (Spanaki et al., 1999), possibly due to reductions in neuronal activity. Moreover, it is possible that these effects may be stronger in specific brain areas, as EST and PROG receptor density vary across the cortex (Barth et al., 2015; Diaz Brinton et al., 2008). However, it is important to note that the vascular effects of sex hormones in animals are largely documented at supraphysiological levels (Pelligrino & Galea, 2001), and it is unclear how these effects translate to humans.

Reproductive life stages are associated with natural changes in the circulating levels of serum EST and PROG, thus providing an excellent avenue to investigate the effects of sex hormones on CBF. Studies evaluating CBF in human pregnancy (Zeeman, Hatab, & Twickler, 2003) and postmenopausal hormone therapy (Ohkura et al., 1995) have yielded conflicting results, yet are confounded by hematological changes (Sanghavi & Rutherford, 2014) and age-related changes in vascular health (Petersen, Zimine, Ho, & Golay, 2006; Thurston et al., 2018). Studies of healthy women across the menstrual cycle (MC) are less susceptible to these biases, and previous studies suggest little to no effect of sex hormones on CBF (Buchpiguel, Alavi, Crawford, Freeman, & Newberg, 2000; Ghisleni et al., 2015; Swihart, Mathew, & Largen, 1989). One limitation of the studies is that serum concentrations of EST and PROG were not evaluated. This may be important as EST and PROG are documented to have opposing effects on neural activity and may exhibit concentration-dependent relationships with CBF. Thus, studying the concentration-dependent-effects of EST and PROG on CBF may be a more sensitive first step to elucidate the potential effects of hormones on blood flow to the brain.

Therefore, the main goal of this study was to directly investigate the link between CBF and hormones while controlling for the size of the large feeding arteries using a multi-modal approach combining ASL and noncontrast enhanced Time-Of-Flight (TOF) magnetic resonance angiography. Additionally, we evaluated if this relationship is dependent on the phase of the MC and if the relationship between CBF and serum concentrations of EST and PROG varied between brain regions. It is hypothesized that EST will have a positive relationship with CBF and arterial diameters while PROG will have a negative relationship with CBF and no relationship with arterial diameters.

2 | METHODS

2.1 | Participant recruitment and selection

Participants were recruited via posters on campuses of Université de Sherbrooke and Bishop's University. Interested volunteers were given a screening questionnaire about their MC, use of hormonal contraception, mental health history, neurological disorders, and use of psychotropic drugs. Participants were excluded if they did not have a regular,

predictable MC (<24 days or >32 days, variability of more than 5 days in the length of one cycle to another), had a history of psychiatric or neurological disorders, use of psychoactive drugs (prescribed or recreational), or use of any medication with known vasoactive properties (i.e., blood pressure medications), history of seizures, presence of metal in their body, chance of being pregnant or breastfeeding, and use of any hormonal contraceptives in the last 6 months prior to participating in the study. The study was approved by the Internal Ethics Review Board of the Centre Hospitalier Universitaire de Sherbrooke (CHUS) and was conducted in accordance with the Declaration of Helsinki of 1975. After reviewing inclusion criteria, participants gave informed, written consent.

2.2 | Study design

Twenty participants were enrolled in the study; three dropped out, and technical issue prevented the inclusion of three participants, leading to a final sample of 14. Only participants who completed both sessions were included in the analysis. Participants were assigned two session dates, one in the follicular phase (FP) and one in the luteal phase (LP) of the MC. Session dates were predicted using calendar-based methods with the onset of menstruation of the previous cycle as day 1 and the average MC length, based on the average number of days between the first day of menstruation and the next start of menstruation. The FP was defined as day ~5–10 post onset of menstruation. This was chosen over day 13–14 (peak EST) because it facilitated scheduling by giving more days available to participants for their FP session. The LP was defined as day ~20–25 post onset of menstruation. Participants were pseudorandomly assigned to FP or LP start group to prevent order effects. FP and LP status were confirmed by serum concentrations of EST and PROG obtained from blood test performed after the MRI acquisition on the day of each experimental session. Participants were scanned between 11:30 a.m. and 2:30 p.m., Monday and Friday.

2.3 | MRI acquisition

MRI data was collected on a 3 T Ingenia scanner equipped with a 32-channel head-coil (Philips Healthcare, Best, Netherlands). A high-resolution 3D gradient-echo T1 weighted image was first acquired (flip angle (FA) = 8°, TR/TE 7.9/3.5 ms, 1 mm isotropic voxels, with a field of view (FOV) of 240 × 240 × 161 mm), total scan time was 6 min and 1 s. Next, images of the cerebral vessels were acquired using TOF sequences; first, a low-resolution TOF composed of four chunks to encompass the entire brain was acquired (FOV = 200 × 200 × 70mm, TR = 23 ms, TE = 3.6 ms, FA = 18°, parallel imaging [SENSE] acceleration factor = 3, acquisition resolution of 0.5 × 0.8 × 1.4, reconstructed resolution of 0.5 × 0.5 × 0.7 mm), total scan time was 2 min and 38 s. This image was used as a scout image to correctly place a high-resolution TOF comprised of one chunk that was centered on the Circle of Willis (FOV = 200 × 200 × 30mm, TR = 23 ms, TE = 3.6 ms, FA = 18°, parallel imaging [SENSE] acceleration factor = 2.8, acquisition resolution of 0.4 × 0.4 × 0.8, reconstructed resolution of 0.4 mm isotropic), the total scan time was 5 min and 32 s. This was followed by a low resolution, magnetic resonance angiography (MRA) of the intracranial arteries (ICA) and the vertebrate arteries (FOV = 150 × 78.75 × 66mm, TR = 23 ms, TE = 3.45 ms, FA = 22°, acquisition resolution of 1.5 × 1.5 × 3mm, reconstructed resolution of 0.47 × 0.47 × 1mm), total scan time was 1 min and 13 s. This image was reconstructed into a 3D-maximum intensity image and used to place the labeling slab for the pseudo-Continuous ASL (pCASL) sequence to improve reliability between sessions. The pCASL sequence was acquired with the following parameters: background suppression = true, label duration = 1650 ms, postlabel delay = 1800 ms, 2D multislice EPI readout, TR/TE 4246/16 ms, 22 4 mm slices, 3 × 3 mm voxels, with a FOV of 240 × 240 mm, and 30 label/control pairs for a total of 60 dynamics. The total scan time was 4 min and 23 s. Lastly, the M0 sequence for CBF quantification was acquired with the following parameters: 2D multislice EPI readout, TR/TE 12000/17 ms, 22 5 mm slices, 3 × 3 mm voxels, with a FOV of 240 × 240 mm, total scan time: 1 min and 12 s.

2.4 | Blood tests & MC phase confirmation

Blood draws were performed after the MRI protocol on each session. The samples were analyzed at the CHUS biomedical laboratory using enzyme-linked immunosorbent assays (ELISA) to determine the serum concentration of EST, PROG which have a coefficient of variation (CoV) of 5%, follicle stimulating hormone (FSH) and luteinizing hormone (LH) which have a CoV of 3%. Upon reception of the serum results, the session was confirmed as FP if the PROG concentration was <5 nmol/L and into LP if PROG was higher than 5 nmol/L. If there was no session in which PROG was higher than 5 nmol/L, the participant was excluded from the analysis. No participant was ovulating at the time of the experiment.

3 | DATA PREPARATION

3.1 | MRI preprocessing

3.1.1 | T1-weighted

T1-weighted images were first converted from DICOM to NIFTI using MRIConvert (Lewis Center For Neuroimaging, 2019) skull stripped and registered to the Montreal Neurological Institute ICBM152 asymmetric brain template (Fonov et al., 2011; Fonov, Evans, McKinstry, Almli, & Collins, 2009) using AFNI (Cox, 1996). This was then segmented using FSL (Jenkinson, Beckmann, Behrens, Woolrich, & Smith, 2012; Smith et al., 2004; Woolrich et al., 2009) to generate gray matter (GM) and white matter (WM) masks (tissue class probability of 50% or more).

3.1.2 | ASL data

ASL images were converted from DICOM to NIFTI using an inhouse conversion program in MATLAB (MathWorks 2019, Natick, MA). Preprocessing (registration, motion correction, tag/control subtraction) was performed using AFNI and FSL. CBF was quantified using the BASIL toolbox from FSL. An example of CBF maps in the FP and LP in one participant are shown in Figure S1.

3.1.3 | TOF data

TOF images were converted from DICOM to NIFTI using MRIconvert (Lewis Center For Neuroimaging, 2019). Lumen diameter values of the ICA and basilar artery (BA) were extracted using a previously published method (Bernier, Cunnane, & Whittingstall, 2018; Bizeau et al., 2018). For ICA, diameter estimates were averaged within the straight portion of the ICA in the imaging slab, this area constituted the region in the blue box in Figure 1. This region was selected as it was the straightest portion of the carotid within our imaging slab and had no branching arteries. The BA diameter was extracted from just below the bifurcation of the superior cerebellar arteries to the bottom of the slab (black box in Figure 1).

4 | DATA AND STATISTICAL ANALYSIS

To quantify the link between serum hormone concentrations and voxel-wise perfusion, we computed Pearson correlation coefficients between EST and CBF in the FP (CBF_{FP}, EST_{FP}) and PROG, EST and CBF in the LP (CBF_{LP}, $PROG_{LP}$, EST_{LP}). Intra-individual effects were investigated by correlating the change in serum EST (ΔEST) and PROG (ΔPROG) with the corresponding change in CBF (ΔCBF) between the FP and LP session. All CBF correlations were computed in the GM only, in which GM was defined as 50% or more of the GM probability map. Due to low serum concentrations of PROG in the FP, CBF correlations were not investigated. Given the relatively small sample size, a data randomization approach was used to evaluate concentration-dependent effects of hormones on CBF. For this, each whole-brain correlation map was subject to a cluster analysis based on two thresholds: First, hormone and CBF data was randomly shuffled (using MATLABs' *randperm*) and correlated. This process was repeated 1000 times to generate a "*shuffled*" distribution for the voxel-wise correlation between each hormone and CBF in each phase. The mean and standard deviation (SD) were then computed and compared to the observed distributions. The first threshold value used for the cluster analysis was ±2 SD of each respective shuffled distribution ($r = \pm 0.56$ for PROG and ±0.55 for EST). Of the remaining voxels, only clusters that made up of at least 1500 voxels whose faces touched (second threshold) were deemed statistically significant and retained for further analysis. Various combinations of different threshold values were used though this did not significantly change the results (results not shown). The size of the clusters were transformed to percentage of GM by dividing the total number of voxels in each cluster by the total number of GM voxels. Next, a partial correlation was performed for CBF_{LP} and $PROG_{LP}$ using EST_{LP} as a covariate, and for ΔCBF and ΔEST with ΔPROF as covariate and for ΔCBF and ΔPROG with ΔEST as covariate (using MATLABs' *partialcorr*). Lastly, the perfusion territories of the Anterior Cerebral Artery (ACA), Middle Cerebral Artery (MCA) and Posterior Cerebral Artery (PCA) were derived by manually labelling the regions in the brain referencing previously published postmortem arterial territory maps (Blumenfeld, 2010; Tatu, Moulin, Bogousslavsky, & Duvernoy, 1998; Van Der Zwan & Hillen, 1991). We used our previously published vascular atlas (https://github.com/braincharter/vasculature; Bernier et al., 2018) to guide the creation of the ROI for each arterial territory. This was used to assess the percentage of correlation clusters lying within each arterial perfusion territory. Serum hormone variables were tested for normality using Anderson-Darling test in MATLAB's (*adtest*). Here, serum EST_{LP} and $PROG_{LP}$ values were normally distributed, while EST_{FP} was not, though this was primarily driven by one participant whose EST was high (690 pmol/l). However, as this value is still within physiological range of the FP (Poromaa & Gingnell, 2014) this participant was not excluded from further analysis.

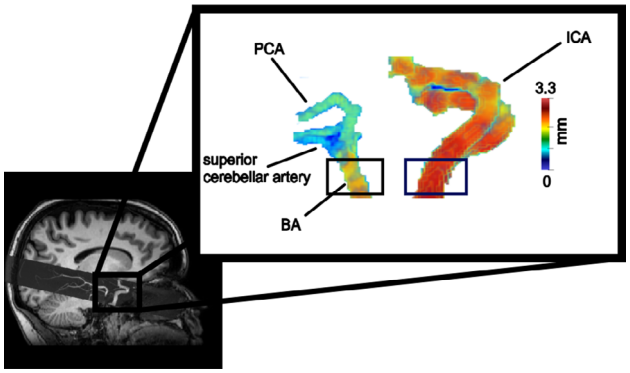

FIGURE 1 Sagittal view of T1-weighted image with overlay of maximum intensity projection of high resolution TOF slab depicting location of the ICA and BA arteries. Black box depicts segmented ICA and BA in sagittal view. The blue box depicts the region where ICA lumen diameters where averaged, the black box denote regions where BA lumen diameters were extracted and averaged

5 | RESULTS

Average serum hormones and demographic information is descripted in Table 1. Nine participants had never taken oral contraceptives, two had stopped taking them at 6 years prior to participating in the study and one had stopped taking them 1 year prior to participating in the study. Two participants did not specify if they had ever taken oral contraceptives prior to 6 months before participating in the study. MC phase and individual serum hormones are presented in Figure 2. Serum hormones were within normal ranges in all participants (Poromaa & Gingnell, 2014). Phase effects on CBF and arterial lumen diameters are summarized in Table S1.

Voxel-wise correlations between EST_{FP} and CBF_{FP} are shown in Figure 3a. In most voxels, EST_{FP} correlated positively, albeit weakly with CBF_{FP} (Figure 3a-1). After thresholding (see description in the methods), most significant correlations were localized to the occipital lobe (Figure 3a-2 and Table S2). EST_{FP} correlation clusters made up 3.36% of GM voxels. The voxel-wise correlations between EST_{FP} CBF were repeated using a spearman rank correlation to address the effects of the outlier. The majority of the voxels remain positively correlated, though no cluster survived the clustering threshold. Voxel-wise correlations between ΔEST and ΔCBF were also weak (Figure S2A-1 and Table S3) and covariate analysis with ΔPROG shifted the correlation distribution towards 0 (Figure S2A-4).

TABLE 1 Overview of participant characteristics and MC, expressed as mean ± SD

	Group average	
Age	24.33 ± 3.08	
Weight (kg)	61.46 ± 12.33	
Average MC length (days)	28.53 ± 1.97	
	FP (average ± SD)	LP (average ± SD)
Cycle day at each session	8.79 ± 6.47	20.27 ± 6.42
PROG (nmol/L)	1.15 ± 0.44	29.85 ± 13.95
EST (pmol/L)	210.29 ± 156.71	513.00 ± 164.44
FSH (UI/L)	5.19 ± 0.98	3.06 ± 1.58
LH (UI/L)	7.43 ± 4.12	5.57 ± 3.26

In stark contrast to EST, CBF was strongly and negatively correlated with $PROG_{LP}$ (Figure 3b-1 and Table S4). Significant correlation clusters were localized to the frontal and temporal lobe (Figure 3b2-3). Correlation coefficients and R^2 of the clusters are reported in Table S4. To control for the potentially confounding effect of high EST_{LP} concentrations, we recomputed $PROG_{LP}$–CBF_{LP} correlations while including EST_{LP} as a covariate (Figure 4a). Controlling for EST_{LP} did not significantly change the results. Specifically, the EST_{LP}–CBF_{LP} correlation clusters (Figure S3 and Table S5), after applications of the two thresholds, made up 1.7% of GM brain voxels (Figure 4b), which is less than what would be expected by chance (2.5%). On the other hand, correlation clusters for $PROG_{LP}$ made up 8.72% (Figure 4b). Similar results were obtained when correlating ΔPROG and ΔCBF, with the majority of voxels negatively correlating with

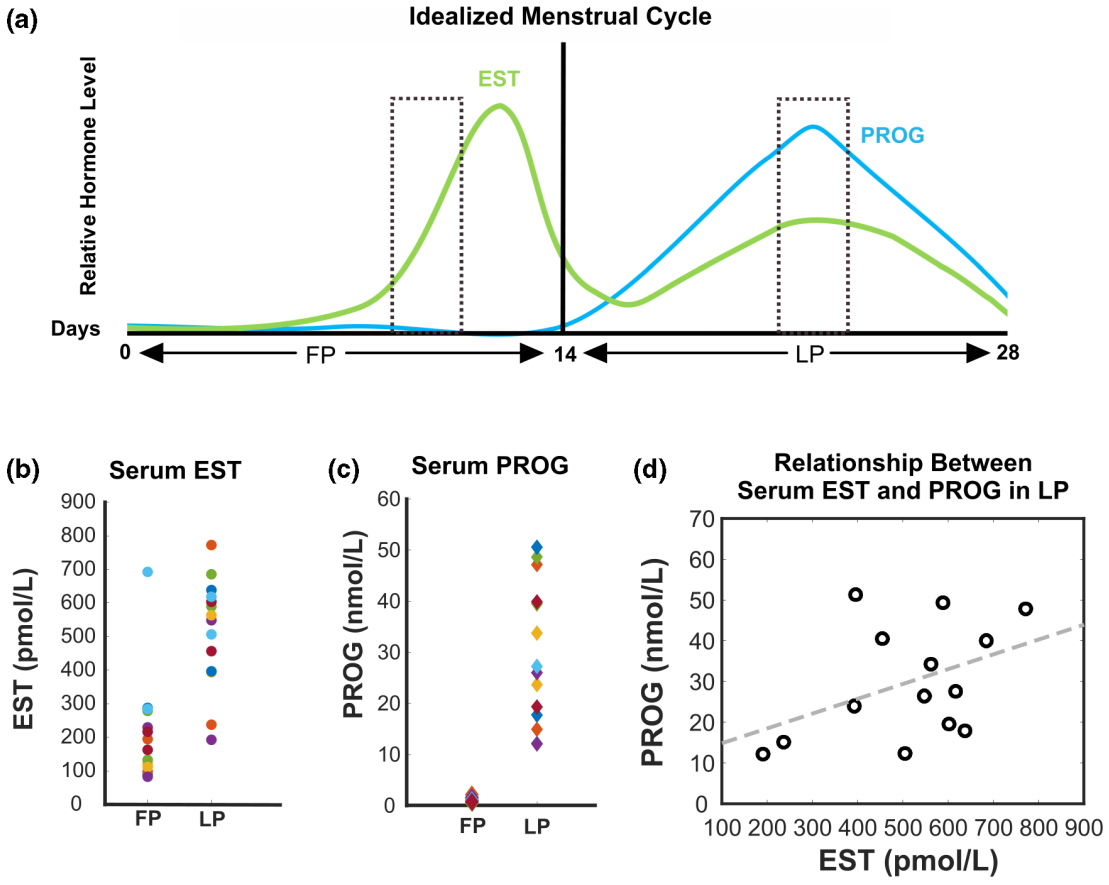

FIGURE 2 Idealized MC is shown in (a). Green line represents expected change in serum EST during the cycle, blue line represents expected change in serum PROG during the cycle. Gray rectangles represent approximately when the participants sessions were scheduled during the FP and LP. EST_{FP} and EST_{LP} in (b) and serum $PROG_{FP}$ and $PROG_{LP}$ are shown in (c). The relationship between EST_{LP} and $PROG_{LP}$ is shown in ([D]; $r = .43$, $p = .13$)

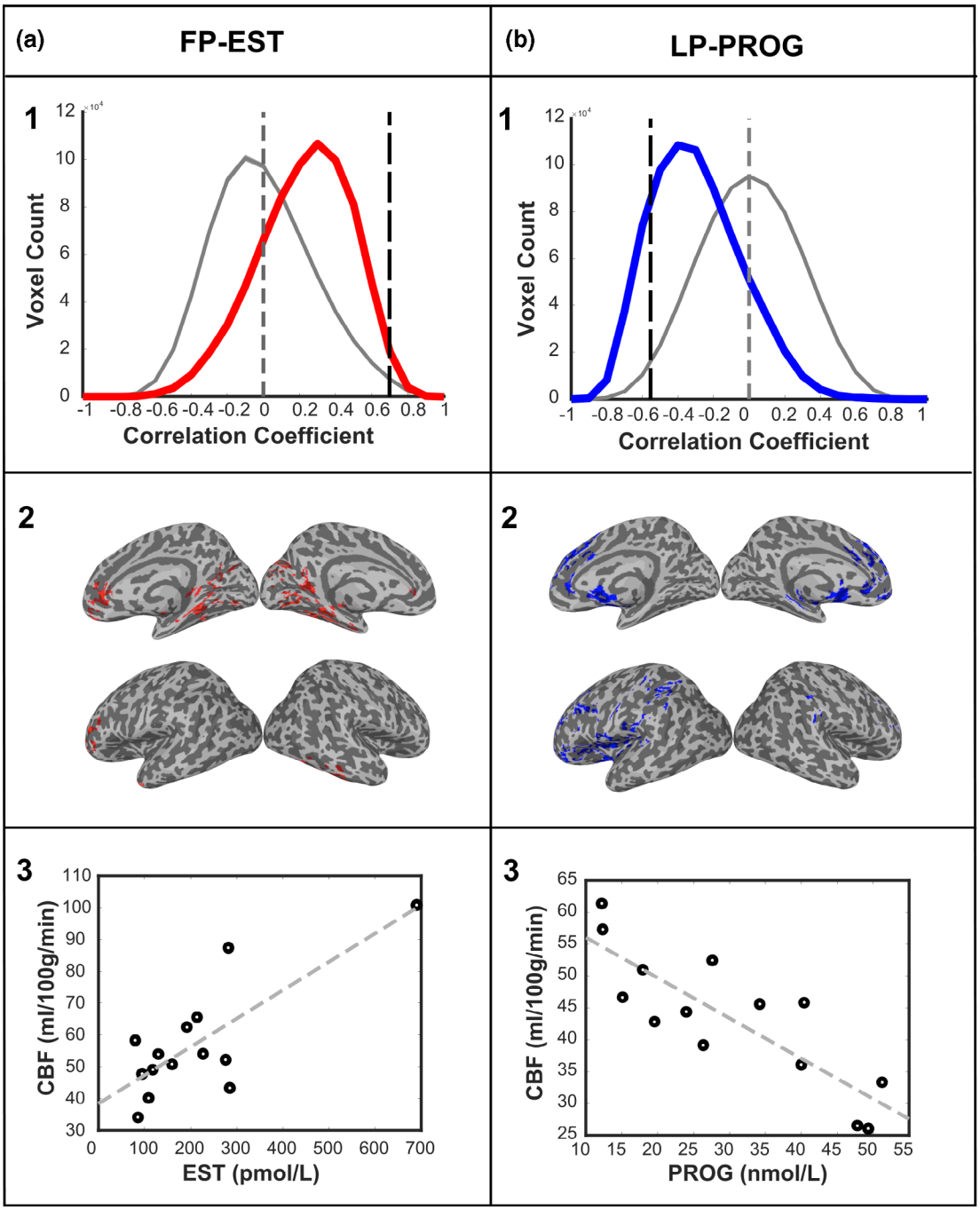

FIGURE 3 Correlation results between EST_{FP} and PROG and CBF. Compared to chance (gray distribution is shuffled correlations), the majority of voxels tended to correlate positively between EST_{FP} and CBF_{FP} (green distribution[A1] Dashed gray line represents $r = 0$, dashed black line is $r = 0.55$ which was used for clustering threshold). The correlation was strongest in the posterior portions of the brain (A.2) and were weakly correlated. PROG correlated negative with CBF compared to chance (B1; gray distribution is the shuffled correlations, dashed gray line represents $r = 0$, dashed black line is $r = -0.56$, which was used for clustering threshold) The correlations were strongest in the anterior circulation (B2) and were strongly, negatively correlated with CBF

CBF (Figure S2A-1–3 and Table S6). Covariate analysis including ΔEST did not change the relationship between ΔPROG and ΔCBF (Figure S2B-4).

Spatial analysis of the clustering pattern in the correlation between CBF and EST_{FP}, EST_{LP} and $PROG_{LP}$ are outlined in Figure 5. EST_{FP} effect was most pronounced in the posterior circulation (Figure 5a,c), these results showed that 46.95% of GM voxels in regions perfused by the PCA positively correlated with EST_{FP}. In contrast, $PROG_{LP}$ effect was strongest in the anterior circulation (Figure 5b,d), with $PROG_{LP}$ clusters making up 19.27% and 34.19% of the GM voxels within the ACA and MCA, respectively. Percentage of EST_{LP} clusters did not exceed that of chance in any of the perfusion

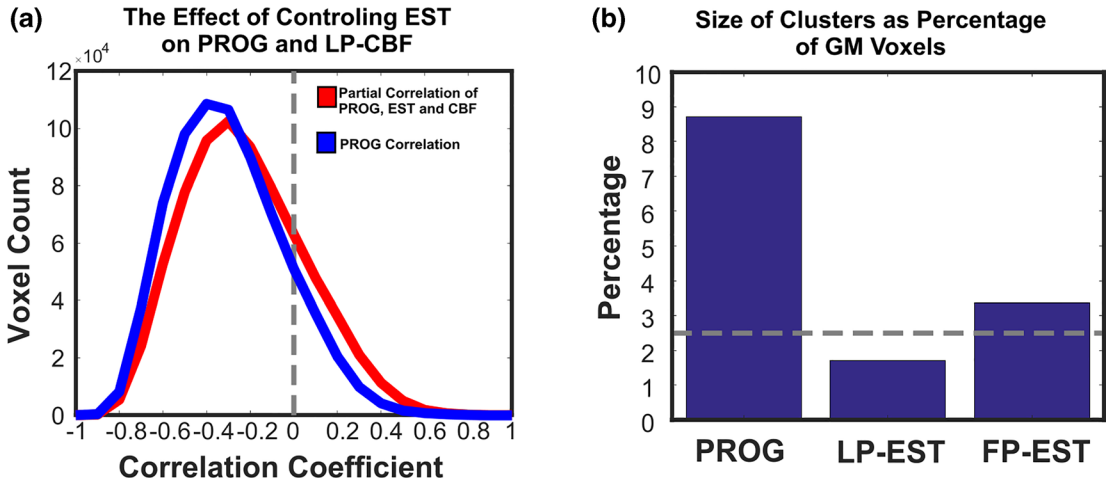

FIGURE 4 Controlling for serum EST in LP does not significantly alter the association between $PROG_{LP}$ and CBF_{LP} (a). $PROG_{LP}$ correlated with more GM-voxels than EST_{LP}. Percentage of EST_{LP} clusters were not more than what would be expected by chance ((b); Black-dashed line represents random chance, set as 2.5% or 2 SD of the shuffle distribution)

territory. Together, these results demonstrate that EST_{FP} has a very weak, positive relationship with CBF in the posterior circulation, while $PROG_{LP}$ has a strong, negative association with CBF in the anterior circulation.

We found no association between ICA or BA diameter in FP and EST_{FP} (ICA: $r = .22$, $p = .45$; BA $r = .32$, $p = .25$) or between ICA and BA diameter and CBF (ICA: $r = -.074$; $p = .8$; BA: $r = .23$, $p = .43$;). Likewise, diameter of intracranial arteries did not significantly correlate with $PROG_{LP}$ (ICA: $r = -.37$, $p = .20$ BA: $r = -.23$, $p = .42$) or EST_{LP} (ICA: $r = -.05$, $p = .87$; BA: $r = -.09$, $p = .76$) or CBF_{LP} (ICA: $r = .23$, $p = .42$; BA: $r = .24$, $p = .40$). The change in Diameter of the ICA and BA did not correlate with the ΔEST (ICA: $r = -.07$; $p = .82$; BA: $r = .24$; $p = .40$) or ΔPROG (ICA: $r = -.11$, $p = .71$; BA: $r = -.15$, $p = -.61$).

6 | DISCUSSION

We observed a relatively strong, inverse relationship between serum $PROG_{LP}$ and CBF_{LP}, while serum EST_{FP} tended to weakly correlate with CBF_{FP}. $PROG_{LP}$'s link with CBF_{LP} was strongest in frontal cortex. Additionally, controlling for EST_{LP} did not impact the relationship between $PROG_{LP}$ and CBF_{LP}, nor did the lumen diameters of the large arteries feeding the anterior and posterior circulation correlate with hormone levels. Taken together, these results show that EST and PROG have strikingly different and independent effects on CBF, and that these effects are likely not driven by large artery morphology.

In terms of $PROG_{LP}$, our observation of its strong, negative, correlation with CBF_{LP} indirectly agrees with the well documented relationship between PROG and GABA (Barth et al., 2015; Del Río et al., 2018). PROG's metabolites are allosteric modulators for GABA receptors (Barth et al., 2015; Bitran et al., 1993; Carver & Reddy, 2013; Del Río et al., 2018; Reddy, 2018) and have been reported to increase neuronal inhibition in a dose-dependent fashion (Belelli et al., 1989; Bitran et al., 1993; Gee et al., 1988; Jeffrey et al., 2014; Puia et al., 2012; Taubøll & Lindström, 1993). Behaviorally, PROG has also been shown to reduce anxiousness in rodents (i.e., increase time spend in open arm of maze; Bitran et al., 1993; Wieland et al., 1991) and to have sedative properties in humans at high doses (Y. V. Wu & Burnham, 2018), supporting its GABA-mediated effects. Interestingly, not all participants experienced a change in CBF in the same direction. Intra-individual differences analysis revealed that women who had small increases in serum PROG experienced increases in CBF while women who experienced large increases in serum PROG experienced decreases in CBF. This may be explained by Puia et al. (2012) finding's in which lower concentrations of PROG's metabolite, allopregnanolone increased firing rates of excitatory and inhibitory neurons which was followed by decreased excitability as concentrations increased (Puia et al., 2012). As CBF is usually coupled to neuronal activity (Iadecola, 2017) and the administration of a GABA agonist typically reduces CBF (Dukart et al., 2018; Spanaki et al., 1999; Wolff, 1990) and metabolism (Spanaki et al., 1999), our observation of a negative correlation between $PROG_{LP}$ levels and CBF_{LP} may reflect a PROG-induced neuromodulatory effect, mediated through GABA receptors. Moreover, it is well documented that local changes in neuronal activity and metabolism have direct impacts on local blood flow via astrocytic end-feet interactions with pericytes in which local changes in capillary tone result in response to local changes in CBF without changes in tone of the large extracranial arteries (Cipolla, 2016). This is concordant with our observation of a weak correlation between BA/ICA diameter and $PROG_{LP}$. These suggests that the relationship between CBF_{LP} and $PROG_{LP}$ is likely not driven by large artery morphology; however, it does not rule out potential interactions between PROG and penetrating arterioles.

In addition to the strong negative correlations, our results revealed that the clusters where $PROG_{LP}$'s effect was strongest primarily

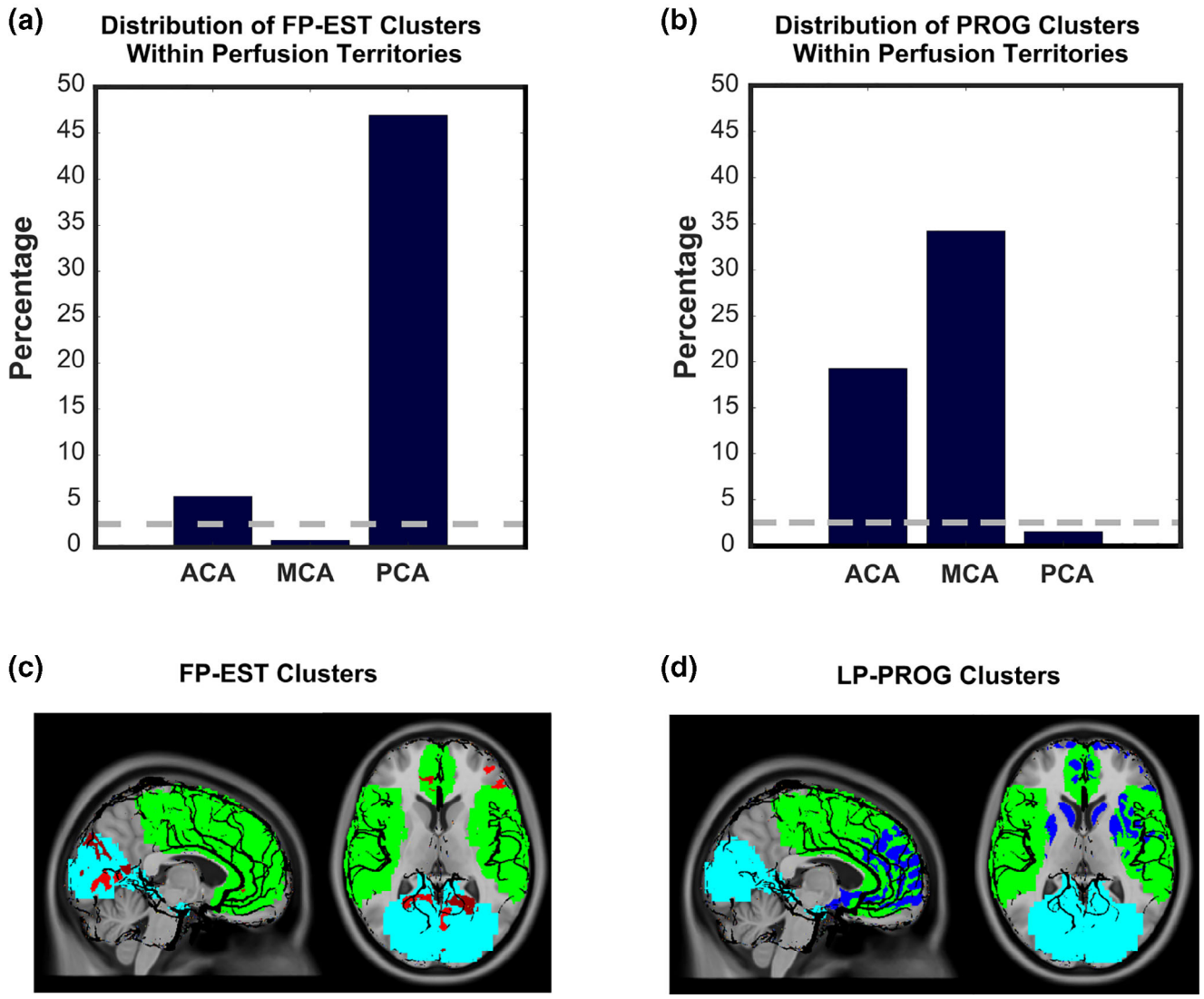

FIGURE 5 Correlations of EST_{FP} are primarily located in the perfusion territory of the PCA (a). PROG clusters are primarily located in the anterior perfusion territories (ACA and MCA; b). EST_{FP} clusters in red (c) and $PROG_{LP}$ clusters in dark blue (d) are shown in relation to a 3D vascular tree from Bernier et al., 2018's vascular atlas. Gray dashed line in (a) and (b) represent 2.5% threshold denoting significance. Green regions in (c) and (d) indicate anterior perfusion territories (ACA and MCA) and light blue in (c) and (d) represent PCA perfusion territory

fell within the territory of the ACA and the MCA, which represent the anterior circulation supplied by the ICA. This is particularly interesting as PROG and its metabolites are well documented $GABA_A$ receptor agonist and $GABA_A$ receptors are widespread throughout the brain (Barth et al., 2015), thus, interactions with the GABAergic system may not be the only explanation for our results. The regional pattern of the $PROG_{LP}$ clusters may be related to regional variations in PROG receptor density. In that regard, animal studies show that PROG receptor density is highest in the prefrontal cortex and limbic system (Barth et al., 2015; Bethea, 1993; Diaz Brinton et al., 2008), regions where we observed the strongest correlations between $PROG_{LP}$ and CBF_{LP}. These regions are important targets of neurotransmitter pathways such as dopamine and serotonin, which have been previously reported to be affected by PROG (Barth et al., 2015; Del Río et al., 2018). Hence, it is possible that the spatial distribution of the $PROG_{LP}$ clusters may reflect interactions between of PROG receptors and these neurotransmitters within these regions. Nevertheless, expression of these genes in the human brain may differ. To compare human gene expression of EST receptor alpha (ERα) and beta (ERβ) and PROG receptor we used the downloadable gene expression maps for each of these receptors from the Allen Human Brain Atlas (Hawrylycz et al., 2012; neurosynth.org/genes/) and compared gene expression in the three vascular territories (Figure S4). We note that expression does not follow our clustering pattern. This would suggest that receptor expression may not be the only explanation for our cluster pattern. As such, the reason why PROG and EST appear to favor different vascular territories remains elusive. Further studies are needed to better understand the regional influences of PROG on CBF, their interactions with receptors, neurotransmitters, and the cerebral vasculature.

We observed a weak, positive Pearson correlation between EST_{FP} and CBF_{FP}. This relationship appeared to be concentrated in the posterior circulation. Nevertheless, caution must be taken when interpreting our EST results as they appear to be strongly driven by one participant whose serum EST was 4 SD above the average EST_{FP}. Additionally, we observed no relationship between EST_{LP} and CBF_{LP} and the intra-individual differences in EST did not correlate with ΔCBF when controlling for ΔPROG. The combination of these results suggests that serum EST is weakly associated to CBF. This observation is supported by a recent study which reported no significant relationship between CBF and serum EST (Ghisleni et al., 2015). Lastly, it is important to note that our participant who was an outlier had serum EST_{FP} within the documented physiological range of serum EST_{FP} (Poromaa & Gingnell, 2014) and may reflect a limitation of our study and the difficulty associated with predicting MC phase and peak estrogen based on MC length (Becker et al., 2005; Mihm, Gangooly, & Muttukrishna, 2010; Sherman & Korenman, 1975). Confirming cycle length and date of last menstruation after completing the last experimental session may help address this limitation. Future studies are needed to clarify the relationship between serum EST and CBF.

We observed no association between either EST or PROG and diameters of the ICA or the BA, nor did we observe associations between CBF and diameter in either the FP or the LP. This result is not striking, as Krezja and colleagues observed no changes in diameter of the ICA during the peak in serum EST_{FP} (Krejza et al., 2003; Krejza, Mariak, Huba, Wolczynski, & Lewko, 2001; Jaroslaw Krejza, Mariak, Nowacka, Melhem, & Babikian, 2004), nor did Nevo et al. (2007) in women undergoing in vitro fertilization with serum EST five and a half times higher and serum PROG more than 7 and a half times higher than our study (Nevo, Soustiel, & Thaler, 2007). Thus, it is possible that serum concentrations of EST and PROG during the MC may not be elevated enough to impact the large intracranial arteries.

There is an inherent variability of the MC that exists both within and across women, notably, large interindividual differences in serum hormones, rapid fluctuations in hormones within each phase and the length of the MC (Becker et al., 2005; Chiazze, Brayer, Macisco, Parker, & Duffy, 1968; Fehring, Schneider, & Raviele, 2006; Mihm et al., 2010; Sherman & Korenman, 1975). These factors result in certain difficulties and limitations when designing studies whose goal is to evaluate women in the LP and the FP (Becker et al., 2005), in particular when predicting when a women will be at her highest serum hormones for both phases. A strength of our study in addressing this limitation, is the use of a within subjects' design and inclusion serum concentrations of sex hormones. This experimental design allowed us to study the effect of the concentration of serum hormone on CBF within each phase rather than relying solely on self-report and calendar-based methods and studying the effect of the phase alone. Another strength of our study is our multi-modal approach and the inclusion of large artery morphology. This component of our study allowed us to gain insight on what may be the driving force behind the relationships between sex hormones and CBF.

Certain limitations of this study should however be noted. While serum concentrations are a strength of our study, our serum values also highlight a limitation regarding the interpretation of the relationship between serum EST_{FP} and CBF_{FP}. Specifically, we only examined our participants once during the mid-FP. This period was chosen for our study because inter-cycle variability, rapid changes in serum EST (~350 mg/24 h; Reed & Carr, 2000) and participant and scanner availability made scheduling sessions for participants at their peak not feasible. Not studying women during their peak serum EST and lowest serum EST resulted in a limited range of EST which may hinder our ability to adequately characterize the concentration dependent relationship between EST and CBF. Future studies are needed which include the early FP (day 2–3; when serum EST is low) and just prior to ovulation (day 13–14; when serum EST is higher) before one can confidently state if there is no relationship between CBF and EST in normally cycling women. Our sample size is another limitation, particularly affecting the reliability of the effect size of our results. Next, while the diameter of large cerebral arteries were assessed, we did not evaluate diameters of the arteries stemming from the Circle of Willis (ACA, MCA, PCA), pial arteries and/or penetrating arterioles which have important influences on the control of CBF (Brown & Marshall, 1985; Cipolla, 2016; Kirkness, 2005; van der Veen et al., 2015). Nor did we evaluate electrical activity of neurons. To elucidate if our results are driven by sex hormones interacting with neurotransmission or small cerebral arteries, multimodal imaging using techniques such as high-field MRI vascular imaging, functional MRI, and EEG, with serological measures are needed.

Other important factors that could influence CBF, and that may be hormone-mediated, are arterial transit time (ATT; Alsop et al., 2015; Dai et al., 2017; MacIntosh et al., 2010; van Osch et al., 2018; Wu, Lou, Wu, & Ma, 2014; W. Wu, St Lawrence, Licht, & Wang, 2011), blood velocity (Gai & Butman, 2019; Krejza et al., 2003; Krejza et al., 2001; Krejza et al., 2004), blood pressure (Clement et al., 2018; Krejza et al., 2003; Krejza et al., 2001; Krejza et al., 2004) and blood properties such as viscosity and oxygen carrying capacity (Brown & Marshall, 1985; Javaid, Hasan, & Naim, 2007; Kotwaney & Shetty, 2014; van der Veen et al., 2015). As our PROG and CBF results were similar in the intra- and inter-women analysis, these factors are likely not the main source of this relationship. The same cannot be concluded for EST as its weak link with CBF vanished in the intra-women analysis. Further research using transit time mapping, phase-contrast-MRI, inclusion of hematological variables, and blood pressure may help clarify if these variables confound the relationship between EST and CBF.

7 | CONCLUSION

In conclusion, the results of our study showed that serum PROG has a strong, negative relationship with CBF, primarily in the anterior circulation while EST tended not to correlate with CBF. These results demonstrate that CBF is dynamic and related to hormonal state in women, highlighting the importance of considering interindividual variations in serum concentrations of sex hormones in women of reproductive age with regards to CBF measurement. Further elucidating the interaction

between CBF, hormones, and brain function may prove pivotal in assessing the long-term impact of hormonal therapies in women, as well as the fluctuation of neurological symptoms and mood across the MC.

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CONFLICT OF INTEREST

All authors declare that they have no conflicts of interest to declare.

AUTHOR CONTRIBUTIONS

Samantha Cote, Adrianna Mendrek, Jean-Francois Lepage, and Kevin Whittingstall conceived the study, analyzed the results and wrote the manuscript. Samantha Cote, Eric Lavallee, and Etienne Croteau collected data. Vincent Michaud and Russell Butler helped with data analysis and interpretation.

ETHICS APPROVAL STATEMENT

This project was approved by the internal Ethics Review Board of the Center Hospitalier Universitaire de Sherbrooke (CHUS) in accordance with the Declaration of Helsinki of 1975.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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