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Annie D. Glessner-Fischer
University of Vermont

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Annie D. Glessner-Fischer[1]

[1]Department of Obstetrics, Gynecology, and Reproductive Sciences, Larner College of Medicine, University of Vermont, Burlington, VT, USA

E-mail: annie.glessner-fischer@uvm.edu

Phone: (973) 934-6462

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Acknowledgements

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Abstract

Uterine vascular adaptations such as vessel growth and vasodilation are needed to facilitate the more than 10-fold increase of uteroplacental blood flow (UPBF) during pregnancy. Adverse adaptations may result in pregnancy complications such as preeclampsia and intrauterine growth restriction. Pregnancy milieu, placentation and the attendant change in wall shear stress are major regulators of uterine vascular adaptation. In this study, we aimed at delineating : (1) the contribution of these regulators in vascular remodeling and (2) the effects of pregnancy milieu (estrogen and progesterone) alone and in combination with wall shear stress on the vascular reactivity. Using Sprague Dawley rats as the animal model, three surgical methods were utilized: (1) unilateral oviductal ligation (OHL) that restricts pregnancy to one uterine horn; (2) cervical-end main uterine artery and vein ligation (VL) that alters the hemodynamic pattern of the UPBF and wall shear stress; and (3) ovariectomy (OVX) with the implant of estrogen + progesterone pellet (0.5 and 100 mg, respectively). A segment of ovarian-end main uterine artery from each uterine horn was dissected, cannulated, and pressurized in an arteriograph system. Lumen diameters in response to phenylephrine (vasoconstrictor) and acetylcholine (vasodilator) were measured. Passive lumen diameters, wall thickness, vessel cross-sectional area, and distensibility were also measured under a microscope. Significant remodeling was seen in OVX rats in response to hormone replacement ($p=0.0457$); however, the extent of remodeling did not reach that seen in the nonpregnant horn of OHL rats. No significant change in wall thickness, cross-sectional area or wall: lumen ratio was found in OVX (+pellet), compared to OVX (-pellet) rats. Estrogen + progesterone had no significant effect on the sensitivity to phenylephrine or acetylcholine. In conclusion, estrogen + progesterone does have a significant effect on vascular remodeling. The presence of other factors, such as placentation, likely augment this process.

I. Introduction

Pregnancy induces significant changes in the body, particularly to the maternal uterine vasculature. These changes are induced in response to the more than 10-fold increase in uteroplacental blood flow (UPBF) that occurs during pregnancy. Normally, placentation promotes invasion of the small uterine arteries by the trophoblast cells of the developing embryo. This results in ablation of the microcirculation, promoting lower resistance in the arteries and allowing the passage of a greater volume of blood to the placenta (Figure 1). The significant increase in UPBF stimulates vasodilation and subsequent remodeling of the uterine vasculature, referred to as outward hypertrophic remodeling (Osol et al, 2014, Osol et al, 2009). By providing a means for more maternal blood to reach the placenta, sufficient nutrient and oxygen transfer to the fetus can occur. This emphasizes the necessity of adequate UPBF and vascular remodeling to support the healthy development of the fetus.

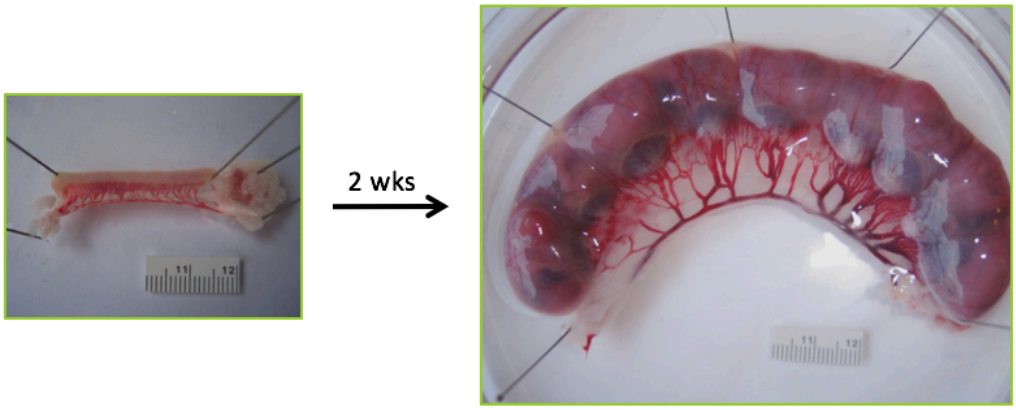

Figure 1 Representative images of a nonpregnant barren uterine horn (left) versus a late pregnant implanted uterine horn (right). Photograph taken by Dr. George Osol's lab.

Two major pregnancy-associated diseases – preeclampsia and intrauterine growth restriction (IUGR) – are characterized by impaired uterine vascular adaptation and compromised blood flow to the placenta (Ong et al, 2005). The uterine vessels lose their capacity for dilation

leading to inadequate growth and remodeling. This leads to reduced transfer of nutrients to the fetus during pregnancy. Understanding the mechanisms underlying uterine vascular adaptation is crucial to treat these diseases. A variety of factors have shown to influence uterine vascular remodeling. These include, but are not limited to, placentation, shear stress from increased UPBF, the presence of nitric oxide (NO), and pregnancy milieu, which is characterized by high circulating levels of estrogen and progesterone.

NO is the most associated with vasodilation during pregnancy. Levels of NO and NOS have been shown to increase significantly during pregnancy, as compared to nonpregnant controls (Nelson et al, 2000). Increased shear stress (the friction resulting from the movement of blood against the wall of the vessel) is the strongest stimulus for endothelial NO production and release; therefore, it is reasonable that shear stress-induced remodeling may occur via a NO-mediated mechanism. During pregnancy, shear stress in uterine arteries is altered by placentation and thought to lead to vessel growth and increased UPBF (Kublickiene et al, 2000).

Humans, as well as rodents, have hemochorial placentation: i.e. when the placenta forms, the microcirculation surrounding the placenta is destroyed, creating a large space for blood to permeate, which reduces distal vascular resistance and stimulates higher velocity of flow in the upstream arteries. The endothelium responds to the wall shear stress and releases vasoactive factors that stimulate the smooth muscle layer of the vessel wall to alter its tone to induce vasodilation, which induces vessel growth and remodeling. It has been hypothesized (Osol, personal communication) that growth continues until shear stress has returned to its "normalized" level. The vessel does not remodel as long as the shear stress is maintained at its normalized level, but is subject to remodeling in response to change in flow, and hence change in shear stress.

Pregnancy milieu (estrogen and progesterone) also has a role in vasodilation and regulation of maternal vascular remodeling. Estrogen levels are significantly increased during pregnancy and has been proposed to stimulate arterial vasodilation (van der Heijden et al, 2005). Like shear stress, the effects of estrogen are also thought to occur via a NO-mediated mechanism, based on the normal vasodilatory response to estrogen and the lack thereof when NOS is inhibited (Rupnow et al, 2001, Maliqueo et al, 2016, Tostes et al, 2003). Inhibiting estrogen, by blocking the estrogen-receptor, has been shown to negatively influence vascular remodeling; however, treatment with exogenous estrogen has shown to restore vascular remodeling (Zhang et al, 2001, Tarhouni et al, 2013) in some regional circulations (but not the uterus).

The goal of this study was to differentiate between the contribution of local placentation versus pregnancy milieu on uterine vascular remodeling. Using Sprague Dawley rats as the animal model, three surgical methods were utilized: (1) unilateral oviductal ligation (OHL) that restricts pregnancy to one uterine horn; (2) cervical-end main uterine artery and vein ligation (VL) that alters the hemodynamic pattern of the UPBF and wall shear stress; and (3) ovariectomy (OVX) with vs. without the implant of an estrogen + progesterone pellet (0.5 and 100 mg, respectively, over 21-day period).

The rat provides a good model for this research because its uterus has two identical horns, allowing for an internal control within the same animal. A segment of ovarian end main uterine artery (MUA) from each uterine horn was dissected, cannulated, and pressurized in an arteriograph system. Lumen diameters in response to phenylephrine (vasoconstrictor) and acetylcholine (vasodilator) were measured. Passive lumen diameters, wall thickness, vessel cross-sectional area, and distensibility were also measured under a microscope. This study

looked at the effects of estrogen + progesterone alone through comparison of OVX^+ and OVX^- animals (+ refers to hormone-containing pellet implant, – refers to sham pellet). It also looked at the effect of pregnancy milieu in combination with shear stress through the comparison of vessels from OVX^+ animals at the ovarian-end with (ligated, ovarian: LO) and without (sham, ovarian: SO) cervical-end ligation. Based on the previously-mentioned findings, and the drastic increase in hormone levels during pregnancy, it was hypothesized that the sex steroid-rich pregnancy milieu is a significant contributing factor to vasodilation and the regulation of uterine vascular remodeling.

II. Methods

Animals. Research was performed using 12-14 week old female Sprague Daley rats purchased from Charles River Laboratories (Saint Constant, Canada) and housed at the University of Vermont (UVM) small animal care facility.

Solutions. HEPES-PSS composed of 141.8 mM NaCl, 4.7 mM KCl, 1.7 mM $MgSO_4$, 0.5 mM EDTA, 1.6 mM $CaCl_2$, 10 mM HEPES, 1.2 mM KH_2PO_4, and 5 mM dextrose in deionized water. The solution is neutralized using NaOH until it reaches pH 7.4 at 37°C. All chemicals for HEPES solution purchased from Fisher Scientific (Hampton, NH, USA). Relaxing solution composed of HEPES-PSS plus 100 μM papaverine (Sigma) and 10 μM diltiazem (Sigma).

Surgical procedures. Three surgical methods were utilized.

(1) Unilateral oviductal ligation (OHL) that restricted pregnancy to one uterine horn, allowing for the generalized presence of estrogen and progesterone in both horns of the uterus, but limited the presence of local influences associated with the presence of fetoplacental units to only the pregnant horn (Figure 2C). Oviductal ligation was performed by Charles River Laboratories.

(2) Ovariectomy (OVX) was performed by Charles River Laboratories 6-9 days prior to animal arrival at UVM small animal care facility and served to establish a sex hormone-deficient state. Implantation (subcutaneous in the periscapular region) of estrogen + progesterone pellets (0.5 mg 17-β estradiol + 100 mg progesterone) or sham (blank) pellets was performed at time of surgery to mimic pregnancy milieu (OVX^+) or maintain a hormone-free control (OVX^-), respectively.

(3) Cervical-end main uterine artery and vein ligation (VL) was performed at UVM day 10 of gestation. Normally the uterus experiences a dual inflow from both the ovarian and the

cervical end (Figure 2A); Ligation, using a 6-0 prolene suture (Ethicon, Somerville, NJ), served to alter uterine hemodynamics by restricting UPBF inflow and outflow to the ovarian end of the uterus, thereby allowing us to know where increased UPBF and shear stress was primarily occurring (Figure 2B). Following the surgery, a simple interrupted suture with 5-0 silk thread (Oasis, Mettawa, IL) was used to close the dermal layer, while a running suture with 5-0 vicryl thread (Ethicon) was used to close the underlying muscle layer. 0.05 mg/kg of buprenorphine was administered immediately after surgery, 4-6 hours post-surgery, and the morning after surgery for pain management.

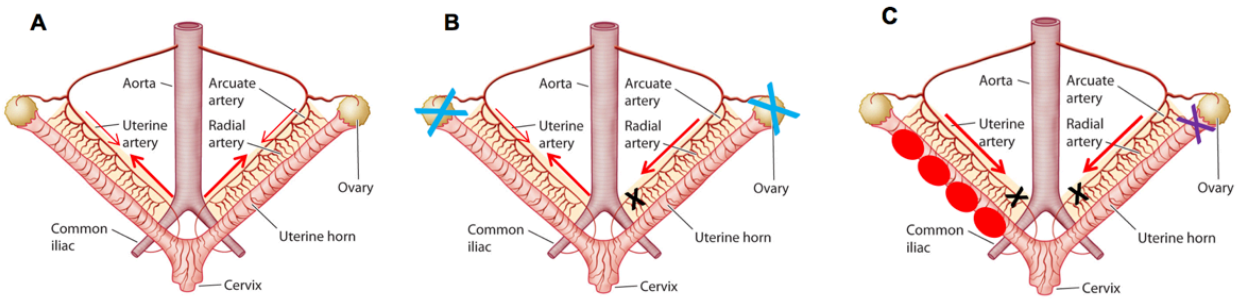

Figure 2 A: Schematic of normal uterine anatomy with bidirectional blood flow from both the ovarian-end and cervical-end (A, red arrows). B: Schematic showing bilateral ovariectomy (blue X's) and the imposition of a single point of inflow/outflow at the ovarian-end due to cervical-end vascular ligation (VL, black X's) of the main uterine artery (MUA) and vein (MUV), as shown here in the right horn. Bidirectional flow remains intact in the left horn. C: Schematic showing unilateral oviductal ligation (OL, purple X) in a late pregnant rat with bilateral cervical end-vascular ligation. Modified from Osol and Mandala, 2009.

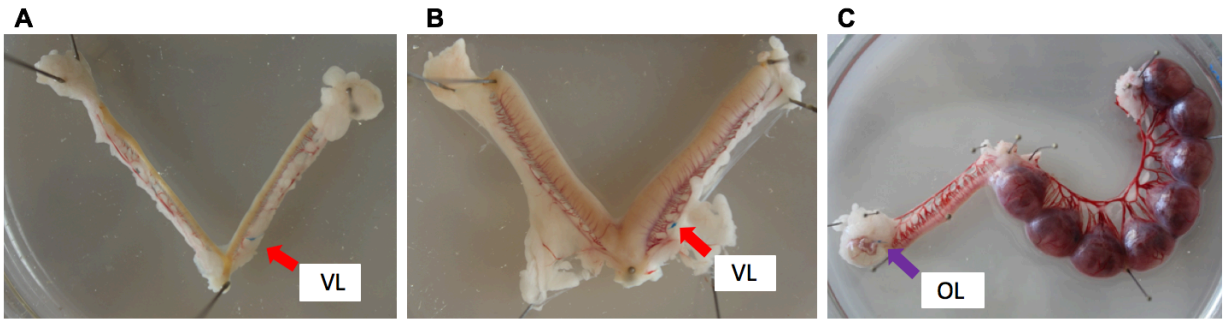

Figure 3 A: Sprague Daley uterus removed from an ovariectomized rat with no hormone replacement (OVX^{-}). B: Sprague Daley uterus removed from ovariectomized rat with estrogen (0.5 mg) + progesterone (100 mg) replacement (OVX^{+}). C: Sprague Daley uterus with unilateral oviductal ligation. Red arrows signify cervical-end vascular ligation (VL) of the main uterine artery and vein. The purple arrow signifies oviductal ligation.

Main Uterine Artery (MUA) Preparation. Experiments were performed 9-10 days (gestation day 20) after surgery. All rats were euthanized using 3% isoflurane and decapitated. The uterus and its vasculature were removed from the abdominal cavity and submerged in HEPES solution (detailed above). A segment of ovarian end MUA from each uterine horn was dissected, cannulated and pressurized to 90 mmHg in an arteriograph system (Living Systems Instrumentation, St. Albans, VT) as shown diagrammatically in Figure 4. Each MUA segment was subjected to 60-minute equilibration period at 37°C prior to experiment. Lumen diameter and reactivity were then recorded.

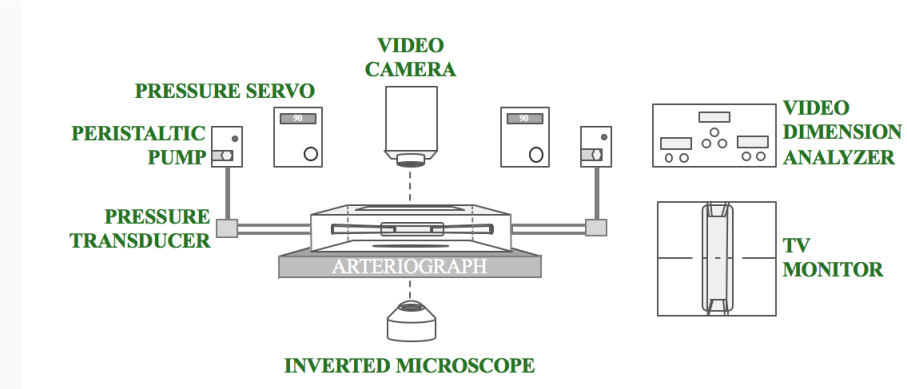

Figure 4 Schematic drawing of pressure system.

Unstressed Measurements. Following dissection and cannulation of arterial segments for experimentation, the uterus was washed and submerged in relaxing solution for a minimum of 15 minutes. MUV and inner/outer MUA diameter (cm) were measured under a microscope (32x magnification) at three matched locations in each uterine horn: ovarian, center, and cervical end (above the ligation site). Measurements were multiplied ×300 based on microscope calibration with a micrometer. Wall thickness ($WT = (D_{outer} - D_{inner})/2$), vessel cross-sectional area ($CSA = \pi r_o^2 - \pi r_i^2,\ r = \frac{D}{2}$) and wall to lumen ratio ($W{:}L = WT/D_{inner}$) were calculated from passive ovarian-end MUA segment measurements.

Distensibility. Following each reactivity experiment, the vessel was bathed in a relaxing solution (detailed above) for a minimum of 15 minutes allowing for full relaxation, and hence maximum dilation, of the MUA segment. The biomechanical properties of each MUA were evaluated by recording the increase in MUA lumen diameter as a function of transmural pressure (from 5-150 mmHg). Distensibility was calculated as $\text{distensibility} = [(\mathrm{D_{relax}} - \mathrm{D_{press}})/(\mathrm{D_{press}})]\times 100$, where $\mathrm{D_{press}}$ = unstressed $\mathrm{MUA_{ID}}$ at specific pressure (x = 5-150mmHg).

Sensitivity. Ovarian-end MUA segments were tested *in vitro* to determine whether there were changes in vessel behavior, specifically, vasoconstriction and vasodilation. These behaviors were induced by a) phenylephrine (Sigma, St. Louis, MO), a synthetic version of noradrenaline that acts as a vasoconstrictor, or by b) U46619 (Cayman Chemical Company, Ann Arbor, MI), a thromboxane agonist that acts as a vasoconstrictor, and c) acetylcholine (Sigma), a neurotransmitter that acts as a vasodilator. The changes in diameter in response to different concentrations of phenylephrine and acetylcholine were measured with video electronic system and WinDaq software (DATAQ Instruments Inc., Akron, OH). Sensitivity was calculated as

$\text{sensitivity} = [(D_{inner} - D_{baseline})/(D_{max} - D_{baseline})] \times 100$, where D_{max} = diameter of MUA with maximal response for a particular drug. By plotting the diameter responses of each vessel to the drug, the concentration predicted to produce the half-maximal response (EC_{50}) was extrapolated.

Data analysis. Data were graphed and analyzed using the statistical program GraphPad Prism 7 (2016). Data were compared via paired and unpaired t-test, one-way analysis of variance (ANOVA) with Tukey's or Sidak's multiple comparisons, two-way ANOVA with Tukey's or Sidak's multiple comparisons and nonlinear regression. Data are shown as means ± SEM, and means were considered significantly different if $p \leq 0.05$.

III. Results

A. Nonpregnant versus late pregnant rats.

In NP rats, there was no significant effect of cervical-end VL on unstressed MUA_{ID} at the ovarian or cervical end, exhibited by the lack of significant difference between MUA_{ID} of SO versus LO MUA segments at either end (Figure 5a). Conversely, there was significant uterine vascular remodeling in response to increased shear stress at the ovarian end in LP rats (Figure 5b). Sidak's multiple comparisons test revealed a significant increase ($p=0.0007$) in MUA_{ID} at the ovarian end between the control (SO, 203.57 ± 18.12 μm) and ligated (LO, 295.71 ± 20.59 μm) MUA segments. Although they trended smaller, no significant difference ($p>0.05$) was found between SO and LO vessels at the cervical end.

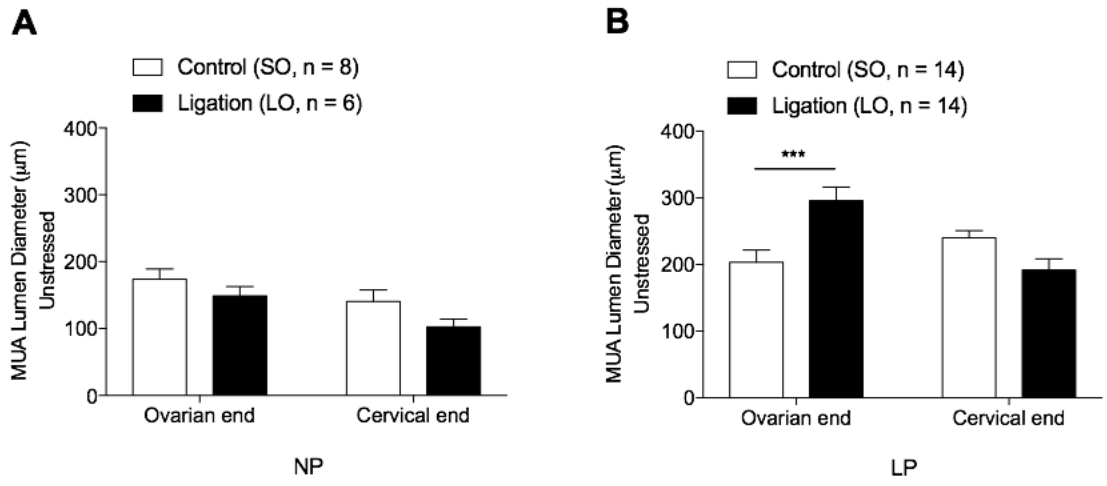

Figure 5 Unstressed MUA lumen diameter (MUA_{ID}) (μm) in NP (A) and LP (B) rats with no ligation (control/SO) or cervical-end ligation (ligation/LO). Measurements were taken at both the ovarian and cervical end of uterine horns. No significant difference ($p>0.05$) in NP MUA_{ID} were noted between the ovarian and cervical ends. A significant difference in control vs. ligated-operated vessels was present at the ovarian end of LP vessels, but not at the cervical-end. Data shown as mean ± SEM. Asterisks convey significance ($p<0.05$).

B. Influence of sex steroids and shear stress in hormone-deficient rats.

No remodeling was observed in hormone-deficient OVX^- rats in response to ligation (2-way ANOVA) (Figure 6a). Analysis of OVX^- rats (control) with post hoc testing with Tukey's multiple comparisons test determined no significant difference ($p>0.05$) in MUA_{ID} of like-vessels between the ovarian and cervical end. At the ovarian and cervical ends, there was also no significant difference in MUA_{ID} between SO versus LO vessels.

In hormone-replaced (OVX^+) rats, there was significant expansive remodeling ($p=0.0457$) in response to hormone pellet implantation such that MUA_{ID} increased significantly in LO (120 ± 0 μm) vs. SO (80 ± 10 μm) vessels at the ovarian, but not the cervical end (Figure 6b). There was also a significant difference ($p=0.01$) between the ovarian-end (120 ± 0 μm) and cervical-end (70 ± 10 μm) MUA_{ID} in the ligated horn of OVX^+ rats such that enlargement took place at the ovarian end, with some reduction in diameter at the cervical end.

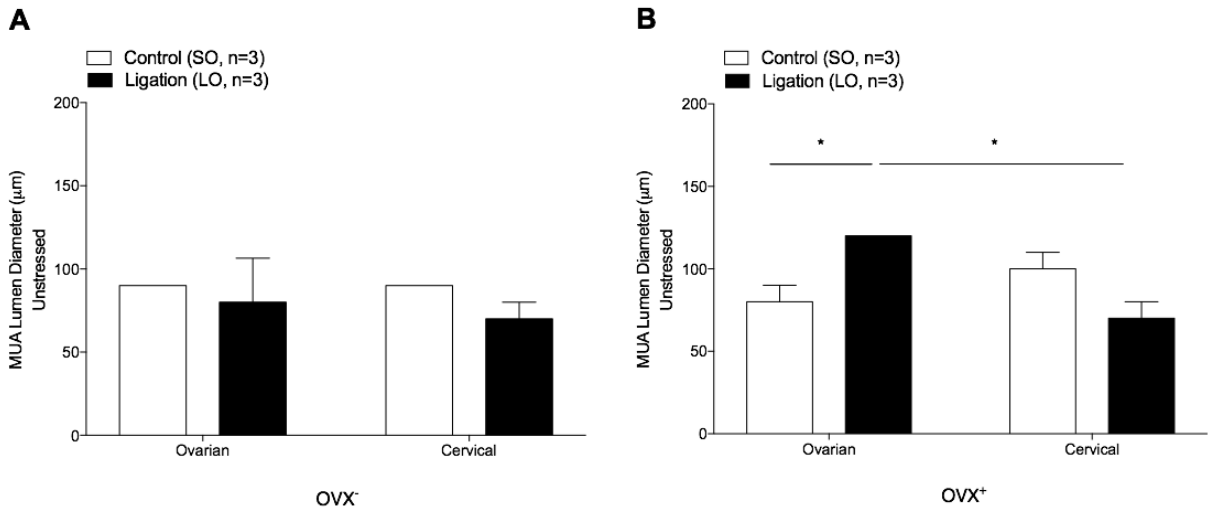

Figure 6 Influence of estrogen (0.5 mg) and progesterone (100 mg) on shear stress-induced vascular remodeling in OVX rats. (A) OVX^- (sham operated) rats. (B) OVX^+ (hormone-replaced) rats.

C. Wall thickness, vessel cross-sectional area, and wall: lumen ratio.

A 2-way ANOVA with Tukey's, Sidak's multiple comparisons tests were performed to analyze WT, CSA and W:L ratio, respectively. There were no significant differences ($p>0.05$) in diameter between groups (OVX^- versus OVX^+) or vessels (SO versus LO) for WT, vessel CSA and W:L ratio (Figure 7).

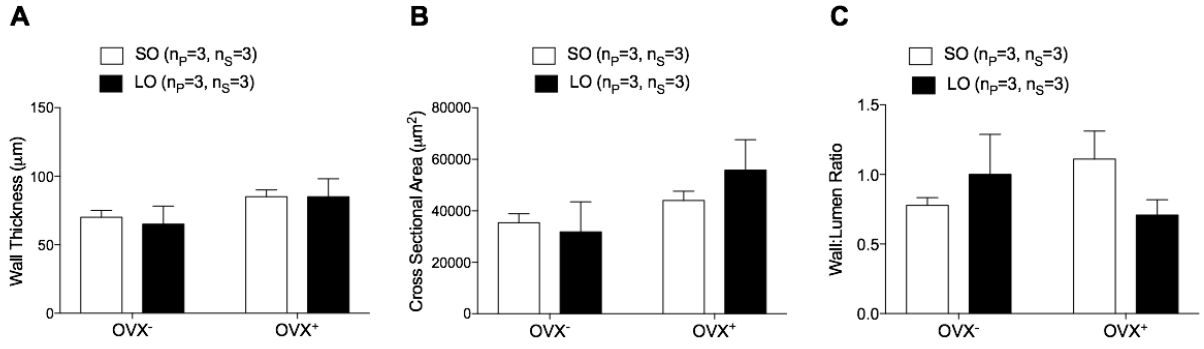

Figure 7 (A) Wall thickness, (B) vessel cross-sectional area and (C) wall: lumen ratio of OVX^- and OVX^+ rats.

D. Hormone replacement vs. natural pregnancy hormones.

Estrogen + progesterone replacement (OVX^+) exhibited a lesser extent of remodeling compared to the nonpregnant horn of OHL rats exposed to a natural pregnancy milieu (Figure 8a). A one-way ANOVA was performed to determine differences for passive MUA measurements. Tukey's multiple comparisons test showed significantly reduced ($p=0.0458$) MUA_{ID} for SO vessels between OVX^+ (80 ± 10 μm) compared to NP (166.5 ± 17.88 μm) rats. Remodeling was present in OVX^+ rats, shown by a significant increase ($p=0.0161$, unpaired t-test) in MUA_{ID} of OVX^+ rats between SO (80 ± 10) and LO (120 ± 0 μm) vessels. No significant difference ($p>0.05$) in MUA_{ID} was found in NP rats in response to cervical-end ligation. A

significant increase (p=0.007, Tukey's multiple comparisons) in MUA_{ID} of SO vessels was found between OVX^+ rats and the nonpregnant horn of OHL rats. No significant difference was found for LO vessels between OVX^+ and the nonpregnant horn of OHL animals (Figure 8a).

Pressurized (at 90 mmHg) measurements were analyzed using one-way ANOVA and yielded similar results (Figure 8b). Tukey's multiple comparisons test showed a significant difference (p=0.0003) between SO vessels of OVX^+ (390.8 ± 10.68 µm) and nonpregnant horn of OHL (532.5 ± 16.32 µm) animals. No significant difference was determined between LO vessels of OVX^+ and nonpregnant horn of OHL animals (Figure 8b).

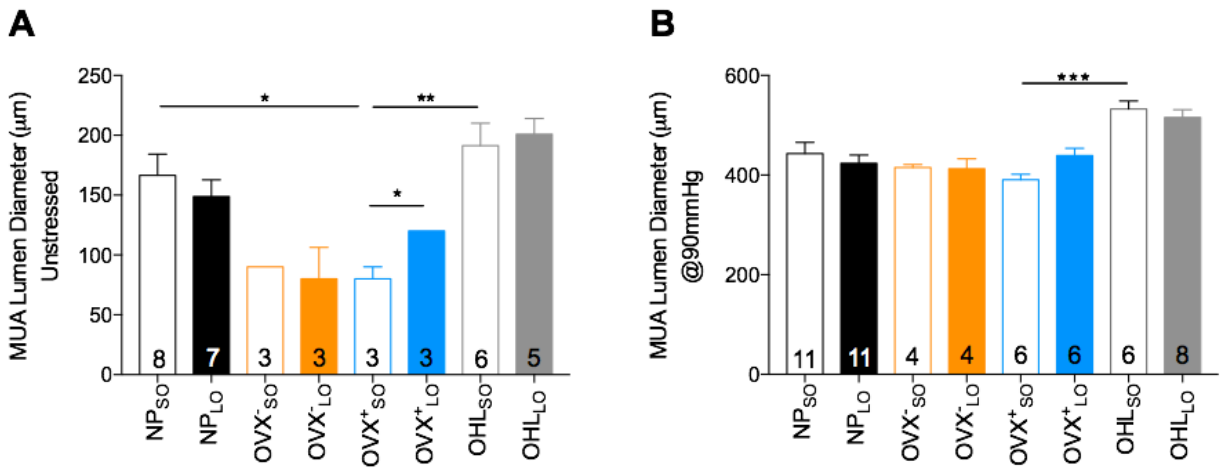

Figure 8 Unstressed vs. pressurized ovarian-end MUA_{ID} of vessels with cervical-end ligation (LO). Comparison of control (SO) and LO vessels of NP, OVX^-, OVX^+ and the nonpregnant horn of OHL animals. Significant difference found between unstressed MUA_{ID} of SO vs. LO vessels in OVX^+ rats (A) and between pressurized MUA_{ID} of SO vessels of OVX^+ and nonpregnant horn of OHL animals (B).

E. Vessel Distensibility.

No significant diameter differences in pressurized MUA_{ID} (Figure 9A) or in distensibility (Figure 9b) were determined between the 4 groups. There is a general pattern of slightly

increased diameter in LO vessels, compared to SO vessels, and a decrease in distensibility of LO vessels compared to SO vessels, but these differences did not reach statistical significance.

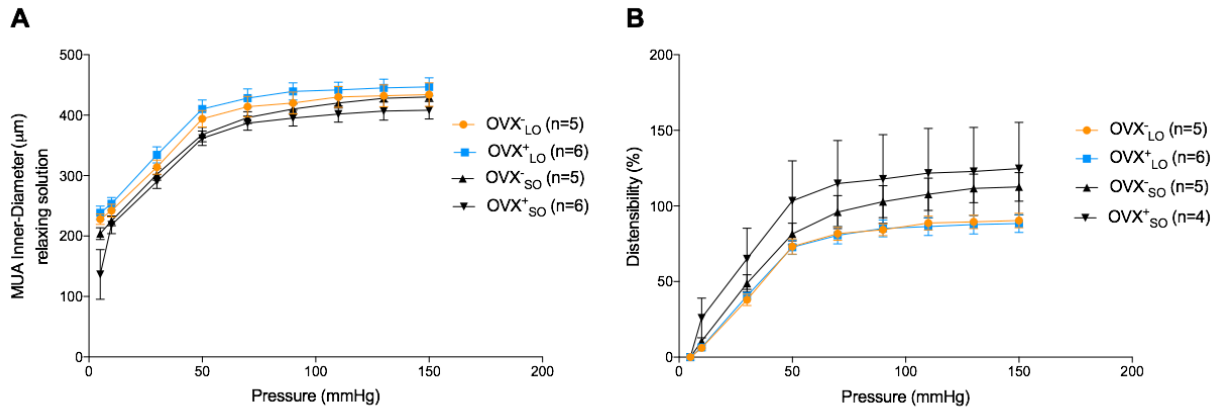

Figure 9 MUA_{ID} and distensibility as a function of pressure, from 5-150 mmHg. No significant differences in pressurized MUA_{ID} or in distensibility between the four groups.

F. Vessel Sensitivity.

Data were analyzed to evaluate the effect of remodeling on MUA sensitivity to phenylephrine and acetylcholine. Sensitivity was measured by calculating EC_{50} values (the extrapolated concentration at which the vessel exhibited 50 percent of the maximal response). A 2-way ANOVA was performed to evaluate the effect of treatment group (OVX^- vs. OVX^+) on sensitivity. Sidak's multiple comparisons test showed no significant difference between treatment groups for SO or LO vessels in response to phenylephrine. No significant difference was found between treatment groups for SO or LO vessels in response to acetylcholine, but the data showed a lowered sensitivity to acetylcholine in OVX^- animals in both vessel types. A 2-way ANOVA was also performed to evaluate the effect of cervical-end ligation on sensitivity. Sidak's multiple comparisons test found no significant difference between the sensitivity of SO

vs. LO vessels for OVX^- or OVX^+ groups in response to phenylephrine or acetylcholine (Table 1). In OVX^+ animals, there appeared to be some increase in sensitivity to phenylephrine in LO vessels compared to SO vessels (Figure 10a), and decreased sensitivity to acetylcholine (Figure 10b). In OVX^- animals, there was a slight increase in sensitivity of LO vessels, compared to SO vessels, for both phenylephrine (Figure 10a) and acetylcholine (Figure 10b). None of the differences between means reached statistical significance ($p>0.05$).

Table 1 Quantification of OVX^- and OVX^+sensitivity to phenylephrine (Phe) and acetylcholine (ACh) using average EC_{50} values (nm).

Drug	**Animal**	**EC_{50} (nM)**	
		SO	LO
Phe	OVX^-	150 ± 33.1	102 ± 10.2
	OVX^+	142 ± 17.2	82 ± 16.3
ACh	OVX^-	330 ± 58.6	248 ± 32.4
	OVX^+	380 ± 39.6	435 ± 28.5

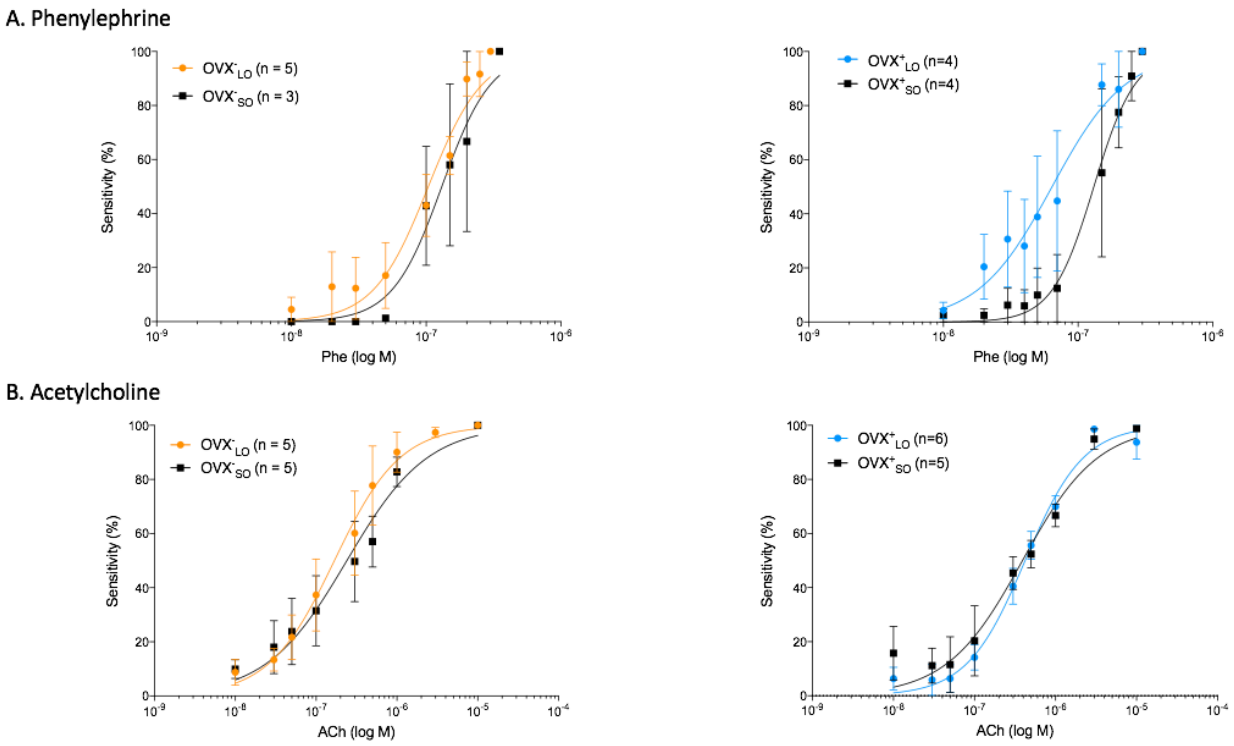

Figure 10 MUA sensitivity (%) as a function of concentration of phenylephrine (A) and acetylcholine (B) in OVX^- and OVX^+ animals. Data are from ovarian-end MUA segments. Colored lines signify ligated-operated (LO) vessels, while black lines signify control (SO) vessels.

IV. Discussion

A. Relative Contribution of Pregnancy Milieu in Regulating Uterine Vascular Remodeling

Sex steroids (0.5 mg estradiol + 100mg progesterone) in combination with wall shear stress induced vasodilation and had a significant effect on uterine vascular remodeling in OVX rats. Passive lumen diameter significantly increased in LO versus SO vessels in OVX^+ animals, and there was a lack of such increase in OVX^- animals. Therefore, the wall shear stress-induced enlargement of MUA segments in OVX animals was a direct effect of the implanted hormone pellet. Other factors may have been involved, but factors related to placentation and the presence of fetoplacental units can be excluded, as animals were all hormone-replaced but not pregnant.

The OVX data are comparable to the remodeling seen between NP and LP rats. The inclusion of these groups was to confirm that there is significant remodeling as a result of pregnancy. Looking at the four groups (NP, LP, OVX^-, OVX^+), our results suggested that wall shear stress has little to no effect on vascular remodeling in the absence of (OVX^-), or when there are only low levels (NP) of circulating estrogen and progesterone. Only when hormone levels are increased by pellet implantation in OVX animals, or by pregnancy (OHL LP), does increased wall shear stress stimulate uterine vascular expansive remodeling. This is not to say that hormone level does not influence baseline vessel size. In fact, the data show that hormone level does influence this given that NP animals had significantly larger vessels than OVX^- animals. Based on earlier studies (Tostes et al, 2003), this difference in baseline vessel diameter is likely carried out by a NO-mediated mechanism, which may be attenuated or possibly nonexistent in animals in which sex hormone levels were low (NP) or entirely absent (OVX^-).

During pregnancy, sex steroids - particularly estrogen- have been found to act via a NO-mediated mechanism (Tostes et al, 2003, Maliqueo et al, 2016). Treatment with estrogen and estrogen + progesterone resulted in increased NOS, along with vasodilation. Estrogen has also been proposed as a physiological mediator of uterine blood flow, in which increased estrogen levels stimulate increased blood flow (Rupnow et al, 2001). In contrast to this, reduced hormone levels have been associated with decreased flow-mediated vasodilation (LeBlanc et al, 2009). The combined effect of increased UPBF and increased NOS could reasonably result in significant vasodilation in the absence of local placentation. As major components of pregnancy milieu, it is likely that estrogen + progesterone replacement in OVX animals stimulated these effects to some extent and contributed to the uterine vascular remodeling observed.

It is important to note that pregnancy milieu in combination with wall shear stress did not produce a significant increase in lumen diameter of MUA segments dissected from the nonpregnant horn of OHL animals. This is interesting considering that this group of animals exhibited natural pregnancy milieu, i.e. high levels of estrogen and progesterone. The reason for the lack in significant vascular remodeling in response to shear stress is unknown, but may possibly be due to differences in hormone level.

Passive lumen diameter of MUA segments dissected from OVX^{+} animals and the nonpregnant horn of OHL animals were expected to be similar in size, given that both groups experienced high level of estrogen and progesterone, but not local factors associated with placentation. The results of this study did not support this prediction. Looking at the effect of sex steroids alone, there was a significant difference in baseline lumen diameter of MUA segments from the control horn of the OVX^{+} animals and the nonpregnant horn of OHL animals. When

combined with vascular ligation, the passive lumen diameters of MUA segments in OVX^+ animals vs. the nonpregnant horn of OHL animals were not significantly different.

There are a few reasons that could account for the difference in vascular remodeling observed between MUA segments from OVX^+ animals and the nonpregnant horn of OHL animals. The first is that hormone levels may be lower in the OVX^+ rats than in the non-pregnant horn of OHL rats. The level of estrogen + progesterone in the implanted pellet was determined based on previous research in which the goal was to mimic pregnancy levels of these sex steroids (Finley et al, 2015, Guzman et al, 1999). However, based on the results of this study hormone replacement in hormone deficient OVX animals may have resulted in hormone levels more closely equal to that of NP, rather than LP animals. MUA diameter in OVX^+ animals in response to shear stress was closer to MUA diameter of NP vessels than the nonpregnant horn of OHL vessels. Given this, it may be interesting to consider administration of the same hormone pellet given to OVX^+ animals to NP animals. This may result in more equal levels of hormones and induce remodeling more comparable to the remodeling seen in the nonpregnant horn of OHL animals. It is also reasonable to consider that more comparable remodeling would be seen if the amount of hormones administered to OVX^+ rats were increased. I was not able to measure circulating sex steroid concentrations in the various groups, and can only speculate on the probable differences.

The second reason for this difference is that hormones do not stimulate NO production to the extent that placentation does (LP rats exhibit increased vessel size compared to the other groups in this study). Fuller et al (2009) discuss the dominating influence of local, rather than systemic, factors in uterine vascular remodeling during pregnancy. Formation of the placenta is of major importance to the remodeling process. As previously mentioned, placentation results in

ablation of the microcirculation and decreased distal resistance. This decreased distal resistance drastically increases the amount of blood flow, and the velocity of blood flow, to the uterus. This is a major regulator of increased wall-shear stress, the strongest stimulus for endothelial NO production. The increase in wall shear stress following placentation is likely responsible for stimulating significantly increased NO production that hormones alone are unable to produce in the absence of this major increase in shear stress.

Lastly, growth factors, such as VEGF (vascular endothelial growth factor) and PlGF (placenta growth factor) are associated with the presence of the fetus. VEGF itself is noted as being produced by trophoblastic cells (Maliqueo et al, 2016). These factors also influence NO synthesis and vasodilation. In addition to increased flow and shear stress from placentation, these factors are also likely contributors to why the greatest vasodilation and uterine vascular remodeling is seen in conjunction with the presence of fetoplacental units. It can be noted that complete formation of the placenta does not happen instantly. In the mouse, the mature placenta was not found to be established until embryonic day 10 (Cross et al, 2002). Therefore, while placentation is a predominating influence, the presence of estrogen and progesterone may facilitate initial uterine vascular remodeling.

B. Effects of Uterine Vascular Remodeling on Vessel Reactivity

Given that changes in vascular tone can influence remodeling, the effects of sex steroids on vessel reactivity to phenylephrine and acetylcholine in OVX animals was evaluated. The reactivity of each vessel was normalized to the maximal response to phenylephrine and acetylcholine to compare sensitivity of each vessel to each drug. This study did not reveal any significant changes in vessel sensitivity as a result of sex steroids alone or in combination with wall shear stress. However, LO vessels from OVX^{-} animals showed increased sensitivity to both

phenylephrine and acetylcholine, whereas LO vessels from OVX^+ animals showed increased sensitivity to phenylephrine, but decreased sensitivity to acetylcholine. The reasons for this are unknown, but it may be worthwhile to further examine the effects of remodeling on vessel sensitivity. The trends observed in the responses to Phe and Ach in vessels from the OVX^+ vs. OVX^- animals suggest that vasoconstrictor (Phe) sensitivity was increased by ligation (increased shear stress), and that vasodilator (ACh) sensitivity was reduced. Since the available literature supports a linkage between vasodilation and outward expansive remodeling (Hill et al, 2003), and vasoconstriction and inward remodeling (Bakker et al, 2002, Martinez-Lemus, 2008), the pattern was opposite to what was expected, and is difficult to explain. An important future direction would be seeing if this trend holds up with a larger sample population.

C. Limitations and Future Directions

This study focused on the effect of estrogen + progesterone (single dose, 21-day release) alone, and estrogen + progesterone in combination with induced wall shear stress, in OVX rats. A major limitation in this study was that circulating hormone levels in OVX and OHL pregnant animals were not measured. We speculated that the difference in vascular remodeling observed between the two groups may have been influenced by varying circulating levels of estrogen and progesterone; however, an accurate comparison between the two groups could not be made. Further research should consider measuring the levels of sex hormones in OVX and OHL pregnant rats. In addition, the administration of estrogen and progesterone alone, and in varying doses is likely to alter effects on vascular remodeling and should be considered for further experimentation. Evaluation of NO production was unable to be measured for this study; however, this would provide a better understanding of what is underlying the changes in vascular remodeling that were observed. NO production is a major stimulator for vasodilation and

subsequent remodeling, but it should be considered that the changes observed in this study could have been due to a different factor. Time constraints and unexpected damage to MUA segments during vessel preparation (dissection and/or cannulation) limited the n number for this study. A larger n number and more experimentation would increase the accuracy of the patterns observed in this study, and provide a better perspective on the effect of pregnancy milieu on maternal vascular adaptation during pregnancy.

D. Conclusions

Pregnancy is a unique physiological state characterized by maternal vascular adaptation (vasodilation and uterine vascular remodeling) in response to increased UPBF. Placentation, involving trophoblast invasion of resistance arteries and subsequent increased UPBF, has been established as a major factor regulating maternal vascular remodeling (Cross et al., 2002, Fuller et al., 2009). As previously mentioned, this is primarily due to the increase in wall shear stress in response to increased UPBF, and the stimulation of NOS in response to shear stress. This study sought to determine the effects of pregnancy milieu (estrogen and progesterone) on maternal vascular remodeling in the absence of the hemodynamic effects of placentation, and of local factors that might be secreted by the placenta to affect neighboring vessels. Considering drastic increases in circulating hormone levels during pregnancy, it was predicted that pregnancy milieu is also a contributing factor in regulating maternal vascular remodeling. This study found that estrogen + progesterone was a prerequisite to the wall shear stress-induced maternal uterine arterial remodeling in OVX animals. These effects are likely augmented by local factors, specifically placentation and signaling from the fetoplacental unit. This information may be beneficial in the future care of patients with preeclampsia, in which there is decreased trophoblast invasion and little reduction in distal resistance. The vasodilatory and increased

blood flow effect of exogenous hormone administration may be influential for these patients in helping to compensate for the reduced vascular remodeling and UPBF characteristic of the disease.

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