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Quantification of dynamic changes to blood volume and vascular flow in the primate corpus luteum during the menstrual cycle

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Abstract

Dynamic contrast-enhanced ultrasound (DCE-US) with intravenous microbubble infusion was performed on the rhesus macaque ovary bearing the pre-ovulatory follicle and corpus luteum (CL) sequentially during the natural luteal phase (n=8) and GnRH antagonist (Antide)-induced luteal regression (n=6). Changes in luteal blood volume (BV) and vascular flow (VF) were observed between stages of the luteal phase. Luteal BV was highest in early stage CL, before decreasing 2.5-fold in late stage CL ($P<0.06$); in contrast, luteal VF peaked at mid luteal stage ($P<0.01$). Two females identified with luteal insufficiency trended toward lower peak BV, compared to typical CLs. Another female was identified with a luteal cyst on the contralateral ovary, and a CL that regressed before P levels declined. After 72 hours of Antide exposure, BV was reduced 2.3-fold ($P=0.03$). DCE-US provides a sensitive, non-invasive measurement of the dynamics of blood volume and flow in dominant ovarian structures.

Keywords

dynamic contrast enhanced ultrasound; ovarian vasculature; luteal blood flow; rhesus monkey; non-human primate

Introduction

Doppler ultrasound is currently the most widely used technique to quantify ovarian blood flow in women (Dickey, 1997). However, conventional Doppler does not quantify blood

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Conflicts of Interest:

All authors have nothing to disclose.

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flow accurately through small capillary bed structures such as the network formed in the corpus luteum [CL; (Lindner, 2004)]. Use of contrast reagents combined with destroy-replenishment imaging [dynamic contrast-enhanced ultrasound (DCE-US)] increases the accuracy of blood flow and blood volume measurements within tissue/organs assessed by ultrasound (Lindner, 2009; Keator *et al.*, 2011). Applying DCE-US technology to quantify changes in ovarian and luteal vascular dynamics could provide a valuable, minimally invasive measurement of luteal function. This method could then be applied for use in both basic research and potentially diagnostic applications in primates and women.

The DCE-US procedure is performed by continuous intravenous infusion of small (~1-2 μM), stable, encapsulated bubbles of gas that circulate similar to red blood cells (microbubbles). Accurate quantification of blood flow to a region of interest (ROI) can be obtained by transiently destroying the existing microbubbles in the field of view with high power ultrasound [(Peak Acoustic Negative Pressure/[frequency]^{1/2}); termed mechanical index, MI], then allowing the tissue to return to saturation with low-MI non-destructive (i.e., low MI) microbubble imaging. A more extensive discussion on MI imaging and effects on microbubbles are provided in a review by Kaul (2002). By plotting relative microbubble intensity as a function of time, one can derive relative microbubble refill kinetics in a tissue of interest: rate of fill (β), relative blood volume (BV), and rate of flow through the ROI [VF; (Lindner and Sklenar, 2004)]. To correct for differences in gain and microbubble intensity between ultrasound sessions, relative intensity is normalized to the blood pool measurement obtained from a ROI defined on a large vessel, often the carotid or brachial artery.

Because quantitative measurements of luteal blood flow would provide valuable information on changes to luteal structure/function not only during the natural menstrual cycle, but also during experimental protocols, it is of interest to evaluate the potential of contrast-enhanced ultrasound to measure luteal vascular parameters. The objective of the current study was to evaluate the effectiveness of DCE-US to quantify luteal vascular function in rhesus monkeys, a valuable nonhuman primate model.

Materials and Methods

All procedures were performed on female rhesus macaques at the Oregon National Primate Research Center (ONPRC) during the 2010-2014 macaque breeding seasons, with approval from the OHSU/ONPRC Institutional Animal Care and Use Committee (IACUC), in compliance with the American Society of Primatologists (ASP) Principles for the Ethical Treatment of Nonhuman Primates, and the Animal Welfare Act (AWA; 1985) of the United States of America governing use of animals in research. All rhesus females were under the direct care of the ONPRC Department of Comparative Medicine (DCM); sedation and monitoring of monkeys during ultrasound examination was performed by nonhuman primate surgical technicians in the DCM Surgical Services Unit (SSU).

Animal Protocols

Hormonal status of adult, naturally cycling female macaques (age 12.6 $\pm$ 0.8 years, range 9.5-14.8; n=14) was monitored throughout imaging protocols beginning 6 days post-mense

until the end of the menstrual cycle (Figure 1), similar to Bishop *et al.* (2009b). The day of the luteinizing hormone (LH) surge (day 0) was defined as a drop in serum estradiol (E) to below 100 pg/ml (Bishop *et al.*, 2009b) with a coincident rise in progesterone (P).

Experiment 1

Female monkeys (n=8) were sedated with Telazol (4mg/kg) for imaging sessions at/near ovulation, and at the early, mid, mid-late and late luteal phase of the menstrual cycle (Bogan *et al.*, 2008). Normal luteal phases were defined, in part, based on previous conventional 3D/4D Power-flow Doppler ultrasound experiments with rhesus monkeys (Bishop *et al.*, 2009b): the observation of one ovary bearing a single large antral follicle that transitioned to a corpus luteum (CL). To account for individual differences in luteal function, we further stipulated that these cycles must have serum P levels peaking ≥ 3 ng/ml, indicating a fully functional rhesus CL (Wilks *et al.*, 1976).

Experiment 2

Female monkeys (n=6) with normal luteal phases (peak serum P ≥ 3.0 ng/ml) were sedated for imaging sessions with Telazol beginning on day 9 of the luteal phase (Time 0). Immediately following this initial imaging session, the gonadotropin-releasing hormone antagonist Antide (3 mg/kg) was administered subcutaneously to induce premature luteal regression as previously described (Duffy *et al.*, 1999). Females were imaged again at 24 and 72 hours post-Antide administration to investigate changes to luteal vascular function during induced luteal regression.

Ultrasonic Imaging

Ovarian morphology was first visualized by ultrasound (US) using a GE Voluson 730 Expert system similar to Bishop *et al.* [(2009b); GE Medical Systems, Waukesha, WI] equipped with a 4D transabdominal probe with color-flow and real-time power-flow Doppler imaging capabilities. Then, transabdominal DCE-US imaging was performed on the ovary bearing the dominant structure (large pre-ovulatory follicle/CL) using a Siemens ACUSON Sequoia system (Siemens Medical Systems, Mountain View, CA) as recently described for analysis of the macaque reproductive tract (Keator *et al.*, 2011) equipped with the contrast-specific imaging modality, Contrast Pulse Sequencing [CPS; (Phillips and Gardner, 2004)]. Use of CPS enhances both the linear and non-linear fundamental and harmonic acoustic signal from the contrast agent at a low MI of 0.18 without significant destruction of microbubbles. Coupled to the ultrasound system was a linear array transducer (15L8) transmitting at a frequency of 7.0MHz. Gain and dynamic range (55dB) was set prior to administration of contrast agent to minimize background tissue signal and held throughout the study.

A single vial (1.5 ml) of microbubble contrast agent, Definity TM (Lantheus Medical Imaging, N. Billerica, MA) was diluted into a total volume of 30 ml in a 0.09% saline solution. A continuous infusion of the diluted contrast agent was then administered using a syringe infusion pump (AS-40, Baxter Healthcare, Deerfield, IL) via peripheral intravenous access to achieve steady state. To establish blood pool acoustic intensity of microbubbles, contrast was administered at a rate of 10 ml/hr and the brachial artery imaged. Digital

acquisitions (~30-60 sec video clips) were captured at a time interval of every 65 msec. After completion of blood pool imaging, contrast administration rate increased to 60 ml/hr and the ovary bearing the dominant structure (large pre-ovulatory follicle/CL) was imaged in the plane of its maximum diameter at the same time interval as blood pool (65 msec). To quantify ovarian vascular flow, a burst-replenishment sequence was initiated to adequately destroy the microbubbles and allow for complete microbubble refill of tissue within the ultrasound beam profile. The destruction of microbubbles was accomplished by sending out high MI (1.9) ultrasound pulses over a period of 2 seconds, which immediately returns to the resting MI of 0.18 for replenishment. Each burst-replenishment sequence was ~30-60 sec to allow for complete replenishment of ovarian tissue. As with the blood pool imaging, burst-replenishment sequences were digitally acquired for future analyses.

Data and Statistical Analyses

Vascular parameters were quantified in DCE-US video captures with custom software [based on mathematical modeling of Wei et al (1997)], using a ROI defined on the dominant pre-ovulatory follicle or CL. This software derives the rate of microbubble replenishment (β) and peak image intensity (VI) within the ROI. These values are then used to derive blood volume (BV) and rate of vascular flow (VF) within the ROI as previously described (Wei et al., 1997); this method was recently applied to investigations into vascular dynamics of macaque uterine tissues (Keator et al., 2011), with the exception of substituting the average value of primate ovarian tissue density (Rosendahl et al., 2010). See Supplemental Figures 1A for example of graph of the data curve used to calculate vascular parameters and 1B for equation used to derive final values. All data from normal luteal phases (Experiment 1) and Antide-induced luteal regression (Experiment 2) were analyzed by Repeated Measures ANOVA mixed models (SAS[®] (version 9.2; SAS Institute Inc., Cary, NC, USA, with individual monkey as subject effect). Differences of Least Squares Means function was then used to interrogate differences between time points of DCE-US sessions. Finally, DCE-US values from individual time points of *Experiment 1* were compared to corresponding hormone values by Correlation procedures (Spearman Correlation Coefficients) of SAS[®] to identify significant differences ($P < 0.05$) and trends ($P < 0.1$) to determine the predictive value of DCE-US scanning for luteal function. Because luteal function is also reflected by the change in both P and E during the luteal phase, the area under the curve (AUC) was determined for both serum E and P levels measured during the luteal phase of each female in Experiment 1 and compared to the change in VF and BV as measured by AUC (Origin[®] version 7.5; OriginLab Corporation, Northampton, MA, USA) again by Correlation procedures of SAS.

Results

Five of eight rhesus females were identified as undergoing normal/ typical luteal phases during serial DCE-US scanning by adequate peak luteal phase progesterone (P) levels (3 ng/ml; Figure 1A), and normal ovarian morphology. Use of contrast reagent during low MI imaging highlighted luteal blood flow even in early phase CL, while virtually no Doppler signature indicating blood flow was detected by conventional Power-flow Doppler within

the ovary (low MI imaging alone, before destructive pulse; Figure 1B and C). Therefore, only DCE-US was used to quantify luteal vascular function.

Experiment 1

During DCE-US, the microbubble refill rate (β) within the CL was <10 sec and did not differ significantly between stages of the luteal phase (Table 1, Figure 2A). Relatively high blood volume and flow rate were observed in CL, and significant changes to luteal BV and VF during the luteal phase were identified by DCE-US (Figure 2B; Table 1). Analysis of individual parameters indicates that, during normal cycles, BV is highest in early to mid-late CL compared to the dominant follicle at/near ovulation, and tended to decline in late CL (Figure 3, Early vs Late; $P<0.09$). In contrast, VF is highest at the time of peak luteal function (Figure 3 Early vs Mid; $P<0.01$; Table 1). To determine the predictive value of DCE-US data with luteal function, correlations were investigated between serum E and P levels measured on the day of scanning, and total changes of each throughout the luteal phase (AUC), and corresponding DCE-US data. The VF of the dominant ovarian structure (peri-ovulatory follicle or CL) positively correlated ($P<0.0001$) with serum P level measured on the day of imaging (Figure 4A), but total VF during the luteal phase did not correlate with overall P produced by the CL over the luteal phase (as calculated by AUC; $P>0.5$, data not shown). While total VF over the luteal phase did not correlate with level of E on day of imaging ($P>0.3$, Figure 4C), it was positively correlated with total E produced during the luteal phase ($P<0.03$, data not shown). The BV of the dominant ovarian structure (peri-ovulatory follicle or CL) correlated positively ($P<0.001$) with serum P levels measured on the day of imaging (Figure 4B). Conversely, while BV only tended to correlate with changes to luteal E (AUC; $P<0.08$), it was negatively correlated ($P<0.04$) with E level on the day of imaging (Figure 4D). Both VF and BV correlations with serum E on the day of scanning are potentially complicated due to the low vascular values measured in the peri-ovulatory follicle when compared to functional CL (Figure 3). Therefore, correlations of changes to VF and BV with changes in luteal E by AUC measurements may be more informative.

Three females did not meet the criteria for normal luteal phases. Two of these females had low serum P levels <3 ng/ml (Figure 1). When their DCE-US values were compared to those from normal P counterparts, these low P females tended to have lower peak BV and peak VF (Figure 5A). One other female presented with a normal CL on the right ovary, which regressed by the mid-late luteal phase, and a luteinized cyst on the left ovary which persisted until the next menstrual cycle (Figure 5B-C). The luteinized cyst was detected as a large ovary with no Doppler signature for a formed CL by 3D/4D Doppler ultrasound analysis (Figure 5D). This female had two significant E peaks during the follicular phase, and two peaks of P during the luteal phase (Supplemental Figure 2), and therefore the structures likely represent two separate spontaneous luteinization events in the same menstrual cycle. Age of macaque female did not seem to correlate with insufficient/abnormal luteal phase in this limited cohort.

Experiment 2

Administration of Antide beginning on day 9 of the luteal phase following the initial 0h scanning session decreased ($P<0.01$) serum P levels to baseline by 24h and levels remained low at 72h; serum E levels significantly declined at 24h and continued to decline to 72h ($P<0.001$; Figure 6A). Within these CL, VF tended to decline ($P=0.07$) by 72h during Antide exposure (Figure 6B). In contrast, BV tended to decline by 24h ($P<0.06$), and was significantly ($P<0.01$) reduced 3-fold by 72h after onset of Antide administration (Figure 6B).

Discussion

Remarkable changes to luteal structure-function occur as the luteal phase progresses during the menstrual cycle in women (Lunn *et al.*, 2002) and nonhuman primates (Christenson and Stouffer, 1996). Primarily, luteal function appears to be dependent on formation and maintenance of a highly vascularized CL with a micro-vasculature (capillary) bed with high vascular flow and blood volume (Niswender *et al.*, 1976; Robinson *et al.*, 2009). Notably, the majority of the blood volume in the ovary is found in within the small capillary bed-structure of the CL during the luteal phase (Niswender *et al.*, 1976). Current Doppler ultrasound methods are not sensitive enough to provide accurate quantification of vascular flow/volume in the small capillary beds of the ovary (pre-ovulatory follicle and CL) or uterus (endometrium) during the menstrual cycle (Lindner and Sklenar, 2004; Keator *et al.*, 2011). Building on a previous investigation of uterine vascular kinetics by DCE-US (Keator *et al.*, 2011), this study was designed to determine the feasibility of using this technique to provide a more sensitive measurement of ovarian vascular flow/volume. This study provides a foundation for further investigations of dynamic changes to blood flow/volume within the dominant structures of the ovary (follicle or CL) during different cycles or treatment intervals using DCE-US.

With a relatively modest number of females displaying normal menstrual cycles ($n=5$), significant differences in luteal vascular parameters were still noted during the luteal phase of the spontaneous menstrual cycle (Figure 3). Use of DCE-US detected a 2.5-fold decrease in luteal BV between the early and late luteal phase, and an 11-fold decrease in luteal VF across the same time frame. Notably, VF and BV increased 2.1-fold and 6.7-fold, respectively, between presumptive ovulation and formation of the CL during the early luteal phase. Furthermore, both VF and BV significantly correlated with functional status of the CL (Figures 4A-D).

Females of Experiment 1 and 2 were separate cohorts, and these were performed in different macaque breeding seasons; the data reported here shows the repeatability of DCE-US measurements, and the utility of these data as baseline values for further experimentation. The modest changes in VF (Figure 6B) following onset of treatment with Antide mimic changes observed in CL from spontaneous luteal phases in Figure 3 (mid-late vs late). It is important to note that the females imaged in Experiment 1 during the mid-late luteal phase ranged between days 9-12 following the LH-surge, which we reported are comparable at the transcriptome level in several previous studies (Bishop *et al.*, 2009a; Bogan *et al.*, 2009). Serum P levels of Antide-treated females from Experiment 2 imaged at day 9 of the luteal

phase are intermediate and have less variation than those of spontaneous cycles (Antide; 3.0 ± 0.1 ng/ml, mid: 3.32 ± 0.5 ng/ml, mid-late; 2.5 ± 0.5 ng/ml). Given the correlation between VF of CL and serum P on the day of imaging, the absolute levels of VF observed in CL of Experiment 2 before Antide administration would be expected to fall in-between those of mid and mid-late CL, which is reported here (Figures 3 and 6B).

The large decrease in luteal BV following onset of exposure to the GnRH-antagonist is also similar to the observed difference in BV between mid-late and late CL during the normal luteal phase. The CL of two of these females were imaged at the day of their peak P level in serum, unlike those from Experiment 1 in which only 1 CL was imaged on the day of peak serum P. Incidentally, 4 of 6 Antide-treated females had peak serum P values >5 ng/ml, and only 2 females from experiment 1 had similar high serum P levels. Therefore, the relatively larger BV values reported for CL of Antide-treated females imaged on day 9 of the luteal phase may be due to greater luteal function in this cohort before onset of treatment.

Doppler ultrasound evaluation of blood flow within macaque CL is complicated by the fact that the macaque ovaries are much smaller than those in women. Therefore, the capillary bed of CL in macaques may not generate enough Doppler signal to accurately measure the resistance index, and lack of transvaginal probes for smaller macaque reproductive tracts further compounds this problem (as discussed by van Diepen *et al.*, 2012). But, a previous investigation on a large cohort of women with normal menstrual cycles ($n=18$) using 3D power Doppler angiography also did not find any significant differences in vascularity index of the ovary between different phases of the menstrual cycle during serial scanning sessions (Hope *et al.*, 2009). A larger ($n=90$) study demonstrated changes in measurements of Doppler impedance (resistance index) to the CL and correlation between serum P and RI, but this study reported data from 61 normal menstrual cycles (Tamura *et al.*, 2008). While there are numerous differences in methodology between these studies and the current report (different primate species, human population differences, ultrasound platform/analysis software, and inclusion criteria), it also is important to note the large sample sizes needed in these previous studies to achieve meaningful results, where dampening of the Doppler signal is not an issue due to the larger size of the CL in women and use of a sensitive vaginal probe. The benefit of the DCE-US method to quantify vascular parameters of CL compared to Doppler-based analyses is clear: the enhanced sensitivity of this method by use of the microbubble contrast reagent results in fewer numbers of research subjects to achieve statistically meaningful data from the complex vascular tissue bed of the primate CL.

In the cohort ($n=8$) of rhesus monkeys that were randomly selected in Experiment 1 to undergo DCE-US imaging procedure, 3 did not exhibit a normal luteal phase. It was determined by post-hoc analysis that two of these females had insufficient luteal phases, (which our research group defines as displaying a luteal phase with peak levels of luteal progesterone < 3 ng/ml). These two females had 26% lower peak VF, and more impressively, 78% lower peak BV values (Figure 5A). Because BV values tended to be highest in the early luteal phase and are relatively stable during the functional luteal phase (Figure 3), this could be a useful diagnostic criteria in future investigations as an early indication of luteal dysfunction. Several Doppler ultrasound studies demonstrated

differences in ovarian blood flow from women with luteal phase defects when compared to their normal counterparts (Jain *et al.*, 2004; Tamura *et al.*, 2008; Hope *et al.*, 2009).

A final female was excluded from all statistical analyses due to the presence of an abnormal luteal phase, displaying potentially two separate spontaneous luteinization events that created one formed CL and one luteal cyst in her ovaries (Figure 5 B-D; hormone profile Supplemental Figure 2). This is not unheard of in rhesus macaque females; in a previous study, our research group documented a similar female undergoing sequential ultrasound analysis that followed the selection and growth of the dominant antral follicle throughout the follicular phase (Bishop *et al.*, 2009b). In that cohort, a female with one presumably ovulated/luteinized and one cystic structure was noted, but no attempt was made to study this female throughout the subsequent luteal phase. Imaging the cystic ovary of the female in Figure 5C-D demonstrates the power of contrast enhanced-US to detect defects in luteal/ovarian vascular structure. Whereas 3D color Doppler image lacked any robust blood flow in the ovary, contrast enhanced-US detected vascularized luteinized tissue surrounding a central avascular cyst. This is similar to early-stage CL in Figure 1B-C, in which a robust vasculature is visible by use of contrast reagent under low MI imaging, but no Doppler signature is visible by 3D/4D Power-flow Doppler. A similar study using wash-out contrast enhanced-US, in which a single bolus of contrast reagent is administered intravenously and imaging recorded until contrast diminishes to baseline, was performed with another primate, the common marmoset (Hastings *et al.*, 2012). This study also identified a cystic-like structure that later formed into a normal CL by histologic analyses. Unlike the current investigation, this previous study was not structured to provide information on differences in ovarian blood flow at different stages of the menstrual cycle.

In summary, these data demonstrate that DCE-US provides a sensitive, minimally-invasive method to detect changes to vascular flow and volume in the dominant structures of the primate ovary (peri-ovulatory follicle and corpus luteum). This method appears to be superior to other methods to detect vascular flow in ovarian tissues, given the statistical significance demonstrated with a relatively small number of research subjects compared to other Doppler-based methods (Tamura *et al.*, 2008; Hope *et al.*, 2009). In addition, abnormalities are readily detected by DCE-US that might be difficult to detect by conventional 3D Doppler ultrasound imaging. Further studies are planned to use DCE-US to detect vascular changes to ovarian and luteal tissue in nonhuman primate models of human ovarian defects.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

- Bishop CV, Hennebold JD, Stouffer RL. The effects of luteinizing hormone ablation/replacement versus steroid ablation/replacement on gene expression in the primate corpus luteum. *Mol Hum Reprod*. 2009; 15:181–93. [PubMed: 19168862]
- Bishop CV, Sparman ML, Stanley JE, Bahar A, Zelinski MB, Stouffer RL. Evaluation of antral follicle growth in the macaque ovary during the menstrual cycle and controlled ovarian stimulation by high-resolution ultrasonography. *Am J Primatol*. 2009; 71:384–92. [PubMed: 19189308]
- Bogan RL, Murphy MJ, Stouffer RL, Hennebold JD. Systematic determination of differential gene expression in the primate corpus luteum during the luteal phase of the menstrual cycle. *Mol Endocrinol*. 2008; 22:1260–73. [PubMed: 18258683]
- Christenson LK, Stouffer RL. Proliferation of microvascular endothelial cells in the primate corpus luteum during the menstrual cycle and simulated early pregnancy. *Endocrinology*. 1996; 137:367–74. [PubMed: 8536637]
- Dickey RP. Doppler ultrasound investigation of uterine and ovarian blood flow in infertility and early pregnancy. *Hum Reprod Update*. 1997; 3:467–503. [PubMed: 9528912]
- Hastings JM, Morris KD, Allan D, Wilson H, Millar RP, Fraser HM, Moran CM. Contrast imaging ultrasound detects abnormalities in the marmoset ovary. *Am J Primatol*. 2012; 74:1088–1096. [PubMed: 22890799]
- Hope JM, Long K, Kudla M, Arslan A, Tsymbal T, Strok I, Timor-Tritsch IE. Three-dimensional power Doppler angiography of cyclic ovarian blood flow. *J Ultrasound Med*. 2009; 28:1043–52. [PubMed: 19643787]
- Jain M, Sinha M, Shukla RC. Transvaginal Doppler ultrasound with color flow imaging in luteal phase defect. *Int J Gynaecol Obstet*. 2004; 84:266–267. [PubMed: 15001380]
- Kaul S. Instrumentation for contrast echocardiography: technology and techniques. *Am J Cardiol*. 2002; 90:8–14.
- Keator CS, Lindner JR, Belcik JT, Bishop CV, Slayden OD. Contrast-enhanced ultrasound reveals real-time spatial changes in vascular perfusion during early implantation in the macaque uterus. *Fertil Steril*. 2011; 95:1316–21.e1-3. [PubMed: 21316046]
- Lindner JR. Contrast ultrasound molecular imaging: harnessing the power of bubbles. *Cardiovasc Res*. 2009; 83:615–6. [PubMed: 19625336]
- Lindner JR. Microbubbles in medical imaging: current applications and future directions. *Nat Rev Drug Discov*. 2004; 3:527–32. [PubMed: 15173842]
- Lindner JR, Sklenar J. Placing faith in numbers: quantification of perfusion with myocardial contrast echocardiography. *J Am Coll Cardiol*. 2004; 43:1814–6. [PubMed: 15145105]
- Lunn SF, Fraser HM, Mason HD. Structure of the corpus luteum in the ovulatory polycystic ovary. *Hum Reprod*. 2002; 17:111–117. [PubMed: 11756372]
- Niswender GD, Reimers TJ, Diekman MA, Nett TM. Blood Flow: A Mediator of Ovarian Function. *Biology of Reproduction*. 1976; 14:64–81. [PubMed: 769853]
- Phillips P, Gardner E. Contrast-agent detection and quantification. *Eur Radiol*. 2004; 14(Suppl 8):P4–10. [PubMed: 15700327]
- Robinson RS, Woad KJ, Hammond AJ, Laird M, Hunter MG, Mann GE. Angiogenesis and vascular function in the ovary. *Reproduction*. 2009; 138:869–81. [PubMed: 19786399]
- Rosendahl M, Ernst E, Rasmussen PE, Andersen CY. True ovarian volume is underestimated by two-dimensional transvaginal ultrasound measurement. *Fertil Steril*. 2010; 93:995–998. [PubMed: 19108822]

- Tamura H, Takasaki A, Taniguchi K, Matsuoka A, Shimamura K, Sugino N. Changes in blood-flow impedance of the human corpus luteum throughout the luteal phase and during early pregnancy. *Fertil Steril*. 2008; 90:2334–2339. [PubMed: 18249380]
- van Diepen HA, Pansier J, Oude Wesselink P, van Drie A, van Duin M, Mulders S. Non-invasive translational Cynomolgus model for studying folliculogenesis and ovulation using color Doppler ultrasonography. *J Med Primatol*. 2012; 41:18–23. [PubMed: 22084982]
- Wei K, Skyba DM, Firschke C, Jayaweera AR, Lindner JR, Kaul S. Interactions Between Microbubbles and Ultrasound: In Vitro and In Vivo Observations. *J Am Coll Cardiol*. 1997; 29:1081–1088. [PubMed: 9120163]
- Wilks JW, Hodgen GD, Ross GT. Luteal phase defects in the rhesus monkey: the significance of serum FSH:LH ratios. *J Clin Endocrinol Metab*. 1976; 43:1261–1267. [PubMed: 826543]

Biography

C. Bishop helped design the study, performed the ultrasonic examinations, analyzed the data, and prepared the manuscript. T. Molskness and F. Xu consulted on study design, assisted with ultrasonic examinations, and approved the manuscript. T. Belcik and J. Lindner provided training and assistance for performing DCE-US experiments, provided ultrasonic equipment for the study, consulted on data analysis, and assisted with manuscript preparation. O. Slayden consulted on study design and manuscript preparation. R. Stouffer helped design the study, assisted with manuscript preparation, and obtained funding for the experiments.

Figure 1.

A) Serum progesterone levels from macaque females with normal (n=5; black squares; values Mean $\pm$ SEM) and insufficient (n=2; open triangles; values Mean only) luteal phases (LP) of spontaneous, natural menstrual cycles. Normal vs insufficient LPs are defined in the Methods. Black rectangles indicate the range of DCE-US scanning for the pre-ovulatory dominant follicle (OV) and CL at specific intervals of the LP. Each female was scanned at all five intervals.

B-C) Ovary with early luteal phase CL surrounded by uterine blood supply (artery/vein) imaged by both contrast enhanced-US with low MI imaging (Panel B; white/grey indicates microbubble oscillation/areas of blood flow, areas lacking contrast reagent are not visible) and 3D/4D Power flow Doppler (Panel C; red to yellow false color indicates areas of blood flow). Boundaries of the ovary are indicated by blue outline. Note how use of contrast reagent identifies a formed CL with blood flow throughout structure (dotted line), while Power-flow Doppler does not indicate any areas of substantial Doppler signature (blood flow) beyond the adjacent uterine artery/vein.

Figure 2.

A) Example of time intervals (summarized to every 130 msec) during microbubble refill after destruction signal (Time 0) in early luteal phase CL. False-color (scale indicated on the right of the montage) demonstrates relative microbubble intensity at individual time intervals (msec) following high-MI pulse to destroy bubbles. Microbubble rupture is not detected in high velocity arteries and veins surrounding the ovary outside the ROI, due to high velocity of blood flow.

B): Parametric maps of the early luteal phase CL from A. False-color heat maps (defined in right side) indicate relative total blood volume (BV) and relative vascular flow (VF).

Figure 3.

Graph of DCE-US values for vascular flow (dark grey bars) and blood volume (light grey bars) through the region of interest (ROI), i.e., the dominant ovarian structure (pre-ovulatory follicle (OV) or corpus luteum) at discrete stages of the luteal phase. Values from Table 1; Different upper case letters denote significant ($P < 0.05$) differences between DCE-US measurements of vascular flow, and different lower case letters denote significant differences between DCE-US measurements of blood volume. Asterisks denote trend ($P < 0.09$) toward significance for blood volume measurements between Early and Late luteal phase.

Figure 4.

A-D): Significant correlations between DCE-US measurements (vascular flow dark grey diamonds; blood volume light grey diamonds) and serum hormone values indicative of luteal function (Correlation procedure of SAS® (version 9.2; SAS Institute Inc., Cary, NC, USA), R^2 ; Spearman Correlation Coefficients). See text for additional details on all correlations performed.

Figure 5.

A) Peak blood volume (BV) and vascular flow (VF) in CL from monkeys exhibiting normal luteal phases (Mean±SEM), versus two females with low serum progesterone (Mean and individual values) during the luteal phase. Due to the small number of females in the Low P group (n=2), SEM is not calculated for these females.

B-D): Contrast enhanced-US of a third female (hormone profile, Supplemental Figure 2) identified a typical CL on the right ovary (Panel B, aqua circle encompasses ovary, white indicates blood flow), but also a luteinized cyst on the left ovary (Panel C). No obvious cyst-like structure was observed using Doppler 3D/4D ultrasound on the same ovary (Panel D). All panels are images captured from the same day/ultrasound scanning session (mid-luteal). On subsequent scans the CL on the right ovary regressed early at mid-luteal phase (no obvious luteal blood flow by DCE-US), while the luteinized cyst persisted to the next cycle.

Figure 6.

A) Serum progesterone and estradiol levels from females exposed to the GnRH-antagonist Antide for 3 days beginning on day 9 of the luteal phase (mid-late CL, n=6; Mean±SEM). Significant declines in both steroids (progesterone, upper case letters; estradiol, lower case letters) were observed within 24h of onset of Antide-exposure, inducing luteal regression (see text for details).

B) Response of luteal BV and VF during Antide exposure to induce luteal regression on Days 9-12 of the luteal phase. Luteal BV declined significantly by 72h ($P<0.01$; lower case letters) and VF trended lower at 72 h ($P=0.07$; asterisks). See text for additional details.

Table 1

Stage of Menstrual Cycle¹

Variable	Pre-Ovulatory Follicle	Early Luteal	Mid-Luteal	Mid-Late Luteal	Late Luteal	P-value ²
Blood Volume (BV) ml/100ml	0.09±0.12	0.59±0.12	0.55±0.13	0.55±0.13	0.24±0.13	0.06
Fill rate (β) S ⁻¹	5.69±2.7	0.11±3.02	1.07±2.7	0.42±2.7	0.12±3.49	0.58
Vascular Flow (VF) ml/min/g tissue	2.88±6.77	6.17±6.77	42.72±7.57	11.74±7.57	3.00±7.57	0.01
Progesterone (P) ng/ml	0.11±0.43	1.45±0.43	3.32±0.48	2.51±0.48	0.48±0.48	0.0009
Estradiol (E) pg/ml	236.80±23.17	45.78±23.17	53.06±25.90	51.18±25.90	52.25±25.90	0.0002

¹) All Values Mean±SEM

²) Repeated Measures ANOVA mixed models procedure of SAS® (version 9.2; SAS Institute Inc., Cary, NC, USA)