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HISTOLOGICAL STUDIES OF THE OVARIES OF SOWS
IV. STEREOGRAPHICAL STUDY OF THE VASCULAR ARRANGEMENT
IN THE VARIOUS STRUCTURES OF OVARIES
BY USE OF NEOPRENE LATEX CASTING SPECIMENS

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INTRODUCTION

REYNOLDS stated the blood vessel reactions of ovarian follicles and of the various structures originated from them may be related closely to the function of the ovary. Therefore, up to this time the ovarian blood vessels in women and many animals, viz., mice, albino rats, guinea pigs, rabbits, cats, dogs and cows, have been described by HIS, CLARK, ANDRES, PETER, DEANSLY, BELOU, BASSET, BABCLAY, BELLMAN et al., KANENO and others. The methods of carrying on the studies were in all their respective ways, for example, there were the pigment injection method, the reconstruction technique, the microangiographical method injecting radio-opaque substances and so on. Recently it has become possible relatively easily to prepare elaborate casting specimens of the vascular system of various organs by the use of highly polymerized substances. So this method has been applied to a study on the vascular system of the ovary in rabbits, dogs, cows and women. Many contributions obtained by the use of this method have been published by REYNOLDS^{23,24,25,26}, DELSON, DELSON et al., BURR & DAVIES, DAITO et al., SHOJI, NISHINA, KOJIMA et al., KAKIMI^{15,16,17,18} and others.

But on the blood vessels of the ovary of the sow only two reports are known. One of them is that of CORNER in brief description; his observations were based on the specimens obtained by injection of india ink. The other report is that of ANDERSON in detailed description; her observations were based on the specimens obtained by injection of GEROTA's prussian blue-ether turpentine-injection masses in combination with SPALTEHOLZ technique.

The present writer improved on the neoprene latex injection method which has been employed on the blood vessels of the kidneys in various animals by TAKAHATA, and applied it to the study of the vascular system of pig ovaries.

MATERIALS AND METHODS

Materials for this research are pairs of ovaries of 21 sows, of which the ovaries of 17 (one case of a young sow, 14 cases of adult and non-pregnant sows which had no career of parturition and 2 cases of adult and non-pregnant sows with careers of parturition) were collected for the writer's third report; new materials were the ovaries of 4 other sows (3 cases of adult and non-pregnant sows which had history of parturition and one case of a pregnant sow).

In 18 left and 3 right ovaries neoprene latex 601 A was injected into their ovarian arteries for the purpose of making neoprene latex cast specimens of the blood vessels in the various structures of the ovaries. Other side of ovaries was used for the purpose of making the routine histological preparations.

For the injection technique one is referred to TAKAHATA's report. Neoprene latex 601 A, diluted with tap water 2 times at use, was injected slowly into an ovarian artery, until the latex began to flow out of the vein. If one follows the original TAKAHATA method, after ligation of the related artery and vein the injected specimens should be left at room temperature for about 2 hours prior to throwing into hydrochloric acid for maceration, but the present writer put them into 10% formalin immediately after the injection and fixed them for 24 hours at least. After fixing, the previously marked structures were cut off and macerated with industrial hydrochloric acid respectively for one or two days. Macerated neoprene casts were washed thoroughly with gently flowing tap water and preserved in 10% formalin again.

According to this method, the loose connective tissues in and surrounding various ovarian structures become hydrolysed and washed off entirely. On the other hand the epithelial tissue, dense connective tissue and smooth muscular tissue remain as transparent gelatinous substances respectively. Therefore various ovarian structures are demonstrable with their original characters. Especially corpora lutea and yellow spots can be recognized easily with the naked eye as semitransparent yellow masses. Blood vessels belonging to each structure can be traced stereoscopically with elastic gummy whitish casts of their lumina. The present writer would like to call such sort of specimens "incompletely macerated specimen"; they are fitted to the study of vascularity of minute structures stereoscopically.

RESULTS

I. Findings on Normal Ovarian Follicles

Findings of blood vessels in normal follicles are as follows:

In "incompletely macerated specimens" many secondary follicles less than about 0.2 mm in diameter are observed close beneath the albuginea ovarii. The theca folliculi and the follicular cavity are not demonstrable. To such follicles the related blood vessels are scarce. The follicles are scattered among the capillary network of the ovarian stroma.

Around the follicles of about 0.3 mm diameter there appears a theca folliculi-like structure. Several small branches springing from the large blood vessels of the ovarian stroma are seen to come into such a layer. These branches form a very poor capillary network around the follicle.

The follicles of about 0.5 mm diameter have a small follicular cavity and distinct *theca folliculi* which differentiated into two layers. In such follicles blood vessels form a wreath

of two layers around them: the blood vessels lying in the theca externa are few and comparatively slightly thicker; they form the loose network of the outer wreath. Arterioles of this wreath send off small branches which reach into the theca interna beneath the granulosa layer, where they make a somewhat coarse capillary network as the inner wreath.

In the vesicular follicles of about 1.0 to 4.0 mm diameter the outer wreath is composed of a looser network of relatively large vessels in comparison with those of the preceding stage. Moreover in this wreath some tortuous vessels are found. The capillary network of the inner wreath is thinner but more dense than in the preceding stage.

In the follicles with a diameter greater than 5.0 mm each layer of blood vessels of the theca folliculi shows conspicuous differentiation. One may easily separate the inner capillary network layer from the outer blood vessel layer artificially. In the theca externa an arteriole network develops markedly, and many venules are found in the demarcation between the theca externa and the theca interna. Moreover, relatively large capillaries which branch from arterioles of the theca externa are observed to run directly through the theca interna up to the striking capillary network beneath the granulosa layer.

In the mature follicles, which have a diameter greater than about 8.0 mm, well-developed capillary network appears here and there in the form of spotted maculae beneath the granulosa layer. In mature follicles also there are no blood vessels developed in the granulosa layer itself.

II. Findings on Atretic Follicles

1) White turbid follicles: The arrangement of blood vessels in the turbid follicles, which are at the second stage of cystic atretic follicles, varies conspicuously according to the stages of the follicular growth and of the degenerating processes.

a) Turbid follicles of about 0.5 mm diameter: In the follicles of this size, which show a second stage of regressive processes, a double blood vessel layer can be distinguished with difficulty in the wall after painful effort. The blood vessels of the outer wreath are thicker and more tortuous than those of the normal follicles of a similar size, and the vessel network is very loose. The inner wreath is composed of a very coarse capillary network. One can not find any capillary network in the parts where histological observation reveals thin hyaline layers with degenerated and collapsed capillaries.

In the follicles at the more advanced stage some thick tortuous blood vessels are found only in the portions, which coincide with the outer wreath. Capillary network of the inner wreath almost disappears. This finding coincides with the histological finding, in which a thick hyaline layer appears in the whole follicular wall and the capillaries contained in this layer are almost completely collapsed.

b) Turbid follicles of about 1.0 to 5.0 mm diameter: In the follicles of such sizes in the second stage of regressive processes, two vascular wreaths can be still distinguished apparently: the outer wreath is composed of a looser and more irregular network than in normal follicles of a similar size. The inner wreath is composed of a very coarse network of a few thin capillaries. Moreover at this stage many blood vessels of the outer wreath and small vessels which branch from them have already ended blindly on the way. In such portions the inner capillary network is not observed. This finding exactly corresponds to the histological finding, in which thin hyaline layers appear in some parts of the follicular wall.

In the follicles at the advanced stage of regressive processes, the blood vessels which coincide with those of the outer wreath are located at one side of the follicles, where the vessels connect with those of the ovarian stroma. These vessels are markedly winding tubes, and end blindly after a short or slightly long course. At this stage the hyaline layer is thick and surrounds the whole follicular wall as found by histological examination. In neoprene specimens no capillary network which coincides with those of the inner wreath are observed.

In the follicles at the more advanced stage, only a few thick and blindly ended blood vessels are found in some portions of the follicular wall.

2) White spots: Arrangement of blood vessels in the white spots, which are cystic atretic follicles from the second to the last stages, varies according to the sizes and the stages of the regressive processes as is also the case in turbid follicles.

In the large white spots with a diameter greater than 1.0 mm, the arrangement of the blood vessels is nearly like to that of the white turbid follicles.

In small white spots shown as scar-tissues, a few small blood vessels are found in the parts corresponding to the outer side of the radial layer. The tortuous capillaries which branch from those vessels end blindly shortly after their penetration into the inner hyaline layer through the radial layer.

At the more advanced stage of regression, some conspicuously tortuous blood vessels are observed in the portions corresponding to the radial layer, while no blood vessels are found in the hyaline layer.

III. Findings on Corpora Lutea of Non-Pregnancy

Vascular arrangement in corpora lutea of non-pregnancy, which were recognized to be at the bloom stage histologically, is as follows:

The corpus luteum at this stage shows a very distinct lobular picture. The corpus luteum is covered with a network of large blood vessels. Small arteries which occur from the large arteries run directly to the base of the lobules, and enter directly into the interlobular spaces. Although a few smaller collateral branches are sent off into the lobular tissues, a main trunk reaches almost to the central core, returns along the margin of the core, forming a single loop, and again runs throughout the substances of the lutein tissue to the periphery of the corpus luteum. The recurrent main trunk sends off several arborizing branches to the lobular tissues. Such small branches still bifurcate several times, and at last branch off into capillary networks. In this stage, on the other hand, small vessels which branch from the main trunk near the top of the vascular loop enter the central core of the corpus luteum and form very fine capillary networks there. Many small veins which connect with capillary networks join to the trunk of the vein lying in the interlobular spaces.

In the non-pregnant corpus luteum which is found as a yellow spot showing slightly heavy hyalinization of the interstitial tissue with signs of the histological hyaline body, there is no lobular picture. In the surface of such hyaline bodies are found many large tortuous blood vessels, especially the arteries with remarkable convolution. In the inside of the hyaline bodies very few tiny arterioles, which are conspicuously distorted and end blindly after a short or a long course, have vestiges of vascular arrangement as observed at the bloom stage. The capillary network almost disappears at this time.

The hyaline bodies at the more advanced stage of regression are reduced in size and

show irregular forms. A small mass of thick arterioles is found in one side of the hyaline bodies, but in the other side only a few vessels are found. A few arteries which remain on the surface of the hyaline bodies are thick and conspicuously convolute. All of small arterioles which remain in the interior are fragmentary.

IV. Findings on Corpora Lutea of Pregnancy

Vascular arrangement in corpora lutea obtained from a pregnant sow, with 9 fetuses of 6.9 cm average body length was as next described.

The pattern of vascular arrangement in pregnant corpora lutea is similar to that of non-pregnant corpora lutea in general feature, but in details there are some differences between the two. The capillaries in the substances of the pregnant corpora lutea are thinner and more numerous in comparison with those of non-pregnant corpora lutea. Therefore the capillary network of the former is far denser in appearance.

V. Findings on Vascular Bodies

Vascular arrangement in vascular bodies is as follows :

In neoprene specimens vascular bodies at the first stage of regression are found as large spherical masses of blood vessels. By manipulation with developing needles there are seen several large arterioles occurring from arteries lying in the surface of the vascular bodies; these arterioles proceed into one side of the bodies and branch off into numerous thick and tortuous or convoluted arterioles, which occupy the greater part of the vascular bodies. In this stage no capillary network is seen within the bodies.

Vascular bodies at the advanced stage of regression are the masses of intricately interwoven blood vessels, which are thicker and more tortuous or convolute than those of the previous stage. Thick and remarkably convoluted blood vessels are found on the surface of these vascular bodies.

Vascular bodies at the more advanced stage are found as the small irregular masses of very thick fragmentary vessels which are interwoven very intricately.

Vascular bodies shown as scar-tissues are the small irregular and flat masses of thick and fragmentary vessels. In this stage a few newly formed blood vessels are found to mingle among the fragmentary vessels.

DISCUSSION

The minute arrangements of blood vessels in the several fundamental structures of pig ovaries were observed on neoprene latex specimens.

Students have come to an agreement in respect to various animals that the primary follicles have no special vascular system of their own, and they lie scattered among the capillary network of the ovarian stroma without direct connection to blood vessels. The stadium of the follicles which acquire a layer of simple capillary networks differs according to investigators or to animal species used.

In SOWS ANDERSON found the youngest wreath around a follicle of 0.25 mm diameter.

In the present observations the secondary follicles of about 0.2 mm in diameter had still no special vasculature. However, a one-layered wreath of capillary network surrounding the follicles was found on the follicles of about 0.3 mm diameter.

In parallel with the differentiation of the theca interna from the theca externa, a double wreath of blood vessels has been proven by many investigators (CLARK, in women; ANDERSON, in sows; KOJIMA et al., in rabbits; KAKIMI, in rabbits, dogs and women). The outer wreath consists of networks of small arteries and veins; the inner wreath consists of a single layer of blood capillaries just on the inner surface of the theca interna. However NISHINA described the vascular network surrounding the follicles as a single layer in rabbits and as three layers in cows.

In the cases of sows the present writer's findings agreed quite with those of CLARK and others.

It has been reported by many authors that no vasculature is found in the granulosa layer of mature follicles as well as in growing follicles. CORNER has reported that in mature follicles of sows capillaries enter the granulosa layer at the base of the cumulus oophorus. Recently KAKIMI observed that in the last stage of mature follicles, only in dogs, does the inner capillary network produce conspicuous capillary sprouts into the granulosa.

The present writer did not find such features in mature follicles of sows histologically nor in the present study. In the previous histological observations it was recognized that in mature follicles, especially in those just before the rupture, the theca interna with conspicuously dilated capillaries infolds partially toward the granulosa. The spotted maculae of capillary networks on the inner surface of the theca interna are thought to correspond with the infolding of the theca interna.

Involution of ovarian follicles should occur at any stage of development and should indicate the various histological pictures with the degree of the regressive processes. Therefore arrangement of blood vessels related to atretic follicles should show different changes corresponding to those affairs. But there is no published description on the alteration of vascular arrangement throughout the atretic processes of ovarian follicles. Accordingly the present writer attempted to identify the histological features of blood vessels of white turbid follicles and white spots, which show atretic follicles of cystic type from the second to the last stages.

Cystic atretic follicles at the second stage still preserve almost similar vascular arrangement to that of the normal follicles. In this stage very tortuous blood vessels are found in the outer wreath as a result of contraction of the follicle wall. Moreover, accompanied by appearance of hyaline layers with collapsed

capillaries in the inner layer of the theca interna, the inner wreath partially loses the capillary network.

In parallel with the advance of atretic processes, hyaline layers become thicker and thicker on the outside of the follicle wall and at last surround the whole follicle wall like a capsule. As if in agreement with such histological findings, capillaries of the inner wreath diminish gradually and at last disappear entirely, and the blood vessels of the outer wreath also diminish gradually. Finally they become masses of conspicuously distorted blood vessels.

The pattern of the blood vessel arrangement of mature corpora lutea of the sow has already been described in detail by ANDERSON.

The present writer's findings in fully-formed corpora lutea of non-pregnancy coincide well with the findings of ANDERSON. It must be emphasized that arterioles occurring from arteries on the surface of corpora lutea run straight between the lobules, return near the central core and throughout their length give off small arterioles, which spread out to form a dense network in the lutein tissue.

On the vascular system of regressive corpora lutea of non-pregnant sows ANDERSON has stated as follows: "As the corpus luteum regresses, the lymphatic system is among the first structures to disappear. The blood capillaries and venules go next. The arterioles are conspicuously too many in relation to the amount of tissue drained. Finally old corpora lutea show as a small uninjected area surrounded by a few tiny arterioles, which still have their old arrangement and look like a hubless old wheel with a few spokes still clinging to its rim".

In the present observation, the writer did not observe lymphatic vessels. However, changes in the arrangement of blood vessels with the regression of non-pregnant corpora lutea were seen in good agreement with the description of ANDERSON. But small hyaline bodies which are old scar-tissues are shown as the small irregular masses of fragmentary arterioles.

It has been reported by ANDERSON that the vascular pattern of mature corpora lutea of pregnant sows is similar to that in mature non-pregnant corpora lutea.

In the cases of the present writer, the vascular arrangement of mature pregnant corpora lutea resembles well that in mature corpora lutea of non-pregnancy essentially, except that the blood vessels, especially capillaries are seen to have developed more conspicuously.

Up to this time, differences of the features of old scar-like corpora lutea of non-pregnancy and pregnancy have not been discussed histologically in any animals nor in women.

The present writer advocated in the previous papers that in sows vascular bodies are old corpora lutea of pregnancy. By means of neoprene specimens

the writer has proved that histological conception of vascular bodies stereo-angiographically. They are found as large masses of numerous, thick and distorted arterioles without capillaries among them. With the regressive processes the arterioles diminish gradually, but are more numerous and distorted in comparison with those found in old non-pregnant corpora lutea.

SUMMARY

The results are summarized as follows:

1. Around secondary follicles in the last stage and also around vesicular follicles in the early stage, a single layer of coarse capillary network is found in the form of a flattened wreath. At the late middle stage of vesicular follicles the vascular wreath become arranged in two layers, consisting of an outer layer of small vessels with arborization branches and an inner of coarse capillary network. The mature follicles have also two vascular wreaths.

2. In mature follicles, especially in ones just before rupture, capillaries of the inner wreath form a remarkable network appearing in spotted maculae on the inner surface of the theca interna.

3. Around cystic atretic follicles at the second stage a double wreath of blood vessels is found, but in the inner wreath capillary network is partially lacking. Along with the atretic process, the capillary network in the inner wreath decreases gradually and at last disappears completely. Meanwhile, arterioles and venules also decrease gradually.

4. The vascular pattern of non-pregnant corpora lutea at the bloom stage and that of the mature pregnant corpora lutea is similar except for a few details. That is to say, the capillary network of corpora lutea is arranged in lobules. The small arteries from large vessels in the outer side of the corpora lutea run straight into the corpora lutea along the interlobular spaces, and near the central core return and stretch again into the substances of the corpora lutea toward the periphery, branching off capillaries which form a dense network. There is no recurrence in veins, unlike arteries, but they take the course of ordinary veins.

5. In regressing corpora lutea the capillaries disappear first. Small vessels remain for a relatively long while and leave the vestige of vascular arrangement of fully-formed corpora lutea.

6. The vascular arrangement of vascular bodies was ascertained stereo-angiographically. It was proved that numerous, thick and distorted arterioles remained as the main constituent of vascular bodies, while most of the capillaries were not found.

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EXPLANATION OF PLATES

PLATE I. A Series of Normal Ovarian Follicles

- Fig. 1. Secondary follicle of 0.2 mm diameter.
- Fig. 2. Growing follicle of 0.5 mm.
- Fig. 3. Growing follicle of 0.8 mm.
- Fig. 4. Showing a part of the wall of a mature follicle which has a diameter of 1.0 mm.

PLATE II. A Series of Cystic Atretic Follicles

- Fig. 5. White turbid follicle of 1.0 mm diameter, which is at the second stage of regression.
- Fig. 6. White turbid follicle of 1.0 mm. which is at the advanced stage of regression.
- Fig. 7. White turbid follicle of 1.0 mm, which is at the more advanced stage.
- Fig. 8. Small white spot showing a scar-tissue.

PLATE III. A Series of Corpora Lutea of Non-Pregnancy

- Fig. 9. Corpus luteum at the bloom stage; View of outer surface.
- Fig. 10. Same as fig. 9; Cross-section view.
- Fig. 11. Yellow spot (hyaline body) showing moderate retrogression.
- Fig. 12. Yellow spot (hyaline body) showing more advanced retrogression.

PLATE IV. Corpora Lutea of Pregnancy and Vascular Bodies

- Fig. 13. Mature corpus luteum; View of outer surface.
- Fig. 14. Same as fig. 13.
- Fig. 15. Vascular body showing the first retrogression; View of outer surface.
- Fig. 16. Same as fig. 15.

PLATE V. Vascular Bodies

- Fig. 17. Vascular body showing moderate retrogression.
- Fig. 18. Vascular body showing more advanced retrogression.
- Fig. 19. Vascular body showing a scar-tissue.









