Lymphology 25 (1992) 90-96

LYMPHATIC VESSELS IN THE BROAD LIGAMENT OF THE UTERUS IN SWINE

B. Gawronska, T. Doboszynska, A. Zezula-Szpyra

Department of Reproductive Endocrinology, Center of Agrotechnology and Veterinary Science, Polish Academy of Sciences, Olsztyn, Poland

ABSTRACT

The lymphatics of the broad ligament were depicted in 24 pigs by multiple interstitial injections of latex into the ovary and subserosa of the mesometrial margin of the uterine horn. Two morphologically different networks of lymphatics emanating from the uterus and ovary were established. Ovarian lymphatics leave the hilus, invariably enter the parovarian plexus, lie superficially under the perimetrium of the mesovarium, and run a parallel course closely intertwined with the blood vessels in this region. Some lymphatics are thus located close to the branches of the ovarian artery and the utero-ovarian vein. Whereas the ovarian and uterine lymphatics in the swine are not directly connected, the uterine lymphatics enter the mesovarium and lie in close proximity to both the ovarian lymphatics and nearby blood vessels.

There have been several detailed accounts of the topography and structure of lymphatic vessels in the reproductive organs of female domestic animals. Most of these studies examine the lymphatic network of the ovary (1-3) and the uterus (4-6), but there is a paucity of data about lymphatics in the broad ligament of the uterus. The anatomical course of the lymphatics from the ovary, the oviduct and the uterus, and the flow of lymph effluent from these organs have been described in the ruminant, mainly in sheep (7-10). Nonetheless, results obtained by different investigators have not been uniformly in agreement perhaps because of different techniques for lymphatic vessel filling. On the one hand, Morris and Sass (2) failed to visualize in sheep direct connections between uterine and ovarian lymphatic vessels. On the other hand, Meckley and Ginther (9) observed 4-8 large lymphatic vessels leaving the cranial part of the uterus and thereafter connecting with ovarian lymphatic vessels, although these communications were apparent only in the distal part of the parovarian plexus. Staples et al (10) supported these findings and also provided more detailed information on the lymphatics in early pregnant sheep and goat. They not only suggested the mixing of uterine and ovarian lymph, but also drew attention in almost all species to the anatomical closeness of these two lymphatic systems with the nearby ovarian artery.

The apparent existence of communications between groups of lymphatic vessels as well as their apparent close proximity to the walls of the blood vessels of the broad ligament of the ovine uterus warrants further morphologic studies to clarify the lymphatic anatomy in this region. Accordingly, we opted to examine the lymphatic system of the broad ligament of the uterus in another species of domestic animal, namely, the swine.

Permission granted for single print for individual use.
Reproduction not permitted without permission of Journal LYMPHOLOGY.

MATERIALS AND METHODS

Studies were carried out in 24 pigs in which the reproductive organs, including the broad ligament of the uterus, the ventral aorta and the posterior vena cava were removed immediately after slaughter. During the transport to the laboratory, the isolated organs were put into warm (37-39°C) physiological fluid containing heparin. The stage of the estrous cycle was assessed from the condition of the ovary according to Doboszynska and Janowicz (11).

To expose the lymphatic network, direct injections of "plastic" into the lymphatic lumen were carried out. In most cases, latex LBS-3041 from Oswiecim (Poland) was used. The procedure of preparation and the way in which latex was colored were processed according to Stefanowski et al (12).

Using a hypodermic needle, latex was manually slowly injected interstitially to fill either the lymphatic vessels exiting the uterus or those exiting the ovary. The uterine injections were done along one or both sides of the uterine horns into the connective tissue of the subserosa at intervals of 1-2cm. To avoid visible blood vessels, care was taken to avoid overly deep insertion of the hypodermic needle. In order to expose all lymphatic vessels, the injections were done both at the dorsal and ventral part of the uterine horn using two different latex colored solutions. After filling the uterine lymphatics, latex was injected interstitially into the ovary with a needle inserted 2-3mm into the connective tissue between the follicles and corpora lutea. Optimal visualization was obtained after 2-3 injections into the parabasal area of the ovary. The tissues with lymphatics filled with latex were fixed in 5% acetic acid and then prepared for stereoscopic microscopy and photographic documentation.

RESULTS

The lymphatics exiting the uterine horns were usually best displayed when the injections were done at multiple sites at the edge of the mesometrium and beneath the serous membrane at intervals not greater than 2cm. To a large extent, the number of injection sites depended on the breadth and length of the uterine horn, and thus was probably related to the age of the swine.

When only the ventral part of the broad ligament was injected, a complete one-layer dense lymphatic network was seen exiting the uterine horn at intervals of 5-10mm *(Fig. 1)*. In general, the greatest density and size of these lymphatics were noted in the widest portion of the broad ligament (i.e., from the isthmus of the oviduct to the uterine body). These features of the uterine lymphatics varied not only among the swine but they also depended on the stage of the estrous cycle. Less numerous and usually narrower

Fig. 1. The follicular stage of the porcine ovary. Lymphatics exit the uterus filled with latex injected subserosally into the uterine wall. The lymphatics that are visible in the broad ligament of the uterus form large trunks that lead into the parovarian plexus.

Permission granted for single print for individual use.
Reproduction not permitted without permission of Journal LYMPHOLOGY.

Fig. 2. Picture of the broad ligament of the uterus from Fig. 1. *Both lymphatics exiting the uterus (ML) and those exiting the ovary (JL) are filled with latex. The lymphatics exiting the ovary form longitudinally "stripes" in the parovarian plexus. Some of them drain to a lymph node (arrow).*

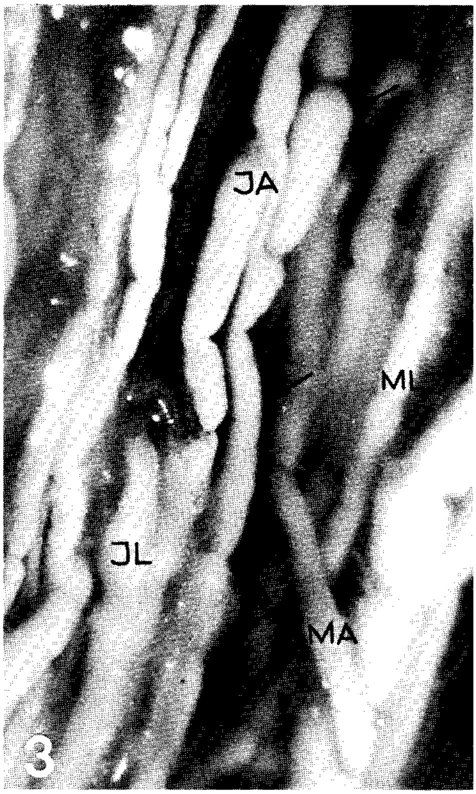

Fig. 3. A portion of lymphatics exiting the ovary (JL) and those exiting the uterus (ML) (magnified x4 from Fig. 2*). Arrows indicate a "separation" line between the two groups of lymphatics. Note branching among lymphatics exiting the ovary (JA) and among those exiting the uterus (MA), but no direct lymphatic inter-communications between these two organ system.*

collecting channels leading to an adjacent uterine lymph node or to lymph nodes in the more caudal part of the broad ligament were consistently observed.

In the second phase, lymphatic vessels exiting the ovary *(Fig. 2)* reached the parovarian plexus, and traversed the mesovarium in two longitudinal layers—dorsal and abdominal. These lymphatics were better displayed after several interstitial injections of latex into the parabasal part of the ovarian cortex. These lymphatics numbered from 15 to 18, involved more than half of the parovarian plexus, and usually terminated in the area of the regional lymph nodes, the number of which varied from one swine to another and even on both sides in the same swine (1-4).

In each swine examined, the collecting lymphatics exiting the uterus were in close proximity to the lymphatics leaving the ovary *(Fig. 3)*. Some of these lymphatics entered the area of the parovarian plexus to drain into the common and sometimes the only lymph node in this region. Whereas no connections were noted among lymphatics emanating from the two different sources (i.e., either ovarian or uterine), many branches were seen among lymphatics exiting either the ovary (short and closely intertwined with two systems of parallel vessels) or exiting the uterus (usually long and connecting more distant vessels) *(Fig. 3)*.

Other latex injections depicted

Permission granted for single print for individual use.
Reproduction not permitted without permission of Journal LYMPHOLOGY.

Fig. 4. Follicular stage of the ovary. At the top of the photograph are lymphatic vessels of the ovarian hilus and lymphatics lying at the anterior part of the parovarian plexus. Note the segmentation and multiple branches between valve imprints (arrows) (magnified x4).

Fig. 5. Late luteal stage of the ovary. "Stripes" of lymphatics from the central part of the parovarian plexus. Note parallel course of these lymphatics with many elongated segments (magnified x4).

lymphatics exiting the uterus both on the mesenteric border at the abdominal and dorsal aspect of the mesometrium. These lymphatics were located in the superficial part of the tissues between two layers of the lamina of the serous membrane. Because of the thin, fragile nature of the mesometrial wall at some sites, the lymphatic vessels of both these layers were more readily seen.

Grouping of lymphatics exiting the uterus and ovary varied as did the course of these lymphatics. Thus, lymphatics were most dense in both layers of the ovarian hilus *(Fig. 4)*. They were often twisted with numerous valves and as a consequence short, thick and oddly shaped segments were detected in these as well as bifurcating and branching lymphatics. Lymphatic vessels, 2cm from the ovary, were composed of long segments well delineated by deep indentations from intraluminal valves *(Fig. 5)*. The diameter to length ratio was approximately 1/2 to 1/8. A similar topography of lymphatics was noted in other portions of the parovarian plexus although the longest and smallest lymphatic segments were seen in the caudal part of the parovarian plexus *(Fig. 6)* especially before

Permission granted for single print for individual use.
Reproduction not permitted without permission of Journal LYMPHOLOGY.

Fig. 6. Late follicular stage of the ovary. Photograph of lymphatics at the caudal part of the parovarian plexus. Note the longer, variable segmental width and characteristic imprints of intraluminal valves in these lymphatics with shadows of adjacent blood vessels on their surface (magnified x6).

Fig. 7. Follicular stage of the ovary. Bunched lymphatic vessels exiting the uterus with thin, wide segments passing near to the ovary (arrows point to branches) (magnified x4).

entering a nearby lymph node. Small blood vessels in close proximity to the dense network of lymphatics were also seen *(Fig. 6)*.

Lymphatics exiting from the uterine horn were less dense than those exiting the ovary. Lobular-like lymphatic segments were stretched and narrowed at the valves. These segments were generally from 1-3 mm wide and 1-4 mm long and often with transverse branching which probably explained the abundant lymphatic network at this site. Approximately 15-20mm away from this network area were longer vessels with a more direct course and numerous bead-like segments.

The longest and largest lymphatic segments were near the uterine body. The dimension of these segments at the mesometrium depended on the stage of the estrous cycle with the larger segments seen during the luteal phase and shorter, narrower segments during the follicular phase. A similar segmental pattern was observed in more distant portions of the lymphatic collectors, which, in general, were composed of long, narrow segments, especially in the bunching uterine lymphatics near the ovary *(Fig. 7)*. The interconnections between these lymphatics were short, thin and the main trunks closely

Permission granted for single print for individual use.
Reproduction not permitted without permission of Journal LYMPHOLOGY.

paralleled one another.

DISCUSSION

These studies are the first attempts to define the swine ovarian and uterine lymphatic pathways in the broad ligament and their variations with the estrous cycle. In recent years, some have suggested a role for lymphatics in the transfer of hormones between the uterus and the ovary (7,8,10,13,14). We therefore paid special attention to the lymphatics emanating from both the uterus and the ovary including their possible interconnections. The most abundant lymphatic network in the swine broad ligament was clearly that emanating from the uterine horns. These lymphatics were primarily located beneath the uterine portion of the broad ligament and under the layers of the serous membrane. They were composed of two layers — dorsal and abdominal. In the more central and more caudal portion of the broad ligament, the number of these lymphatics gradually diminished. Some branching intercommunications transported uterine lymph to lymphatic collectors, the number (from 7-15) varying among the swine. On this account, some differences apparently exist between species. For example, in the swine, there were 15-18 lymphatics leaving the ovary whereas Morris and Sass (2) and Meckley and Ginther (9) found in sheep only 4-8 lymphatics.

From our study in swine and from other studies in other animal species (7-10,13), there are two groups of lymphatic vessels in the uterine broad ligament. First are lymphatics that emanate from the uterus and second, lymphatics that separately exit the ovary. The latter vessels drain into the ovarian hilus and invariably enter the region of the parovarian plexus. In swine most of these lymphatics are located superficially beneath the serous membrane. Because of their parallel course and close relationship, these lymphatics are coated and intertwined by blood vessels both on the dorsal and abdominal surface of the mesovarian. Some of these lymphatics are intimately associated with the spiral branches of both the ovarian artery and utero-ovarian vein in the parovarian plexus (11).

These studies confirm that in the swine, lymphatics exiting the ovary and those leaving the uterus are separate and not in direct communication with one another. On the other hand, the network of lymphatics exiting the uterus lead to the parovarian plexus and are situated in close proximity to lymphatics derived from the ovary and to nearby blood vessels. In the ewe (7,10) and in the cow (13), a similar close relationship exists between lymphatics and blood vessels (mainly the ovarian artery), which supports the possibility of a countercurrent transfer of uterine $PGF_{2\alpha}$ to the ovary via the bloodstream or lymph system.

Heap et al (8) reported that ^{3}H-$PGF_{2\alpha}$ concentration was considerably higher in the ovary when ^{3}H-$PGF_{2\alpha}$ was injected into uterine lymphatic vessels than when it was injected into the uterine vein. Connections between lymphatics exiting the uterus and those from the ovary in ewe as previously described (7-10) made the transport of substances theoretically possible from the uterus via lymphatics into the parovarian plexus. However, other investigators differ on this issue (2). Moreover, Heap et al (8) further suggested that the countercurrent transfer might be a two-way exchange if $PGF_{2\alpha}$ from uterine lymph, for example, is transported first into venous blood and subsequently into ovarian arterial blood. Alternatively, countercurrent transfer could be one-way exchange if, for example, $PGF_{2\alpha}$ from uterine lymph is transferred into arterial blood through the walls of closely allied lymphatics and arteries in the parovarian plexus.

In swine, a network of lymphatics exit separately from the uterus and the ovary and often differ in their course, the length and width of their segments and the arrangements of their valves. These lymphatics may also theoretically participate in the countercurrent transfer mechanism as suggested by Kotwica et al (15), and supported by the scanning studies of the parovarian plexus (Doboszynska et al,

Permission granted for single print for individual use.
Reproduction not permitted without permission of Journal LYMPHOLOGY.

16), where lymphatics draining to nearby lymph nodes are coated by a dense network of very small arteries which compose the lymphatic vasa-vasorum.

Although our studies failed to demonstrate direct intercommunications between ovarian and uterine lymphatic pathways in the broad ligament, the close proximity of these lymphatics and adjacent blood vessels especially in the region of the parovarian plexus makes a countercurrent transfer of substances between lymph and blood possible and worthy of further investigation.

ACKNOWLEDGMENT

This work is supported by Polish Academy of Sciences (project RPBP 05.2.6). The authors thank A. Penkowski, M.Sc., for preparing the photographs.

REFERENCES

1. Andersen, DH: Lymphatic and blood vessels of the ovary of the sow. Contrib. Embryol. 88 (1926), 107-123.
2. Morris, B, MB Sass: The formation of lymph in the ovary. Proc. R. Soc. V. 164 (1966), 577-591.
3. Riccardi, MP, P Marroni, F Giannessi, et al: A contribution to knowledge of lymphatic vessels morphology in the ovary of the cat. Boll. Soc. It. Biol. Sper. 5 (1989), 381-384.
4. Fabian, G: The cyclical changes in the uterine lymphatic of the pig. Investigations on the perimetrium. Lymphology 14 (1981), 17-23.
5. Fabian, G: Die zyklischen Veranderungen der Lymphefabe in Uterus des Schweines. Untersuchungen am Myometrium. Z. Angew. Zool. 71 (1984), 489-497.
6. Fabian, G: Zyklische Veranderungen der Lymphefabe im Uterus des Schweines. Untersuchungen am Endometrium, Lymphologie 12 (1988), 36-39.
7. Abdel Rahim, SEA, KP Bland: The lymphatic drainage of the cranial part of the sheep's uterus and its possible functional significance. J. Anat. 140 (1985), 705-709.
8. Heap, RB, IR Fleet, M Hamon: Prostaglandin $F_{2\alpha}$ is transferred from the uterus to the ovary in the sheep by lymphatic and blood vascular pathways. J. Reprod. Fert. 74 (1985), 645-656.
9. Meckley, PE, OJ Ginther: Lymphatic drainage of uterus and ovaries in the ewe. J. Anim. Sci. 1 (1969), 195.
10. Staples, LD, IR Fleet, RB Heap: Anatomy of the utero-ovarian lymphatic composition of the afferent lymph in relation to the establishment of pregnancy in the sheep and goat. J. Reprod. Fert. 64 (1982), 409-420.
11. Doboszynska, T, K Janowicz: Attempts at determination of the phases of maturation and involution of the sow corpus luteum. Folia Morphol. 39 (1980), 363-377.
12. Stefanowski, T, T Doboszynska, K Janowicz, et al: Zastosowanie polskiego lateksu syntetycznego LBS 3041 w anatomicznych badaniach naczyn krwionosnychi chíonnych. Polimery w Medycynie 9 (1979), 215-220.
13. Hein, WR, JN Shelton, MW Simpson-Morgan, et al: Flow and composition of lymph from the ovary and uterus of cows during pregnancy. J. Reprod. Fert. 83 (1988), 309-323.
14. Magness, RR, SP Ford: Estrone, estradiol-17 and progesterone concentrations in uterine lymph and systemic blood throughout the porcine estrous cycle. J. Anim. Sci. 57 (1983), 449-455.
15. Kotwica, J: Mechanism of prostaglandin $F_{2\alpha}$ penetration from the horn of the uterus to the ovaries in pig. J. Reprod. Fert. 59 (1980), 237-241.
16. Doboszynska, T, A Zezula-Szpyra: Morphological basis of the vascular paraovarian plexus functioning during the oestrous cycle in pig and sheep. II. SEM studies of corrosion casts of the arterial, venous and lymphatic vessels with special attention on side microcirculation network in the vasa-vasorium network of the paraovarian vacular plexus. Roczniki Nauk Rolniczych, PWN, 223 (1990), in press.

Barbara Gawronska, M.Sc.
Polish Academy of Sciences
Dept. of Animal Reproduction
Endocrinology
10-718 Olsztyn Kortowo 32
P.O. Box 55
POLAND

Permission granted for single print for individual use.
Reproduction not permitted without permission of Journal LYMPHOLOGY.