

The Cyclical Changes in the Uterine Lymphatics of the Pig. Investigations on the Perimetrium*

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Summary

The orthograde demonstration and outlining of the uterine lymphatic system in the pig, during the sexual cycle was successfully achieved by the resorption of patent blue violet and Japanese ink.

During oestrus the lymphatics in the perimetrium are markedly widened and variable in size.

The subsequent phases of the cycle are characterized by a reticulate arrangement so that the prooestrus resembles the picture of oestrus.

The removal of the dyestuff takes place through the myometrium in a matter of seconds into larger vessels in the mesometrium which have a typical beaded appearance.

Our knowledge concerning the structure and function of the lymphatic system has been greatly enriched during recent decades. The pure morphological demonstration of the lymphatic system has become changed to a functional morphology of the lymphatic circulation (57) and it is only on this basis that any pathological occurrence can be understood and explained.

The first description of the lymphatic system of the uterus was given by *Nuck* (1692) (37) although the anatomy of the uterus had already been described in the first century A.D. by *Hippocrates and Galen*. Since the time of *Nuck* (37) many research workers have occupied themselves with this subject and in the period that has followed, the angioarchitecture of the vascular system of the uterus has been investigated in man and other mammals, even during the endometrial cycle.

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However, up to the present time little is known (25), about the lymphatic vessels and capillaries of the uterus although investigations in man, domestic animals and in laboratory animals have enabled further information to be gathered. There have been investigations into the lymphatics of the human uterus, also during the recurring cyclical changes in the uterine mucosa, and even during pregnancy (2, 4, 5, 7, 10, 18, 26, 29, 30, 31, 32, 33, 34, 38, 39, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 54, 55, 56, 57 u.a.). Investigations have taken place into mammalian uterine lymphatics, for example in cattle (3, 19, 22, 23, 24, 25), in pigs (1), in horses and sheep (21), in cats (27), in rhesus monkeys (58), in rabbits (35, 52), and in the mouse (13, 14, 15, 16).

In the latter species the investigations have taken place predominantly on the lymphatics of the endometrium in young animals up to the onset of sexual maturity (15), as well as in nulliparous (13) and multiparous (14) animals in the recognized five stages of the sexual cycle, and also in older nulliparous and multiparous mice (16).

There have been no reports of similar investigations in the pig, according to the literature which is available to us. In the course of this research project on sexually mature nulliparous pigs an effort was made to elucidate the cyclic behaviour of the lymphatics in the perimetrium during the sexual cycle.

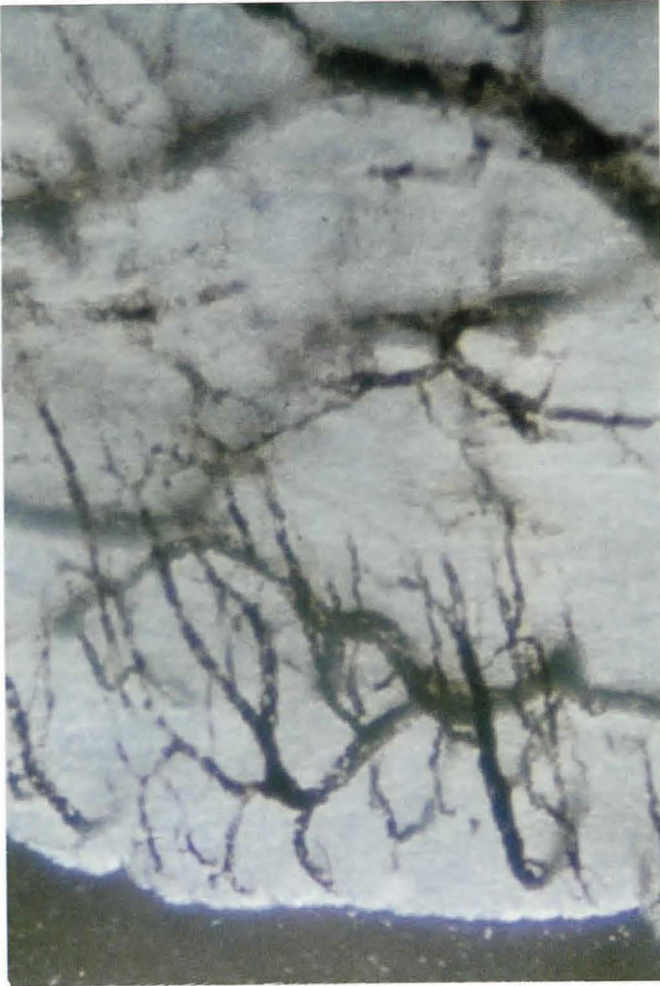


Fig. 1 Oestrus. Lymph capillaries in the perimetrium (below) with a larger vessel at the border of the myometrium running in the long axis of the uterine cornu. Absorption of Japanese ink, cleared in oil of wintergreen. Negative magnification 51 x. Positive magnification 288 x

Material and Methods

The investigations were carried out on 193 uteri obtained from sexually mature, nulliparous pigs (pure-bred German „Landschwein“) aged about five months, which were in various stages of the sexual cycle. As in the previous investigations (13, 14, 15, 16) patent blue violet (from Byk-Gulden, Constance) was again chosen, because it is known to have an affinity for the lymphatic system (6,

12, 53) as also has Japanese ink (20). The dye was applied to the surface of the mucous membrane of the left cornu of the uterus which was opened under the stereo-microscope slightly laterally, by the broad ligament (mesometrium). Retrograde and orthograde injections of dyestuff were also made. Subsequently the organ was fixed and put through the routine histological processes.



Fig. 2 Early postoestrus. Tortuous reticulate pattern of the lymph capillaries in the perimetrium. Absorption of Japanese ink, cleared in oil of wintergreen. Negative magnification 80 x. Positive magnification 434 x

Results

The following findings apply to all phases of the cycle, the total duration of which is normally 21 days.

After application of Japanese ink to the uterine mucosa there appears immediately, on its surface a black pattern which fades away into the depths of the mucous membrane and to some extent can be traced further peripherally, that is, towards the perimetrium (the

serosal surface). Under the peritoneal surface there appears, plainly outlined a network of lymphatic capillaries, which in the further course of the removal of the dye subsequently fades away in the direction of the myometrium. In general it appears that in the serosal layer (perimetrium), running in the long axis of the uterine cornu, there are two parallel lymph capillaries of larger size, which are joined together by transversely or obliquely running anastomoses.

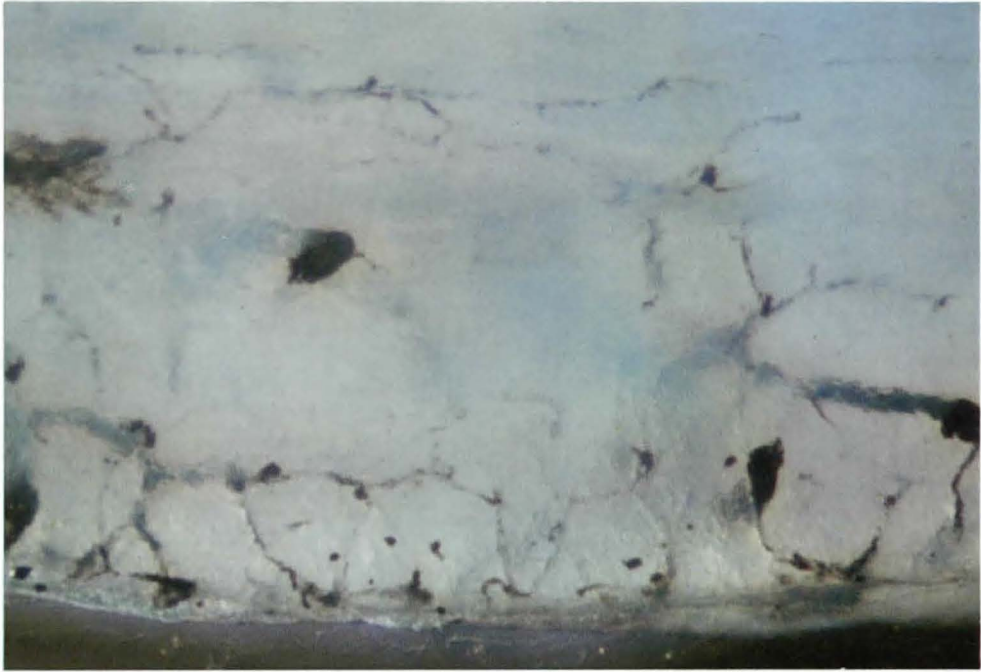


Fig. 3 Interoestrus. Regularly arranged network of lymph capillaries with dilatations in the perimetrium. Absorption of Japanese ink, cleared in oil of wintergreen. Negative magnification 64 x. Positive magnification 347 x

During *oestrus* these capillaries in the perimetrium can reach an enormous size, similar to those in the endometrium of the mouse (13) which, although solitary can reach ten times the size of the predominantly very fine branches which measure up to 3 μm . Coming off from these branches one can see a larger lymph capillary running in the perimetrium to the border of the myometrium, in the long axis of the uterine cornu (Fig. 1). In addition to this a second lymph capillary runs parallel nearly at the base of the perimetrium, which however is only seen intermittently during oestrus. Both lymph capillaries show a narrow, irregular reticulate communication one with the other.

We were unable to demonstrate in this or in the following phase of the cycle, the irregular network with numerous blind ramifications coursing in various ways towards the surface of the uterus, which had been identified in the bovine perimetrium by Jelínek (22). How-

ever, as regards the demonstration of these "blind" branches it must be borne in mind that by an appropriate injection of Japanese ink into these blind ends (or beginnings), lymph capillaries of very fine calibre (1–6 μm) are seen to fill. Evidence of this kind can be produced by a similar injection into the subcutaneous tissue of the lower leg in the rat (12).

In the next phase of the cycle – *early post-oestrus* – the lymph capillaries are found to be more tortuous and closely packed in their general appearance. It is the picture of a more uniform reticulate character (Fig. 2) whereas the pattern of the lymph capillaries in the earlier phase shows them to be lying relatively close together and drawn upwards.

In *late post-oestrus* the picture changes, to the extent that the arrangement of the lymph capillaries is much more homogeneous although on account of their great extensibility, the capillaries acquire a slightly wavy appearance.

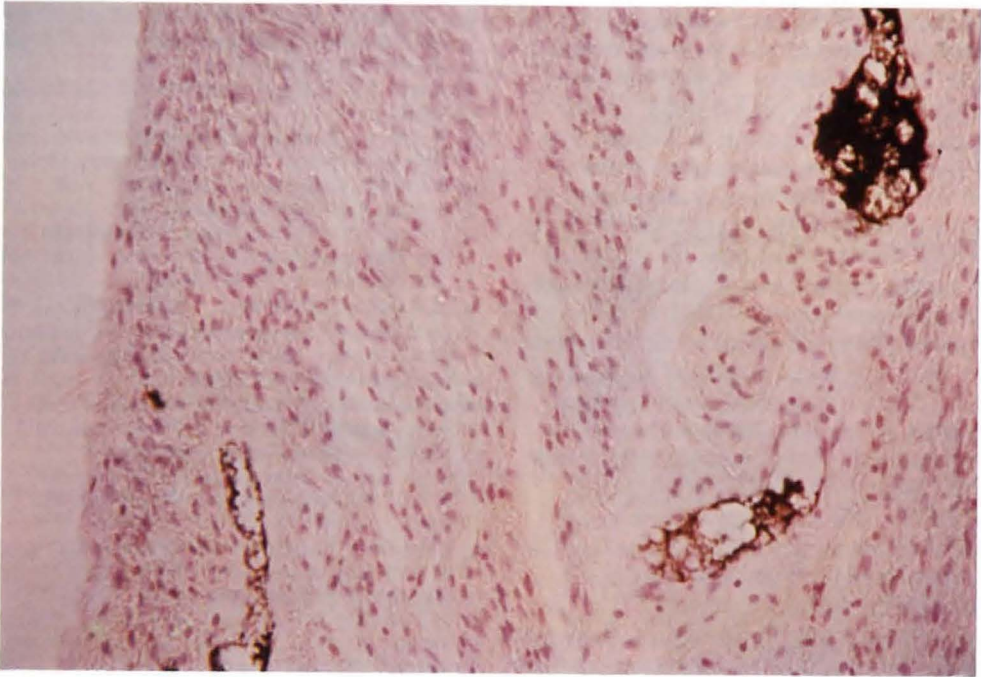


Fig. 4 Preoestrus. Branches of lymph capillaries visible in the perimetrium (lower part of figure) which are extending into the lymph capillaries at the border of the myometrium and from here are identifiable deeper in the myometrium (upper part of figure). Haematoxylin-Eosin. Negative magnification 160 x. Positive magnification 867 x

The *interoestrus* also characterized as the phase of recovery shows the lymph capillaries still slightly wavy but in the form of a uniformly arranged network. This often exhibits dilatations at the confluence of several lymph capillaries (Fig. 3) similar to the larger lacunae found in cattly by Jelínek (22) in the proliferative phase of the uterine cycle and also in multiparous cattle.

The *preoestrus* follows as the last phase of the cycle and is continued into the stage of oestrus, in which the lymph capillaries in the perimetrium begin to take on an elongated course. Otherwise it would seem to approach the picture as it exists later in oestrus. That is to say, that the size of the lymph capillaries corresponding to the *interoestrus* has increased, likewise there also appears an increase in fine coursing lymph capillaries which seem to be striving to advance to the border of the

myometrium (Fig. 4) in order to joint on to larger lymph capillaries, which in turn communicate with lymphatics in the myometrium.

Finally the removal of the dyestuff from here takes place very rapidly (in a matter of seconds) into the mesometrium where the lymphatics have a beaded appearance and run between the serosa and the blood vessels in the so-called stratum lymphaceum (28).

Discussion

The perimetrium — the serous covering of the uterus — is the continuation of the double layer of peritoneum which forms the broad ligament of the uterus (i.e. the mesometrium) and it is intimately attached to the outer aspects of the myometrium (8). The wall of the uterus from without in-

wards consists of the perimetrium, the myometrium, and the endometrium where functionally the most intense changes are found, as determined by the sexual cycle. Similar findings have been recorded in the pig (11, 40, 41) and changes associated with the sexual cycle have also been demonstrated in the blood vessels of the pigs uterus by *Engemann* (9). Investigations have likewise been done on the lymphatics (17), in which cyclical changes have been identified in the separate phases. Even the perimetrium is involved in the total processes, as will be shown. *Jelínek* (22) has undertaken investigations into cattle, with particular reference to the lymph capillaries of the perimetrium. If one uses the method of displaying the lymph capillaries by light microscopy it is to be expected with present day methods, that these are quite prominent. This has already been achieved by the technique employed (12, 13, 14, 15, 16) and enabled on to decide whether the the removal of the dyestuff takes place through the lymphatics or the veins. In the isolated organs which we have been using, removal of the dyestuff can still be guaranteed more than 15 hours post mortem, as a result of the intrinsic contractility of the lymphatics. *Mislin* (36) was also able to confirm experimentally the inherent (autochthonous) automatism of the lymphatics. It is possible to exclude completely any transport of the dyestuff through the vascular system as the contractility of the blood vessels is abolished soon after death and hence no removal of dyestuff is possible by this route. It is thus easy to differentiate the blood vessels from the lymphatics and lymph capillaries, a point which must again be particularly emphasized in these investigations.

Our findings are in agreement with the observations of *Jelínek* (22) on the lymph vessels in the bovine perimetrium and it thus appears that cyclic changes in the lymphatic system are also demonstrable in the pig in connexion with the sexual cycle.

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