

# Structural changes in the uterine artery of the domestic pig (*Sus scrofa domestica*) in the first trimester of pregnancy

## Abstract

**Background:** Adaptation of the uterine artery in pregnancy involves changes in anatomy that include widening of the lumen, medial thickening, elastic lamina degradation, and smooth muscle cell hypertrophy. Early remodeling of the uterine vascular system is needed to maintain pregnancy and ensure desired pregnancy outcome. The trigger mechanisms for uterine artery remodeling are uncertain. Also unclear are the regional variations in the uterine artery in the first trimester of pregnancy.

**Methods:** 10 domestic pigs (*Sus scrofa*) 5 in the first trimester of pregnancy and 5 non gravid were sacrificed to examine the structure of the uterine artery. The specimens were prepared for paraffin wax embedding and stained with Masson's Trichrome, Weigert's Resorcin-Fuschscin counterstained with Van Gieson stain and Hematoxylin and Eosin. Slides were examined with a light microscope at x40, x100, and X400 magnification. Observations made were recorded and photomicrographs taken.

**Results:** The Uterine artery of the domestic pig in the first trimester showed marked reduction in the thickness of the tunica intima, a prominent internal elastic lamina and elastic fibres. The tunica media had hypertrophied vascular smooth muscle with zonation of the media into inner circular and outer longitudinal smooth muscle layers in the proximal regions of the artery. There was also prominence of the Vasa vasorum in the adventitia of these uterine arteries that reduced distally along the artery.

**Conclusion:** The proximal sections of the uterine artery shows unique physical-mechanical adaptation in the first trimester of pregnancy related to increased demand of blood to the feto-placental unit.

**Keywords:** vasoactive substances, paraffin wax, non-gravid uterine artery, smooth muscles, artery, female sex steroids

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**Abbreviations:** TUA, terminal uterine artery; TCE, trichloroethane; IEL, internal elastic lamina; eNOS, endothelium nitric oxide synthetase; nNOS, neuronal NOS

## Introduction

The gravid and nongravid uterus receive blood supply from the paired uterine arteries and uterine branches of the ovarian arteries. These arteries anastomose giving rise to arcuate arteries which enter the myometrium to form the radial arteries and finally the spiral arteries within the decidua. In normal pregnancy, there are multiple physiological changes that take place to nurture the developing fetus. Maternal cardiovascular system, for example, undergoes adaptation such as a fall in systemic vascular resistance with dramatic increase in uterine blood flow to meet the fetal metabolic demands.<sup>1,2</sup>

In early pregnancy, the endovascular trophoblast invade the inner third of the uterus and the spiral arteries converting them into large caliber channels that are well adapted for the uteroplacental exchange.<sup>3,4</sup> Thus presumably, in a normal pregnancy, the limiting factor to the uteroplacental blood flow would be upstream within the conduit vessels.

Adaptation of the uterine artery in pregnancy involves changes in both histology and morphometry that include widening of the

lumen, media thickening, elastic lamina degradation, and smooth muscle cell hypertrophy which reverse after the termination of the pregnancy.<sup>5,6</sup> Early remodeling of the uterine vascular system contributes to the increased blood flow.<sup>7</sup> Since the change in the uterine blood flow occurs after vessel remodeling, there is need to maintain this state throughout the pregnancy. The trigger mechanisms for uterine artery remodeling are unclear. Several mechanisms have been proposed including, mechanical force exerted by increased blood flow,<sup>8,9</sup> increased level of circulatory vasoactive substances,<sup>10</sup> the conceptus and effect of estrogen acting through estrogen receptors have been suggested.<sup>11-13</sup> In complicated pregnancies such as diabetes, preeclampsia, obesity, there is inadequate remodeling of the uterine arteries which may lead to uterine artery dysfunction impairing the fetal growth and development.<sup>14-16</sup>

It is unclear whether there are regional variations in the adaptation of the uterine artery in the first trimester of pregnancy and what the changes (if any) would imply.

## Methods

A total of 10 domestic pigs (*Sus scrofa*) 5 in the first trimester of pregnancy (less than 1 month 1 week in pregnancy) and 5 non gravid were sacrificed to examine the structure of the uterine artery. Only animals that were verified to be healthy by a veterinary doctor and

did not have any recognizable cardiovascular disease were included in this study.

After weighing the pigs, they were anesthetized with sodium pentobarbital (50mg/kg IV) intravenously. With complete immobilization the animals were opened through a mid-line abdominal incision and thoracotomy to expose the heart. For complete clearance of blood, a canula were inserted into the left ventricle and saline, introduced from the perfusion kit 1.5metres above the heart and the right auricle punctured to drain out the blood followed by 10% formaldehyde solution perfused for 30 minutes using gravitational trans-cardiac perfusion kit.

The aorta, common iliac, internal iliac and uterine artery were identified sequentially. The uterine artery was dissected in three parts; the segment just after the internal iliac artery named main trunk uterine artery (MTUA), A. The segment within the broad ligament named broad ligament uterine artery (BLUA), B and the terminal part named terminal uterine artery (TUA), C.

The specimens were fixed in 10% formaldehyde solution and dehydrated in increasing concentrations of alcohol of 70% to absolute alcohol each for one hour. They were prepared for paraffin wax embedding by clearing in Trichloroethane (TCE) for two hours and infiltrated with wax for 12 hours. They were then embedded in fresh molten wax for 12hours. Seven micrometer thin serial sections were cut using a Leitz Wetzlar sledge microtome, floated in warm water and thereafter mounted and then dried in hot air oven at 40°C overnight.

The sections were stained with Masson's Trichrome and Hematoxylin and Eosin. Slides were examined under Leica Light

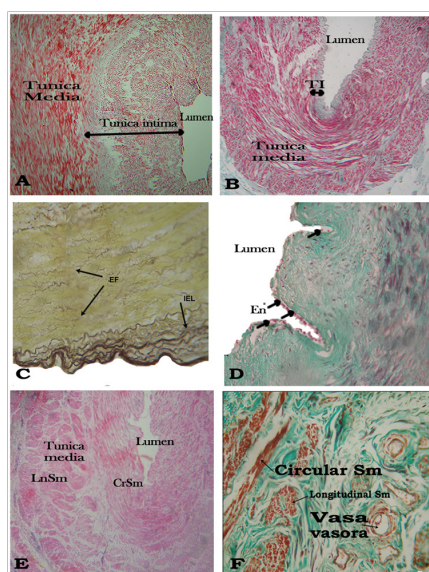
microscope at x40, x100 and x400 magnification. Observations made were recorded on the preformatted data sheet and photomicrographs taken.

## Results

During the first trimester pregnancy, the tunica intima reduced in thickness compared to the tunica intima from a uterine artery of a non-gravid uterus (Figure 1A & 1B). The tunica intima was composed of the endothelium, the subendothelial layer and a prominent, continuous but folded internal elastic lamina with some areas of duplication (Figure 1C). The endothelial cells were cuboidal in shape and exhibited prominent nucleus (Figure 1D). There was a marked reduction in thickness of the subendothelial layer and the fibromusculoelastic content were poorly discernible.

The tunica media was prominent with enlarged and elongated circular smooth muscles with prominent longitudinal muscle fibre bundles (Figure 1E) and reduced volume of collagen fibres but increased amount of elastic fibres (Figure 1C) compared to non-gravid uterine artery. The elastic fibres were interspersed within the elongated smooth muscles.

The tunica adventitia was composed of collagen fibres loosely and circumferentially arranged. Vasa vasora in the adventitia were more prominent than in non-gravid uterine artery (Figure 1F). These changes were consistent within the main trunk and broad ligament segments. The terminal segment had all the other features seen in the MTUA and BLUA except that the tunica media was composed of only circular smooth muscles and lacked longitudinal bundle seen in the other segments.



**Figure 1** Histology of the uterine artery of the domestic pig during the first trimester pregnancy. The uterine arteries biopsies were taken from three regions of the uterine artery the main trunk (MTUA), the broad ligament (BLUA) and the terminal segment (TUA) then prepared for paraffin wax embedding, sectioned and stained with either Hematoxylin & Eosin, Masson Trichrome stains or Weigert's Resorcin-Fuschscin counterstained with Van Gieson stain.

- (A) Uterine artery from a non-gravid adult domestic pig used for comparison purposes. Note the tunica intima showing a thickened morphology, X100.
- (B) Main trunk uterine artery in the 1st trimester pregnancy. Notice the thickness of the tunica intima, TI, markedly reduced compared to A. Note also the prominent internal elastic lamina, X100.
- (C) Main trunk uterine artery showing tunica intima and media. Note the prominent internal elastic lamina, IEL and elastic fibres, EF in the media., X100.
- (D) Broad ligament uterine artery showing the endothelial cells, (En). Notice that their shape varies from squamous to cuboidal, X100.
- (E) Tunica media of main trunk uterine artery. Notice the zonation of the media into inner circular, (CrSm) and outer longitudinal smooth muscle, (LnSm) layers, X100.
- (F) Tunica adventitia of broad ligament segment of uterine artery. Note the prominent vasa vasora in the adventitia, X400.

## Discussion

The tunica intima was markedly reduced in thickness compared to uterine artery from a non-gravid uterus with distinct endothelium, the subendothelial layer and a prominent, continuous and folded internal elastic lamina with some areas of duplication. This was more marked in the proximal section of the uterine artery. The reduction in intimal thickness has been described before and could be due to exposure to high quantities of circulating estrogen<sup>17-20</sup> and increased volume of blood flowing through the artery.<sup>21-23</sup> These factors are said to inhibit intimal hyperplasia. Indeed, it has long been postulated that sex of an individual has a role in the development of intimal hyperplasia with a greater female resistance. This has been attributed to direct or indirect beneficial effect of the female sex steroids.<sup>24,25</sup>

The Internal elastic lamina (IEL) represents a flexible barrier between the endothelium and tunica media and may have a role in physical barrier properties. It also allows for communication between the endothelium and the smooth muscle cell layers of the uterine artery through vasoactive substances and direct structural interaction.<sup>26,27</sup> The increased prominence of the internal elastic lamina is at variance with previous reports that suggest that internal elastic lamina degenerates during pregnancy.<sup>28</sup> The IEL has also been suggested to play a role in modulating smooth muscle cell migration from the media to the intima. For such smooth muscle cell migration to occur, it has been suggested that IEL fenestrations must be greater than 3–4µm wide. A prominent internal elastic lamina as observed in this case may be in part designed to provide increased mechanical strength to withstand the force of increased blood volume during the first trimester of pregnancy and may also explain the reduced intimal thickness of the intima during pregnancy.<sup>29</sup>

We observed that the smooth muscle cells were elongated, prominent and with multiple nuclei. There is insufficient information on the mechanism of medial remodeling during pregnancy. Some factors have been implicated including mechanical forces due to blood flow, increased levels of circulating vasoactive substances, higher amount of nitrous oxide and estrogen.<sup>30-32</sup> The elongation of the smooth muscle cells results from the direct effect of the endothelium nitric oxide synthetase (eNOS) and cell hypertrophy.<sup>33</sup> In addition, the neuronal NOS (nNOS) also causes outward remodeling of the media leading to vascular smooth muscle hypertrophy.<sup>34</sup>

The increase in the number and sizes of the vasa vasorum is in keeping with increased demand of oxygen and nutrition to the hyperplastic and hypertrophied tunica media. This is similar to other reports.<sup>35-37</sup> Failure of the uterine vessels to properly adapt may lead to uterine artery dysfunction marked by increased vessel resistance to blood flow to the fetoplacental unit. The consequences may involve fetal intrauterine growth restriction and other undesired pregnancy outcomes.

In conclusion, the proximal sections of the uterine artery shows unique physical-mechanical adaptation in the first trimester of pregnancy related to increased demand of blood to the fetoplacental unit.

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## Conflicts of interest

The authors declare there are no conflicts of interest.

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