DEVELOPMENT OF ARTERIOVENOUS ANASTOMOSES IN THE SKINS OF RATS DURING THE FETAL PERIOD

Desarrollo de anastomosis arteriovenosas en la piel de ratas durante el período fetal

Mehmet Çimen

Dept. of Anatomy, Faculty of Medicine, Cumhuriyet University, 58140- Sivas / TURKEY
E-mail: mcimen@cumhuriyet.edu.tr
Tel: +90 346 2191010/ 1081. Fax: +90 346 2191155

ABSTRACT

In order to describe the development of AVAs in *Rattus norvegicus* species, the fetus were followed up for 10 days after coitum. The serial sections obtained from the fetuses of 10-20-day rats were subjected for examination under the light microscope. Various staining processes were applied on the preparations and the simple types of AVAs determined in the skin of fetuses were observed until birth every day. The regions with AVAs were then photographed by photomicroscopy and evaluated. An increase in the smooth muscles in the wall structure of simple anastomoses was observed in 10-day embryos until birth.

Key words: Arteriovenous anastomoses, rat, fetus, development.

RESUMEN

Para describir el desarrollo de AVAs en especies de ratas (*Rattus norvegicus*), los fetos fueron evaluados hasta 10 días posterior al coito. Las secciones seriadas obtenidas a partir de los fetos de ratas de 10-20 días, estuvieron sujetas a examen bajo el microscopio de luz. Varios procesos de coloración fueron aplicados sobre las preparaciones y los tipos simples de AVAs fueron determinados en la piel de los fetos, los cuales fueron observados cada día, hasta el momento del nacimiento. Las regiones con AVAs fueron fotografiadas mediante microfotografías y avaluadas. Un incremento de los músculos suaves en la estructura de la pared de anastomosis simples fue observado en embriones de 10 días hasta su nacimiento.

Palabras clave: Anastomosis arteriovenosa, rata, fetos, desarrollo.

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INTRODUCTION

Arteriovenous anastomoses (AVAs) are large vessels through which the blood may be shunted directly from arterioles to venules without passing through the capillaries. Thus, the AVAs allow a high level of local blood flow without overburdening the capillary net. In contrast to capillaries serving the tissue nutrition, it has been that AVAs have a thermoregulatory role in most skin areas. They are classified in two groups as simple and glomus anastomoses [2, 20, 22, 23].

Some detailed studies on bird skins have also been reported [9, 10, 13, 14]. In these studies, no researcher mentioned AVAs although some defined glomus or an aggregation of blood vessels in the period of incubation. It is then surprising that the presence of AVAs has not been mentioned earlier. It may probably be because the AVA morphology was unknown to the researchers [16].

The AVA morphogenesis in the rabbit ear [3, 4, 18, 19], kittens [12] chickens [15, 17] and rats [7, 8] has been investigated, but the studies related to the AVA development and density are unsatisfactory and insufficient.

It was investigated the development of the simple and glomus anastomoses in the mesenchymes of developing rat fetuses in a first study [7], and the development and morphology of the glomus type anastomosis in the rat skins during the development in the second study [8]. The aim of the present study is to follow up our earlier studies and attempt to determine the presence and development of the simple anastomoses in the skins of rat embryos.

MATERIALS AND METHODS

In this study, 20 *Rattus norvegicus* species obtained from the Experimental Animals laboratory of the College of Medicine, Cumhuriyet University, Sivas were used. At least two

animals were used for each day of gestation. Normal females were mated with experienced males and the day on which spermatozoa were detected in the vaginal smear was denoted as day 0 of gestation. Fetuses followed up in the rats during the period of 10-20 days post-coitum (pc) were placed in physiological serum following separation under ether anaesthesia. The parts separated from the fetuses in coronal, sagittal and transverse sections were kept in Bouin's fixative for 18 h. Later, they were dehydrated in 50-100% ethanol solution and embedded in paraffin. The serial sections obtained at 5-7 µm from the paraffin blocks using a Reichart microtome were subjected to various staining techniques such as haematoxylin-eosin and toluidine blue. In these preparations, the regions with identified AVAs were evaluated under a Jenamed 2 photomicroscope.

RESULTS AND DISCUSSION

Simple types of AVAs were observed in the skins of 10 day-old rat fetuses. Anastomoses were easily distinguishable from the surrounding vasculature by their irregular lumina and thicker walls. During these fetal days, the tunica media of AVAs were formed by one or two smooth muscle cell layers (FIG. 1). These smooth muscle cells increased to three layers in some skin areas by 12 days p.c. (FIG. 2). In all the AVAs, the arterioles and venules of 14-day old rat fetuses had a well-developed morphological appearance. The smooth muscle cells in the AVAs wall, had a circular arrangement in the outer part of the wall especially close to the venule, and longitudinally or irregularly to the lumina in the inner zone of the tunica media (FIG. 3).

In 16–day fetuses, the AVA wall, formed mainly by multi-layered smooth muscle cells were arranged circularly in the outer part (FIG. 4).

The most important feature of the AVA wall in the skins of 18-day fetuses was the maturation of the smooth muscle cells. They were arranged longitudinally in the inner part of the tunica media and irregulated the lumina. The internal elastic membrane was not observed (FIG. 5).

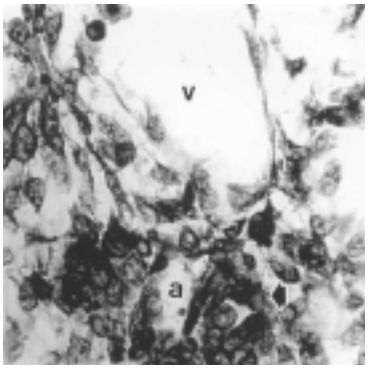

FIGURE 1. ARTERIOVENOUS ANASTOMOSIS (ARROW) IN THE SKIN OF A 10-DAY FETUS. ARTERIOLE (A), VENULE (V). HEMATOXYLIN -EOSIN. X 100.

In the skins of prenatal 20-day fetuses, it was observed that both the anastomoses and the vessels connected by anastomoses were mature. While the venule had a wide lumina and a thin wall, artery had a thicker wall with a typical internal elastic membrane. The AVA was easly distinguishable with its irregular lumina and numerous smooth muscle cells. With this morphological structure, it was seen that the AVAs began to mature (FIG. 6).

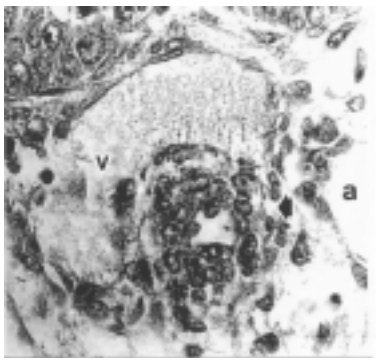

FIGURE 2. ARTERIOVENOUS ANASTOMOSIS (ARROW) IN THE SKIN OF A 12-DAY FETUS. ARTERIOLE (A), VENULE (V). HEMATOXYLIN -EOSIN. X 100.

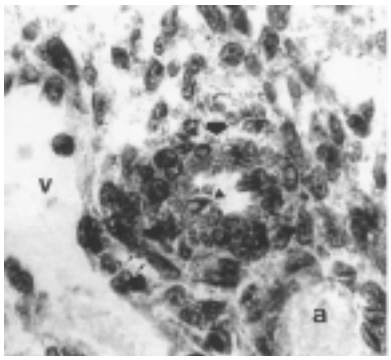

FIGURE 3. ARTERIOVENOUS ANASTOMOSIS (ARROW) IN THE SKIN OF A 14-DAY FETUS. ARTERIOLE (A), VENULE (V), CIRCULAR SMOOTH MUSCLE CELL (DOUBLE ARROW), LONGITUDINAL SMOOTH MUSCLE CELL (ARROW HEAD). TOLUIDINE BLUE. X 100.

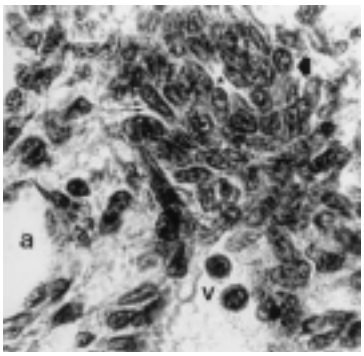

FIGURE 4. ARTERIOVENOUS ANASTOMOSIS (ARROW) IN THE SKIN OF A 16-DAY FETUS. ARTERIOLE (A), VENULE (V), CIRCULAR SMOOTH MUSCLE CELL (DOUBLE ARROW), SLIT-SHAPED LUMEN (ASTERISK). HEMATOXYLIN -EOSIN. X 100.

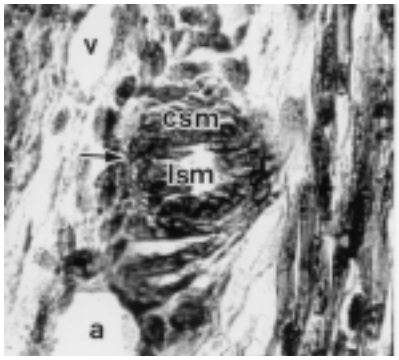

FIGURE 5. ARTERIOVENOUS ANASTOMOSIS (ARROW) IN THE SKIN OF A 18-DAY FETUS. ARTERIOLE (A), VENULE (V). CIRCULAR SMOOTH MUSCLE CELL (CSM), LONGITUDINAL SMOOTH MUSCLE CELL (LSM). HEMATOXYLIN -EOSIN. X 100.

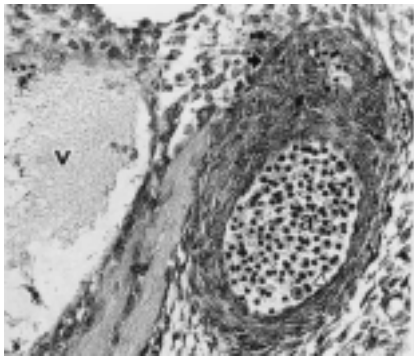

FIGURE 6. ARTERIOVENOUS ANASTOMOSIS (ARROW) IN THE SKIN OF A 20-DAY FETUS. ARTER (A), VEN (V), INTERNAL ELASTIC MEMBRANE (ARROW HEAD). HEMATOXYLIN-EOSIN. X 40.

AVAs consisted of basic capillary tubes, surrounded by the mesencymal tissue [2, 21]. The presence or absence of AVAs at specific times of ontogeny has been reported for various tissues in several species. In some of these studies, the structure of vascular bed in the ear of the rabbit has been demonstrated during the birth period by Clark and Clark [3, 4]. On the other hand, Morris and Bevan [18] showed the development of vasculer bed in the rabbit ear from 20 days of pregnancy to adult, but they couldn't observe AVAs in the young animals before postnatal 8 days. Similarly, Jasinsky and Miodonski [12] couldn't observe AVAs in 4-day-old kittens. In another study, Morris and Bevan [19] observed that the number of the AVAs increased after the ligation of the nerve fibers in the rabbit ear. Midtgard [16] quantifed the number of AVAs in the several areas of the skins of eyelids. In contrast, a few of AVAs were observed in the wattles and in the thorarcic skin. He also reported simple types of AVAs in the incubation patch of Herring gulls. In a study carried out on the effects of temparature and environment on the development of AVAs in the skins of chicken, the number of AVAs increased [17].

Çimen et al. [7, 8] observed the presence of simple and glomus anastomoses in the rat embryos. It has been shown that the number of the smooth muscle cells layers increased gradually from days 10 p.c. to birth. The presence and development of the embryos were also investigated. In the present study, the morphogenesis of the AVAs has been investigated in the skins of rat fetuses and observed that the smooth muscle cell layer also increased gradually from fetal 10 days to birth (FIGS. 1, 6). The findings of the presence of both simple and glomus anastomoses in the mesenchymes and skins of rat fetuses were found to be in conformity with the results obtained by Hale and Burch [11]. They observed some of the AVAs in the skin complex glomus organ. The increase of the smooth muscle cell layers in the AVA wall was consistent with the findings indicated by Midtgard [17]. He showed that the number of cell layers was two in hatching chicks whereas it was 4-5 in five-month old chicks [17]. The arrangement of the smooth muscle cell layers circularly in the outer zone and longitudinally in the inner zone of the AVA wall has also been shown as FIGS. 3, 5 in this study. This also was indicated as a characteristic of the skin AVAs by Amevo and Molyneux [1]. In addition the AVAs morphology in the skins of prenatal 18-20 days rat fetuses (FIGS. 5, 6) was similar to the finding suggested by Çimen and Gürsoy [5, 6] in the adult rat uterus and ovary at the light microscopical level, this had not developed completely. It may be suggested that the morphogenesis continues during the postnatal period. These features of AVAs are similar to those in the newborn human skins which were underdeveloped and insufficient [22].

CONCLUTIONS AND RECOMMENDATIONS

In order to determine the maturation time of rat AVAs, further researches should be undertaken to enlighten the postnatal AVA development.

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