DISTRIBUTION OF SPERMATOZOA IN THE OVIDUCT AND FERTILITY IN DOMESTIC BIRDS

VII. INNERVATION AND VASCULAR SUPPLY OF THE UTEROVAGINAL SPERM-HOST GLANDS OF THE DOMESTIC HEN

A. B. GILBERT,* MARGARET E. REYNOLDS
AND F. W. LORENZ

*Department of Animal Physiology, University of California,
Davis, California, U.S.A.*

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Summary. The innervation and vascular supply of the uterovaginal sperm-host glands of the domestic hen were investigated. No nerves were associated with these glands, although nerves were demonstrated in other regions of the oviduct.

The glands have a complex blood supply consisting of a capillary network connected to the arterial and venous systems and to the epithelial capillary plexus. The capillaries of the glands are in intimate contact with the cells over relatively great lengths.

The possibility of the vascular system being in some way related to the control of the function of the glands is discussed.

INTRODUCTION

Recently sperm-host glands have been identified and described in the uterovaginal region of the oviduct of the domestic fowl (Fujii, 1963; Fujii & Tamura, 1963; Bobr, Lorenz & Ogasawara, 1964a; Bobr, Ogasawara & Lorenz, 1964b). They are located within a circular strip, about 2 to 5 mm wide, just distal to the so-called uterovaginal sphincter. The function of the tubular glands appears to be related to the survival of spermatozoa within the oviduct, thus allowing sequential fertilization of ova for a period of about 10 to 21 days. It is unknown how this survival is achieved. Likewise, little is known about the mechanisms involved in the control of glandular function and the release of spermatozoa at the correct time.

Hodges (1965) reported in detail on the vascular supply to the uterus, but did not consider the region where the host glands are found. Gilbert, Reynolds & Lorenz (1966) gave brief details of the vascular supply of the glands; this paper reports on a more extensive investigation of the blood system, together with a study to determine the innervation.

* Present address: A.R.C. Poultry Research Centre, Edinburgh, Scotland.

MATERIALS AND METHODS

The birds used were Single Comb White Leghorns (SCWL), of the Department of Animal Physiology, University of California, Davis, and Brown Leghorns of the Agricultural Research Council's Poultry Research Centre, Edinburgh. Experimental birds, 12 to 52 weeks old, were chosen at random from each of the above flocks. Some of the adults were inseminated (Lake, 1962) and left for 48 hr or more, until it was confirmed that fertile eggs were being laid; others were inseminated and left for 10 to 21 days, until infertile eggs were being produced again; still others were used in the virgin state. In this way it was hoped to relate any possible effects of insemination to the vascularization and innervation of the cells of the host-glands.

To investigate innervation, birds were killed by an overdose of Nembutal (Abbott Laboratories) and the uterovaginal region dissected out. Nerves were stained by one of the following techniques (Gilbert, 1965a):

- (1) the method of Holmes (1947);
- (2) the Ranson bulk impregnation (1914);
- (3) modified Methylene Blue technique of Schabadasch (Gilbert, 1961);
- (4) triple silver impregnation (Gilbert, 1965b).

The presence of myo-epithelial cells was studied by the methods of Richardson (1949) and Linzell (1952).

The vascular system was demonstrated by adopting one of the following procedures:

- (1) the bird was killed with Nembutal, and a solution of Indian ink (Pelikan; Günther Wagner) was injected through the abdominal aorta under slight pressure to ensure adequate distribution;
- (2) the bird was anaesthetized with Nembutal, injected with Indian ink (C11/1431a; Günther Wagner) in the wing vein and, after 2 min (to allow complete circulation), was killed by an overdose of Nembutal;
- (3) either acetylcholine (1.0 ml of 1:5000 solution) or adrenaline (0.5 ml of 1:200,000 solution) was injected into the wing vein, and after 30 sec procedure 2 was carried out.

After death, the required tissue was dissected out as quickly as possible after clamping the larger blood vessels in order to prevent drainage, movement or loss of the injected material. Then, either the tissue was dropped into an isopentane-solid CO₂ mixture (approx. -75° C) and sections were cut on a cryostat at -15° C (10 μ) or on a freezing microtome (40 to 60 μ), or the tissue was fixed in 10% formalin for paraffin embedding (5 to 10 μ) or for sections to be cut on the freezing microtome (20 to 30 μ). Thus, sections were available for study with a range of thickness from 5 to 60 μ . In some cases it was possible to see the tissue in better detail after the sections were stained lightly in a haematoxylin solution (diluted 1:1) for 30 to 60 sec. Some small pieces of tissue were also mounted whole so that the general distribution of the vascular system might be seen under a low-power, binocular microscope.

Histological examination of the normal micro-anatomy of blood vessels was carried out on tissue taken from uninjected birds. Paraffin sections were cut and stained with haematoxylin and eosin.

RESULTS

Since no differences were found between SCWL and Brown Leghorns in vascularization and innervation of the sperm-host glands, the two breeds were considered together. The general anatomy and histology of this part of the oviduct, an area a few millimetres wide at the junction of the uterus and vagina, has been described briefly by Bobr *et al.* (1964a) and by Gilbert, Reynolds & Lorenz (1968). The mucosal surface is thrown into a series of folds or small leaflets (Pl. 1, Fig. 1) and is covered by a ciliated and glandular

TEXT-FIG. 1. Diagram of a leaflet of the uterovaginal mucosa showing the venous system only.

epithelium. The sperm-host glands are to be found within the leaflets. This study was concerned only with the innervation and vascular supply of the host glands within the leaflets, since studies have been made on the broader aspects of the innervation (Gilbert & Lake, 1963) and vascular supply (Hodges, 1965) of the oviduct.

Despite an extensive search and the use of a wide variety of stains, no nerves were found to be associated with the glands. Although many nerves were found in the sections, they were all in the muscular or serosal layer; none entered

the mucosa. No evidence was found of myo-epithelial cells associated with the tubular glands.

In general, the vascular system of the leaflets was similar to that described by Hodges (1965) for the folds of the uterus. The venous system (Text-fig. 1) consisted of one main drainage venule, running more or less mesially through the corium of the leaflet (Pl. 1, Fig. 2). At the base of the fold this passed laterally to join with venous systems from other leaflets (Pl. 1, Fig. 3), and in some cases it also passed through the muscle layers to join the general venous drainage of the oviduct. At the apex of the leaflet, the main venule divided into a series of smaller vessels (Pl. 1, Fig. 4); branching also occurred along its entire length. The branches linked up with a complex and very extensive epithelial plexus (Pl. 1, Fig. 5) which appeared to be equivalent to, and homologous with, the peripheral capillary network described by Hodges (1965). The arterial supply to the fold was essentially similar to the venous drainage and usually consisted of one small, central arteriole which ran alongside the main venule (Pl. 1, Fig. 6) and branched into the capillary network.

The blood supply of the glands was extensive (Pl. 2, Figs. 7 to 12) and in some respects resembled a miniature portal system. Branches arose from the central arteriole and joined a fine network of capillaries surrounding the glands. This capillary bed was emptied by small vessels into both the main drainage vessel (Pl. 2, Fig. 7) and the capillaries of the epithelial plexus. It is therefore possible for blood to flow from the central arteriole through the epithelial plexus to the glandular network as well as from the central arteriole to the glandular plexus and thence to the epithelial capillary bed. What is remarkable about the plexus of the glands is not only its extent, but also its close proximity to the gland cells. In many sections lengths of blood vessels of 100 μ or more were in direct contact with gland cells (Pl. 2, Figs. 9 and 10). It was evident that, for most of the capillaries of this plexus, only the capillary wall separated the blood from the cell walls of the glands (Pl. 2, Figs. 11 and 12).

In immature birds, the vascularization of the region where the sperm-host glands exist was identical with that reported for the folds of the uterus (Hodges, 1965); a central main arteriole and venule supply the peripheral capillary network of the epithelium. At this age, there was no sign of the adult glandular capillary network deeper in the corium. The host glands first make their appearance as slight invaginations of the epithelium, becoming progressively deeper. These depressions gradually become tubular and take on the appearance seen in the adult. The adult blood supply does not, however, seem to be

EXPLANATION OF PLATE 1

FIG. 1. A low-power view of a section through the oviduct to show position and appearance of the host glands. Haematoxylin and eosin.

FIG. 2. Leaflet showing the main drainage venule. Indian ink, haematoxylin. Frozen section.

FIG. 3. Showing the central venule passing through the muscle layers (M) and also joining a vessel from an adjacent fold. Indian ink, unstained. Frozen section.

FIG. 4. Terminal branching of main venule. Indian ink, unstained. Frozen section.

FIG. 5. Epithelial plexus. Indian ink, unstained. Frozen section.

FIG. 6. Arteriole (A) and venule (V). Indian ink, unstained. Frozen section.

PLATE 1

(Facing p. 308)

PLATE 2

(Facing p. 309)

established at this time; up to this point, the host gland appears to be essentially avascular.

With the methods used, no changes in the vascular epithelium were observed following injection of acetylcholine or adrenaline. Similarly, no effects on the vascular supply were noted after insemination, nor could we distinguish between virgin hens and those artificially inseminated either 24 hr or 10 days before dissection and examination.

DISCUSSION

Failure to discover any nervous tissue associated with the host glands was probably not the fault of the methods used, since nerves were stained in other regions of the same sections, and since all of the methods used have previously stained nerves reliably in avian tissue (Gilbert, 1961, 1965a, b). Therefore, it must be concluded that the uterovaginal sperm-host glands are not innervated.

The case for stating that myo-epithelial cells are absent is not as strong, for the staining methods were not previously used and tested on avian tissue. Our negative findings, however, agree with those of Van Krey, Ogasawara & Pangborn (1966), who were unable, in light- and electron-microscope studies, to demonstrate any contractile tissue elements associated with the glands.

The nature of the extensive vascular system suggests a fine control of blood flow within the area; it appears likely that blood may flow either directly to the gland from the central arteriole or, alternatively, first pass through the epithelial capillary network. The latter might allow the host glands to be influenced by secretions from the adjacent epithelium, and the former would allow hormones to reach the glands from the systemic circulation unvitiated by contact with a capillary bed. Evidence for a role of any hormone or secretion in the function of these glands is as yet very meagre, although oestrogen was shown to be important for their development (Lorenz, Reynolds & Howard, 1966). It is possible that this or other secretions may aid in the control of such glandular functions as secretory activity, the survival of spermatozoa in them, and/or release of spermatozoa from them. The negative results obtained after injection of acetylcholine and adrenaline must be interpreted with caution, since systemic injection of these substances is physiologically crude.

Whatever the exact role, the nature and extent of the blood supply, and its intimate contact with the cells of the host glands, suggest a significant relation

EXPLANATION OF PLATE 2

FIG. 7. Capillary network surrounding host gland (g). Note connection to drainage venule (v). Indian ink, unstained. Frozen section.

FIG. 8. Transverse section of gland. Note capillaries lying adjacent to the gland cells. Indian ink, haematoxylin. Frozen section.

FIG. 9. Longitudinal section of host gland. Note the length of blood vessels in contact with gland cells. Indian ink, haematoxylin. Frozen section.

FIG. 10. Transverse section of gland. Blood vessel can be seen completely encircling the gland. Indian ink, unstained. Frozen section.

FIG. 11. Host gland and associated capillary. Haematoxylin and eosin.

FIG. 12. Host gland and associated capillary (c). Haematoxylin and eosin.

to their function; and the absence of both neural and contractile elements strengthens the impression that controlling mechanisms are to be sought in blood-borne agents. Conversely, since the glands appeared to be fully formed histologically before the capillary network developed, it seems likely that the glands themselves exert a local influence on the growth and formation of the blood supply, and possibly also on the blood flow.

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