The integrity of uterine circulation is essential for normal pregnancy outcome. The clinical relevance of maternal uterine vascular adaptation during pregnancy is underscored by the fact that its aberrance is associated with several common gestational pathologies, including intrauterine growth restriction, gestational diabetes, and preeclampsia [1]. Preeclampsia is a major cause of maternal and neonatal mortality and morbidity [2]. It is known that the vascularization of the uterus is of primary importance in the pregnancy success [3], and vascular disorders may play a role in pathological pregnancies [4]. Furthermore, normal uterine vascular development may have critical effects on the growth and development of the fetus and insufficient uterine vascular establishment is associated with increased risk for cardiovascular morbidity in adult life [5].

The lymphatic circulation plays an important role in regulating content of interstitial fluid and adaptive immunity [6] and lymphatic vessels could also play a role in maternal and fetal immunity [7]. The uterine lymphatics during pregnancy have been scantily studied.

Rabbit has a duplex uterus, with the main uteroovarian arteries and veins running parallel to, but well outside of, the uterine wall within the mesometrium. The vessels of the mesometrium are perfused by arterial blood coming from either the uterine or the ovarian end. Secondary vessels analogous to the arcuate arteries in humans may form redundant loops with the main artery, and tertiary radial arteries connect the arcuate loops with the uterine wall. These radial arteries divided into premyometrial arteries. The premyometrial arteries enter the uterine wall supplies the myometrium. These radial arteries divided into premyometrial arteries. The premyometrial arteries enter the uterine wall supplies the myometrium [1]. A better understanding of the basic morphological structure of the blood vessels of the uterus of the rabbit may guide the clinicians to the design of strategies to improve their reproductive efficiency and help in solve pregnancy problems which related to the blood vessels.

The aim of the present investigation was to study the different types of vessels in the uterus at critical early stages of pregnancy. The current study focused on arteries, veins, lymphatics, arterioles, venules, blood vessels of special structure.

### RESULTS

Mesometrial arteries were congested and demonstrated in varies directions

### MATERIAL AND METHODS

#### Sample collection

The study was approved by the Ethics Committee of Assiut University, Egypt. The material included in this work was originated from the female genitalia of 6 healthy New Zealand rabbits at 0 and 7 days of pregnancy. The uterus was taken immediately after slaughtering.

#### Histological preparation

The collected materials were dissected as soon as possible and immediately fixed in Bouin’s fluid for 22 hours. The fixed materials were dehydrated in ascending series of ethanol, cleared in methyl benzoate and then embedded in paraffin wax. Transverse paraffin sections at 1-7 μm in thickness were cut and stained with Harris haematoxylin and eosin for general histological examination, Crossmon’s Trichrome for identification of collagenous and muscle fibers and Wiger’s resorcin fuchsine for identification of elastic fibers. For carbohydrate histochemistry, sections were stained with Periodic Acid-Schiff (PAS) technique. For demonstration of neutral mucopolysaccharides, combined alcin blue-PAS technique were used [8].

#### Semithin sections and transmission electron microscopic preparations

Other small specimens of the uterus were fixed in a mixture of 2.5% paraformaldehyde and 2.5% glutaraldehyde in 0.1M Na-cacodylate buffer, pH 7.3 for 4 hours at 4°C. They were washed in the same buffer used and then post-fixed in 1% osmic acid in 0.1M Na-cacodylate buffer for further 2 hours at room temperature. The samples were then dehydrated in ethanol and embedded in Araldite-Epon mixture. Semithin sections (1μm in thickness) were cut and stained with Toluidine blue and examined under a light microscope. Thin sections, obtained by a Reichert ultramicrotome, were stained with uranyl acetate and lead citrate [9] and examined with a Philips EM 400.
cross and longitudinally oriented arteries (Figure 1A and 1B). The wall of these arteries was formed mainly of 3-5 layers of smooth muscle fibers tunica media, intermingled by elastic fibers. The tunica intima consisted of a thin layer of flat endothelial cells separated from the coating muscular media by a well-developed highly tortuous internal elastic membrane. The adventitia contained abundant elastic fibers and rich in collagenous fibers (Figure 1A-1D). In addition, there were arteries recorded in the mesometrium with spirally oriented smooth muscle fibers in the tunica media (Figure 1E).

Myometrial arteries possessed a wide lumen and surrounded by a 2-4 layer of smooth muscle fiber. In addition, the adventitia showed several blood vessels. These arteries demonstrate PAS-positive granules in their wall and well developed internal elastic membrane (Figure 1F-1H).

Mesometrial veins were numerous wide thin-walled vessels of different size. The tunica intima was surrounded by a thin tunica media composing of a 1-2 layer of smooth muscle fibers incircled by a relatively thicker adventitia rich in collagen and elastic fibers (Figure 2A and 2B). At the myometrial region, veins showed PAS-positive reaction in their wall (Figure 2C).

Several thin-walled wide-lumened lymphatics were recorded within the mesometrium. Their wall is composed of an intimal tunica media surrounded by a thick fibrous layer consisting of collagenous, elastic fibers and connective tissue cells. Some of these vessels demonstrate valves (Figure 2A).

Myometrial lymphatics were composed of an intimal tunic surrounded by a fibrous layer of collagenous and elastic fibers. The valves of these vessels were mainly thin and consisted of a single cellular layer. The wall of myometrial lymph vessels showed a positive reaction with PAS-AB combination (Figure 2D and 2E). The lymphatic vessel in the endometrium appeared as wide irregular thin walled vessels (Figure 2F).

Several arterioles were demonstrated within the mesometrium. These vessels were composed of 1-2 smooth muscle fiber-thick tunica media encircling a thin tunica intima with interrupted internal elastic membrane and surrounded by tunica adventitia which composed mainly of collagen fibers (Figure 3A-3C). In addition, arterioles of the myometrium were composed of 1-2 smooth muscle fiber-thick tunica media surrounded a thin intimal with interrupted internal elastic membrane and was surrounded by fibrous adventitial tunic which mainly consisted of collagen fibers (Figure 3D and 3E). Many arterioles were observed within the endometrium with 1-2 smooth muscle fiber-thick tunica media encircling a thin tunica intima with interrupted internal elastic membrane and surrounded by tunica adventitia.
which contained mainly elastic and collagenous fibers. In addition, few PAS-positive granules observed on the wall (Figure 3F-3I).

Venules wall consists of an intimal tunica surrounded by one cell-thick tunica media of circularly arranged smooth muscle fibers coated by thick adventitial tunic consisting of collagenous fibers and connective tissue cellular elements. However, venules of endometrium appeared as dilated thin wall vessels engorged with blood (Figure 3A and 3G).

Spiral artery also demonstrated and characterized by the wide lumen and spiral course. Its wall consists of tunica intima of endothelial cells surrounded by a thin layer of smooth muscle fibers and thin adventitia (Figure 4A). In addition, arterial sinuses demonstrated and characterized by wide irregular lumen, one layer of smooth muscle tunica media and unevenly disrupted elastic fibers (Figure 4B-4D).

Anastomosis between arterioles and venules was recorded in mesometrium and showed an abrupt change from the arteriole to a venule. Another arteriovenous anastomosis in the mesentery with several glomus cells and its lumen is closed (Figure 4E and 4F). Anastomosis between arterioles and venules was also recorded in the myometrium and (Figure 4C).

Blood vessels of a special structure were demonstrated within the mesometrium presenting a vascular wall of variable thickness. The thicker portions of the wall showed a tunica media of 2-3 layers of smooth muscle fibers. However, the thin portions of the wall showed tunica media consisting of an interrupted layer of smooth muscle fibers (Figure 5A). In addition, another vessel showed 2-3 layers of longitudinally-arranged smooth muscle fibers at one side only (Figure 5B).

Various special structures arteries observed within the mesometrium. Characterized by the wide lumen and 2-3 layer of glomus cells (Figure 5C). Another special type of artery characterized by smooth muscle fibers arranged at various directions and fibrinoid deposition (Figure 5D).

Various special structures veins observed within the mesometrium and characterized by a wide irregular lumen. The flattened endothelial cell lining was surrounded by 2-3 cell thick muscular media composing of circularly arranged smooth muscle fibers. These muscle cells were oriented into several protrusions, within the consecutively irregular lumen, bearing as muscular pads. The relatively thick adventitia mainly consists of collagenous and elastic elements. In addition, several islets of longitudinally-arranged smooth muscle fibers surrounded these vessels and characterized by PAS-positive granules (Figure 5E and 5F).

Small arterioles of a special structure were also observed and lined with 2-3 endothelial cells and surrounded by smooth muscle fibers of tunica media with few glomus cells (Figure 5G). Another arteriole with an occlusive lumen and one layer of glomus cells also demonstrated (Figure 5H).

Blood vessels of the special structure were demonstrated also within the myometrium. Our observation revealed that there were arteries with triangular shape lumen and surrounded by tunica media of spirally oriented smooth muscle fibers (Figure 6A). In addition, some arteries demonstrate 2-4 layer of circularly arranged smooth muscle fibers and patches of longitudinally smooth muscle fibers in between (Figure 6B).

Spirally oriented arteries were present within the myometrium with a wide lumen, clear internal elastic membrane and elastic fibers distributed at a various layer of the vessels wall (Figure 6C). Another spirally oriented arterioles observed and characterized by clear glomus cells in the tunica media (Figure 6D). In addition, there was another special type of arteries which characterized by the narrow lumen, thick wall and unevenly distributed elastic fibers in the wall (Figure 6E). Although, there was another type of arteries characterized by the wide irregular lumen and unevenly distributed elastic fibers in the wall (Figure 7A).

Veins of special structure could also be observed within myometrium bearing an irregular lumen and relatively thick fibrillar adventitia mainly consists of collagenous and elastic elements (Figure 7B and 7C). Small arterioles of a special structure were lined with 2-3 endothelial cells and surrounded by glomus cells (Figure 7D).

Transmission electron microscopic observations of uterine blood vessels at 0 day pregnancy showed that there were endometrial arterioles with endothelial cells surrounded by two smooth muscle layers. Endothelial cells and the inner smooth muscle cells gave cytoplasmic processes to each other and numerous filopodia-like protrusions of the endothelial cell toward the lumen (Figure 8A). Capillary with overlapping endothelial cell ends also observed and characterized by many caveolae of different size distributed singly or in small groups, numerous small vesicles, many free ribosomes,
Some arteries in the myometrium contained batches of longitudinal smooth muscle in between circularly arranged smooth muscle (black arrow) (Harris haematoxylin and eosin). (B) Artery with patches of longitudinally smooth muscle (black arrow) in between circularly arranged smooth muscle (black arrow) (Harris haematoxylin and eosin). (C) Spinally oriented artery (A) with a clear internal elastic membrane (green arrow) (Wigert’s resorcin fuchsin). (D) Spinally oriented arterioles with glomus cells in the tunica media (red arrows) (Wigert’s resorcin fuchsin). (E) Artery of a special structure with the narrow lumen (red arrow) and unevenly distributed elastic fibers in the wall (green arrows) (Wigert’s resorcin fuchsin).

At this stage of pregnancy transmission electron microscopic observations of uterine blood vessels showed that endothelial cells of the arteriole appeared to a plump with abundant mitochondria, some mitochondria, and myaline figure (Figure 8B). In addition, spirally oriented capillary demonstrated and characterized by abundant ribosomes and rich in caveolae which small in size and arranged in rows or large in size and distributed singly (Figure 8C and 8D)

7 days of pregnancy

The most prominent characteristic features of this stage of pregnancy were the observation of numerous vessels of special morphological character. Some arteries in the myometrium contained batches of longitudinal smooth muscle in between the circular smooth muscle of the tunica media (Figure 9A). In some arteries, smooth muscle of the tunica media appeared in different directions (Figure 9B). Other arterial tunica media showed different thickening of the smooth muscle from 1 to 3 layer (Figure 9C). In addition, there was arteria showed inner circular and outer longitudinal epitheloid cells in tunica media and thick adventitia (Figure 9D).

Our observation also revealed that Internal elastic lamina of some arteries appeared less developed but the external elastic lamina clearly well developed (Figure 9E) and in other arteries the elastic fibers distributed in one side of the wall only (Figure 9F). PAS-positive granules and PAS-positive material demonstrated in the wall of the blood vessels in the large amount (Figure 10A and 10B). Moreover, our observation also revealed that some arterioles surrounded by numerous venules (Figure 10C). Other arterioles of different size characterized by a very narrow lumen and some of them with dumbbell-like appearance (Figure 10D and 10E).

At this stage of pregnancy Arteriovenous anastomosis also observed with thick fibrous adventitia. The arterial side of the anastomosis may show narrow lumen and thick media (Figure 10F). However, another artery tunica media formed from epitheloid cells (Figure 10G).

Endometrial arteries showing special character one of them showed extensive venous or arterial profiles at the tunica adventitia of many arteri (Figure 11A). Spirally oriented arterioles and venules also observed at the endometrium (Figure 11B and 11C). In addition, arteriovenous anastomosis observe at the endometrium (Figure 11D). Extensive capillary network observed in lamina propria and take strong PAS positive reaction (Figure 11E).

At this stage of pregnancy transmission electron microscopic observations of uterine blood vessels showed that endothelial cells of the arteriole appeared to a plump with abundant mitochondria, numerous cytoplasmic filopodic extensions toward the lumen and few microvilli. The nuclei of these endothelial cells change to large nuclei (Figure 12A). However, the endothelium of the venule characterized by the presence of abundant free ribosomes, many mitochondria, some caveolae, few rough endoplasmic reticulum, scant microvilli and several filopodic extensions toward the lumen (Figure 12B-12D). Endothelial of the capillary contained caveolae and free ribosomes and large microvilli resemble the microvilli which present in the nearby telocyte (Figure 13B).
A histological, histochemical and ultrastructural characterization of uterine vessels at early stages of pregnancy

Figure 9) Arteries at 7 days pregnancy. (A) Myometrial artery showed batches of longitudinal smooth muscle (blue arrows) in between the circular smooth muscle of the tunica media (TM) (Harris haematoxylin and eosin). (B) Myometrial artery with smooth muscle of the tunica media appeared in different directions (blue arrows) (Harris haematoxylin and eosin). (C) Myometrial artery tunica media showed different thickening of the smooth muscle (blue arrows) (Harris haematoxylin and eosin). (D) Myometrial artery showed inner circular epitheloid cells (blue arrow) and outer longitudinal epitheloid cells (red arrow) in tunica media and thick adventitia (TA) (Harris haematoxylin and eosin). (E) Myometrial artery showed less developed internal elastic lamina (red arrow) and well developed external elastic lamina (green arrows) (Wigert's resorcin fuchsine). (F) Myometrial artery with elastic fibers distributed in one side of the wall only (green arrows) (Wigert's resorcin fuchsine).

Figure 10) Myometrial vessels. (A and B) PAS-positive granules (black arrow) and a large amount of PAS-positive material demonstrated in the wall of the blood vessels (blue arrows) (figure A and B stained with PAS). (C) Arteriole (blue arrow) surrounded by numerous venules (black arrows) (Harris haematoxylin and eosin). (D and E) Semithin sections of arterioles of different size characterized by the very narrow lumen (black arrows) and some of them with dumbbell-like appearance (red arrows). (F and G) Arteriovenous anastomosis showed the arterial side of the anastomosis (black arrow) and epitheloid cells (red arrows) (figure F and G stained with Harris haematoxylin and eosin).

Figure 11) Endometrial vessels. (A) Artery with extensive venous and arterial profiles at the tunica adventitia (black arrows) (Harris haematoxylin and eosin). (B and C) Semithin sections showed spirally oriented arterioles (black arrows) and venules (red arrows). (D) Arteriovenous anastomosis showed the arterial side of the anastomosis (blue arrow), venous side of the anastomosis (black arrow). (E) Extensive capillary network observed in lamina propria showed strong PAS positive reaction (black arrows) (PAS).

Figure 12) Digital colored TEM micrographs of uterine blood vessels at 7 days pregnancy. (A) Arteriole endothelium (E), nucleus (N), mitochondria (blue arrows), cytoplasmic filopodic extensions (black arrows) and microvacuoles (red arrow). (B) Venule endothelium (E), nucleus (N) and filopodic extensions (black arrows). (C and D) Venule endothelium (E), nucleus (N) and filopodic extensions (black arrows), free ribosomes (black arrowheads), mitochondria (red arrows), caveolae (green arrows), rough endoplasmic reticulum (blue arrows), scant microvacuoles (red arrowheads).

Figure 13) Digital colored TEM micrographs of uterine blood vessels at 7 days pregnancy. (A) Capillary (C), telocyte (T), telopoedes (black arrows) and microvacuoles (red arrow). (B) Capillary (C), telocyte (T), nucleus (N), caveolae (green arrows) and free ribosomes (blue arrow), microvacuoles (red arrows) and rough endoplasmic reticulum (black arrows).
DISCUSSION

Rabbit is considered one of the widely used laboratory animals. Rabbits have been used extensively for basic research in drug and bacteria, toxicology, healing, tissue and organ culture, mycology, skin sensitivity, immunology, ophthalmology, oncology and reproductive biology [10]. The rabbit is believed to be a helpful model for comparative biology in humans, concerning sperm capacitation and the general reactivity of the female genital tract during the reproductive cycle [11].

In this study, we established for the first time a detailed investigation of the arterial, venous and lymphatic mapping of the uterus at early stages of pregnancy in the rabbit. The viability of this vessels were very important for the female reproduction and fetus maintenance. It was recognized in the present study that arteries were congested and demonstrated in various directions in the mesometrial region.

Rabbit has uteroovarian arteries running within the mesometrium and giving secondary and tertiary vessels (radial arteries). These radial arteries give the premyometrial radial arteries enter the uterine wall supplies the myometrium. The uterus is drained by a venous system that parallels the arterial tree, with closely apposed arterioles and veins [1].

The present study demonstrated that arteries of mesometrial and myometrial regions were characterized by well-developed elastic lamina which helps the vessel to bear changes in blood pressure during pregnancy.

Large increases in uteroplacental blood flow during gestation are essential for normal foetal growth and survival and occur in every mammalian species studied, including human beings [12]. During pregnancy, the passive (fully dilated) diameter of the human uterine artery is approximately doubled [13], with similar changes reported in rodents, sheep, pigs and guinea pigs [14,15].

The myometrial arterial supply that course between the outer and middle thirds of the myometrium and this zone is referred to as the ‘vascular zone’. Because of their semicircular course, these arteries are referred to as ‘arrested’ arteries [16]. Our observation revealed that myometrial arteries characterized by its wide lumen, thin muscular coat, and vascularized adventitia.

Arteries and veins are arranged in close apposition in a number of species [17]. Our observation revealed that mesometrial and myometrial veins characterized by its thin wall encircled by a thick adventitia rich in collagen and elastic fibers.

The uterus is an immunologically unique organ in that it must be able to protect itself effectively from invading pathogens, but at the same time is faced with periodic exposures to foreign cells and tissues, namely allogeneic spermatozoa and the feto-placental unit. Even though the uterus seems to be an effective site of immunization for some antigens [18]. In rats, mice, and similar small mammals, there is only one main lymphatic plexus lying between the circular and longitudinal muscles. In addition, Endometrium possessed few or no lymphatics [19].

Our observation revealed that several thin-walled wide-lumened lymphatics were recorded within the mesometrium. Their wall is composed of an intimal tunic surrounded by a thick fibrous layer consisting of collagenous, elastic fibers and connective tissue cells. Some of these vessels demonstrate valves. The rat showed similar observation [20].

Myometrial lymphatics were composed of an intimal tunic surrounded by a fibrous layer of collagenous and elastic fibers. The valves of these vessels were mainly thin and consisted of a single cellular layer. The wall of myometrial lymph vessels showed a positive reaction with PAS-AB combination. The lymphatic vessel in the endometrium appeared as wide irregular thin walled vessels similar observation obtained in the rat [21].

Rabbit arterial sinuses as arterial device affecting the pulse pressure and regulate optimal condition for systemic blood pressure. Similar observations were recorded in camel ovaries [26] and camel skin [30].

In our study, Various vessels of special structure detected at different parts of the uterus. The vessels of the special structure are supposed to possess an important regulatory function for the peripheral circulation. They exert an active function on the blood flow and pressure regulation. This function is attained either through the contraction of the smooth muscle fibers or by the presence of glomus cells which cause a reduction in the diameter of the lumen by their ability to swell [25].

Ultrastructure study revealed that numerous filopodia-like protrusions at the arteriolar endothelium toward the lumen. The formation of filopodial structures described under inflammatory conditions and in response to the inflammatory mediators [31]. Mild but significant inflammatory activity is involved in the development of normal pregnancy, which might have important physiological roles [32,33]. The maternal inflammatory response is supposed to be modulated to allow the establishment and maintenance of a viable pregnancy.

Abundant caveolae of different sizes and different arrangement were observed in the endothelial cells of the capillary. The permeability of endothelial cells is usually described in electron microscope studies as related to the presence of caveolae [34]. With the advancement of pregnancy, endothelial cells of some vessels appeared swell to a plump and Similar observation obtained at early stages of human pregnancy [35].

Our data revealed that telocyte encircled the capillary with similar vacuoles in both. The telocyte discussed as play a role in juxta and parocrine signaling [36]. In addition, telocyte expressed estrogen and progesterone receptors and act as a hormonal sensor [37,38]. However, it always needs to be noted the exceptions cases out of such rules, and cutaneous horns at the gross level should prompt consideration of biopsy for the definitive diagnosis. Our case #10 also seems to support such speculation.

REFERENCES
