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The three-dimensional architecture of the myosalpinx in the rat (*Rattus norvegicus*) as revealed by scanning electron microscopy

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Summary. The three-dimensional (3-D) architecture of myosalpinx in the rat has been investigated by means of scanning electron microscopy after microdissection and removing interstitial connective tissue with 6N NaOH digestion. In the extramural portion of tube-uterine junction the myosalpinx shows circularly arranged fibers originating from the uterus, together with oblique fibers typical for the salpinx, which occur more frequently in the deeper layers. As fibers approach the mucous folds they assume a plexiform arrangement, which is maintained through all tubal segments. In the isthmus surface fibers form wide muscle rings around the elbow of loops, peculiar to the rat tubal morphology. Surface fibers in the ampulla and pre-ampulla have an even circular course. Our 3-D results reveal that the muscular architecture of rat tube is mainly organized in concentric, monolayered shells with a plexiform arrangement tightly fastened together. Functionally, this muscular arrangement seems to be capable of stirring rather than pushing the embryo and gametes. Finally, such a plexiform network might work as a mechanism of «tube locking» in proximity of isthmic loops as well as at the level of the ampullary-isthmic junction.

Key words: Salpinx, Smooth muscle cells, rat, Ovum transport, Scanning electron microscope, *Rattus norvegicus*

Introduction

A review of the literature on the architecture of tubal musculature (myosalpinx) in the rat as well as in other mammals reveals inconsistencies with respect to the arrangement of smooth muscle cells (SMC). According to Nilsson and Reinius (1969) the SMC of myosalpinx in the rat are arranged into one or two longitudinal layers at the ampullar level, two or three longitudinal layers at the pre-ampullar level and into a coat consisting mostly of circularly arranged fibers in both isthmus and tubo-uterine junction (TUJ). In the latter, an outer longitudinal layer arising directly from the uterus occurs. On the other hand, Beck and Boots (1974) reported two thin longitudinal layers enveloping a third intermediate circular layer at both ampullar and isthmic level, with the outer longitudinal layer lacking in the infundibulum.

Such inconsistencies in the literature may arise from different interpretations of inadequate histological data obtained from bidimensional observations by means of light microscopy, which have been used to describe a three-dimensional (3-D) structure such as the myosalpinx (Muglia et al., 1992).

Knowledge of the myosalpinx structure is important not only from a merely structural point of view, but also for its possible correlation with the functional role played by the tube and, particularly, its contractile power, as demonstrated by a number of papers dealing with the mechanism of tubal transport of gametes and early embryos (Verdugo, 1986; Hunter, 1988).

The present paper reports new data on the 3-D architecture of myosalpinx in the rat, obtained from direct observation with scanning electron microscopy (SEM). This was made possible by a recent technique involving maceration and microdissection of the tube which allows removal of masking connective tissue (Takahashi-Iwanaga and Fujita, 1986; Muglia et al., 1991a,b).

Materials and methods

Our investigations were carried out on 15 female Wistar rats in estrous aged 4 months, and weighing 400 g on average. Salpinges were excised from 10 rats by laparatomy under anaesthesia (100 mg Ketamine + 4 mg Xylazine/kg), dissected under a stereo microscope, gently stretched and mounted for their whole length by

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means of thin needles on a silicon plate in Kreb's solution. Such a procedure was followed in order to make observations possible throughout the whole salpinx, respecting its topography. After dissection, Kreb's solution was replaced by 2.5% glutaraldehyde in 0.1M phosphate buffer for 48 h. Salpinges were excised from the remaining 5 rats upon perfusion, fixed by immersion and processed as the microdissected samples. Perfused samples were compared to the microdissected ones in order to detect any structural change of the myosalpinx caused by microdissection and stretching. The following segments were isolated from the stretched salpinges, according to the current anatomical and physiological classification (Nilsson and Reinius, 1969): TUJ, isthmus, ampullary-isthmic junction, ampulla and pre-ampulla. All segments were processed according to the Takahashi-Iwanaga and Fujita's maceration technique (1986). The digestion time was determined by trial and error. Samples were shaken for 2-3 min during digestion until they were visibly fragmented, then they were put into 0.1M phosphate buffer (pH 7.4) in order to stop digestion. Fragments were then washed in running tap water for 3 h for mechanical dissociation after chemical treatment, dehydrated in graded alcohols, critical point-dried, coated with 20 nm gold-palladium and examined under a Cambridge Stereoscan 240 scanning electron microscope operating at 20 kV. A number of samples were further microdissected by ultrasonication during dehydration (Low, 1989) for 3-5 min at 20 kHz to detect the deepest muscular bundles. Some segments were embedded as a whole in historesin and transverse sections were cut, staining with Sirius Blue and H/E and observed under the light microscope (LM).

Results

The salpinx in the rat is 25 mm long on average. It is a coiled organ consisting of approximately 10 loops. Consistent with the classification of Nilsson and Reinius (1969), the following segments can be distinguished along its length: TUJ, which is located in the extra-mural portion at the level of the 1st loop; isthmus, between the 2nd and 7th loop; ampullary-isthmic junction (AIJ), at the level of the 7th loop; and ampulla and pre-ampulla, corresponding to the 8th and 9th loop, respectively. The myosalpinx architecture, as revealed by our observations, will be described starting from the TUJ towards the ampulla, following the gradual decrease in its thickness.

Two muscular components could be distinguished within the myosalpinx in the rat. 1) a musculature typical of the serous membrane enveloping the salpinx, which ran within subperitoneally connective tissue (SCT) of mesosalpinx (heterologous musculature); 2) a musculature peculiar to the salpinx itself (autologous musculature). Such components were independent from each other and will be described separately for each of the above segments.

Tubo-uterine junction

Heterologous musculature

By means of LM, the myosalpinx showed two main surface arrangements. The first one was characterized by sturdy muscle bundles that ran within the SCT going from the tubal periphery towards the myosalpinx surface. In these bundles, SMC fibers appeared oriented perpendicularly and/or obliquely with respect to the major axis of the tube. The second arrangement was characterized by bundles partially enveloped by the SCT, circular or oblique in section at the periphery of autologous musculature (Fig. 1).

Parallel transverse sections of myosalpinx viewed by SEM revealed SMC running obliquely with respect to the major axis of the salpinx, forming an incomplete wide spiral layer that enveloped the salpinx. The SMC were elongated, regular in shape and always ran parallely with the serous membrane (Fig. 2).

Autologous musculature

Under the LM an unevenly circular coat could be observed and, in some cases, an underlying unevenly longitudinal layer (Fig. 1).

In transverse sections observed by SEM the myosalpinx architecture gradually changed as the uterine horn merged into the isthmus. At the most superficial level, the SMC fibers followed a circular course which is typical of the uterine structure. Such SMC fibers were regular in shape, elongated, ribbon-like and showed the same morphology as the uterine SMC fibers. Among them, oblique SMC fibers were detected which were irregularly shaped. Oblique SMC fibers were found in increasing numbers at the deepest levels, where they

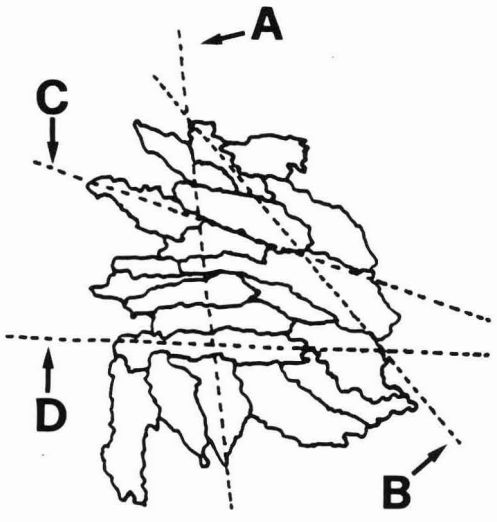

Diagram 1. This diagram shows the general orientation of autologous SMC fibers in different directions according to their own irregular shape. Major axis (broken lines) of A, B, C, D fibers.

appeared arranged into a plexiform structure (Fig. 3). The plexus consisted of fibers, either single or gathered. They were articulated with each other fitting their own profile and were oriented in different directions (Diagram 1). Gathering of several cells oriented towards the same direction could give rise to bundles of different thickness whose extremities merged into the plexus (Fig. 4). The SMC were packed together and arranged into concentric, monolayered shells, which were tightly assembled. A fine basked made up by collagen fibers enveloping each SMC could be seen in partially digested samples.

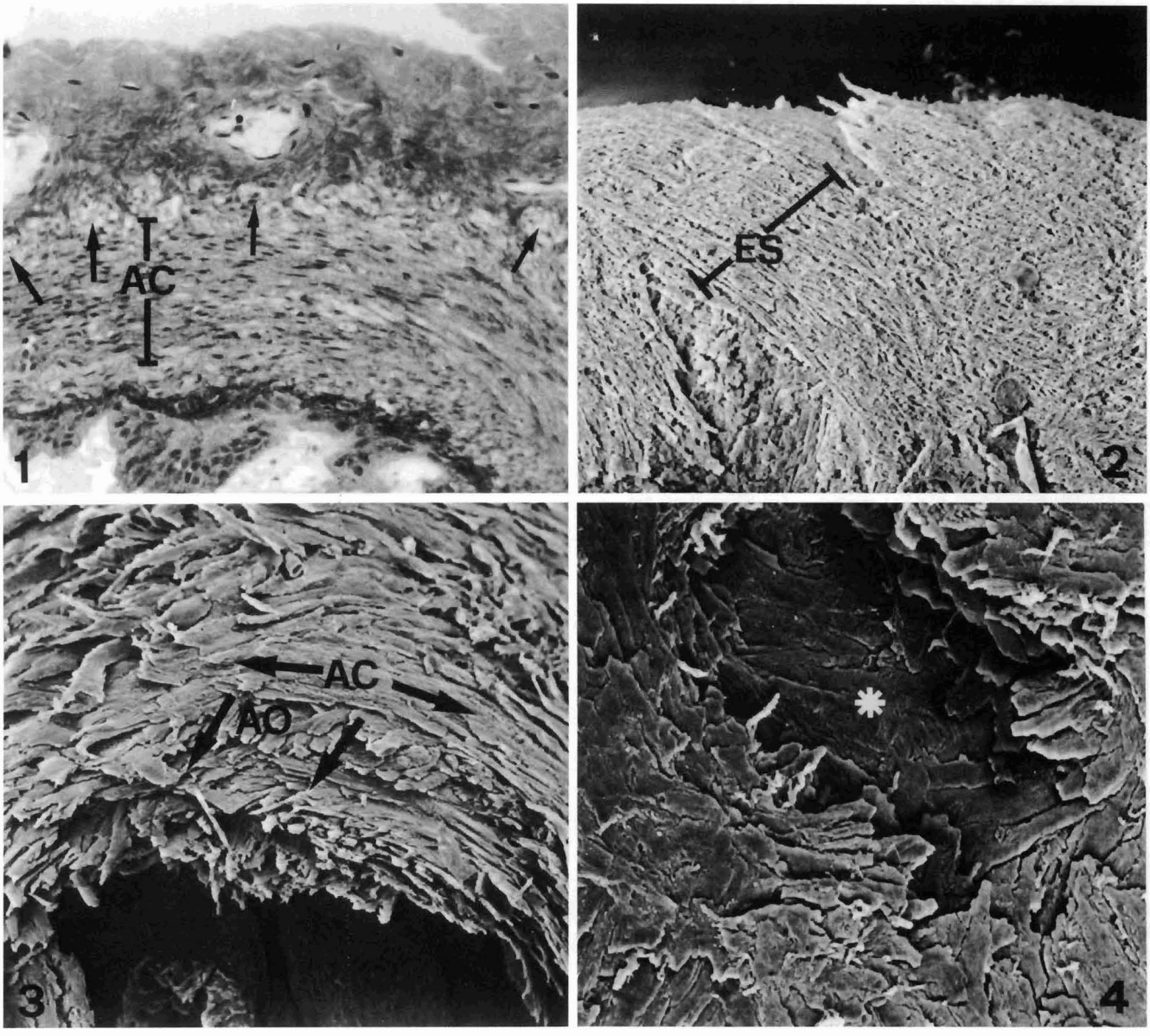

Fig. 1. Transverse section of the extramural portion of the TUJ. Heterologous musculature: SMC bundles circular or oblique in section (arrows), more or less enveloped by the serous membrane. Autologous musculature: unevenly circular coat (AC). LM, Sirius blue. x 330

Fig. 2. Extramural portion of the TUJ. Heterologous musculature: incomplete wide spiral SMC fibers (ES) enveloping the salpinx. SEM. x 400

Fig. 3. Extramural portion of the TUJ. Autologous musculature: Oblique fibers (AO + arrows) in an oblique fashion and in increasing numbers at the deepest levels of the myosalpinx. Circular fibers (AC + arrows). SEM. x 600

Fig. 4. Extramural portion of the TUJ. Autologous musculature: plexiform arrangement in the deepest level of the myosalpinx (asterisk). Arrangement of the SMC as in diagram 1. SEM. x 970

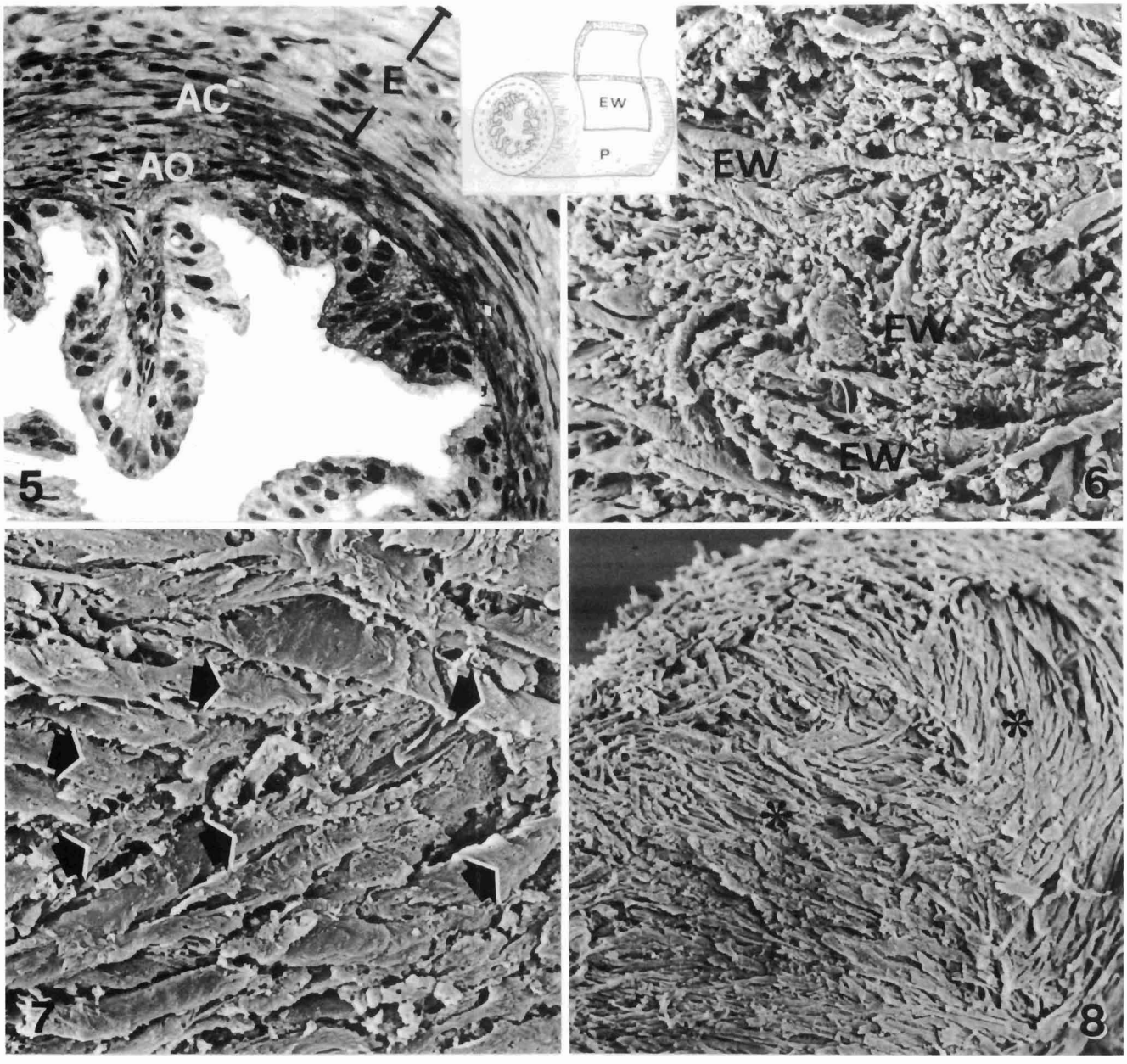

Fig. 5. Transverse section of the isthmus. Heterologous musculature: muscle fibers sectioned along the same plane show a different orientation forming various bundles within the SCT (E). Autologous musculature: outer circular muscular coat (AC) and inner oblique SMC fibers (AO). LM, Sirius Blue. x 340

Fig. 6. Isthmus. Heterologous musculature: whorl-like fibers (EW) merging after maceration of the outer serous membrane. SEM. x 1,380. Inset: the schematic drawing shows the panoramic view of the tube wall and the level (just under the peritoneum) where the SEM observation was made to obtain figure 6. P: peritoneum.

Fig. 7. Isthmus. Autologous musculature. Inner levels: the SMC fibers converge forming acute angles (arrows). SEM. x 2,660

Fig. 8. Isthmus. Autologous musculature. Outer levels: ring-like systems (asterisks). SEM. x 425

Isthmus

Heterologous musculature

By LM, muscle fibers sectioned along different cutting planes were present within SCT (Fig. 5).

By SEM, elongated irregularly spindle-shaped SMC with thin extremities were clearly visible within the SCT. Here they formed bundles of three or more cells. The bundles showed either bifurcated or ravelled extremities

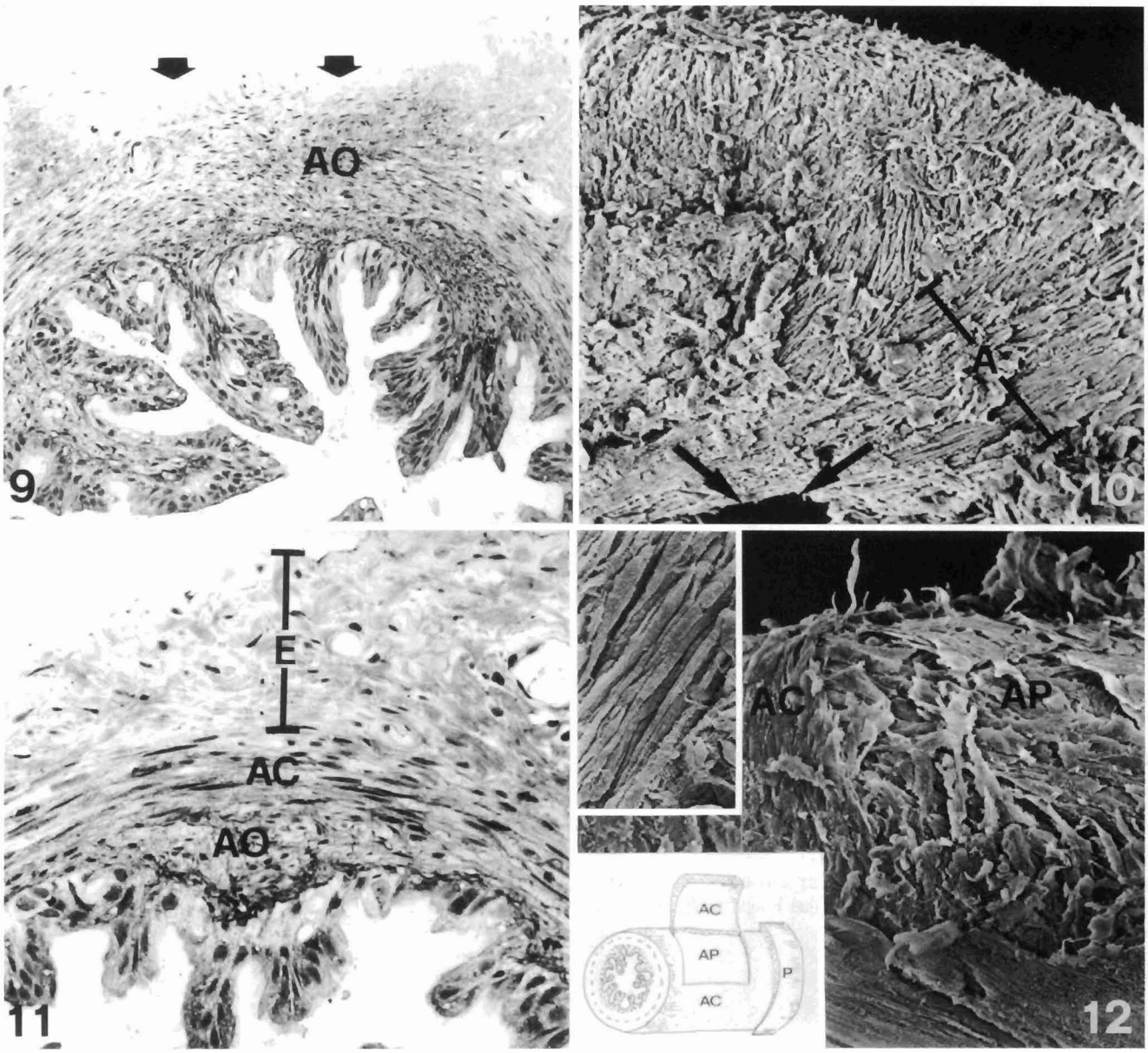

Fig. 9. Transverse section of the ampullary-isthmic junction (AIJ) (7th loop). Autologous musculature. Inner edge of the AIJ (arrows): fibers irregularly oriented (AO). LM, Sirius Blue. x 187

Fig. 10. AIJ. Autologous musculature. Fibers run obliquely from the inner curve (arrows) of the loop towards the opposite arm of the loop itself (A). SEM. x 264

Fig. 11. Transverse section of the ampulla. Heterologous musculature: sectioned SMC fibers show different orientation within the serous membrane (E). Autologous musculature. AC: Outer circular SMC fibers. AO: Inner oblique fibers. Sirius blue. x 360

Fig. 12. Ampulla. Autologous musculature. Outer circular SMC fibers (AC); inner plexiform arrangement of SMC fibers (AP). SEM. x 417. Inset: circular rod-like and clavate muscular cells. SEM. x 1,100. Drawing: the scheme shows the spatial relationships in the thickness of tube wall of the structures (AC, AP and P) seen by SEM. in Fig. 12.

which enveloped or were articulated with other bundles following different directions, thus originating whorl-like structures (Fig. 6).

Autologous musculature

Under the LM, in transverse sections, an outer circular coat and an inner oblique layer were observed (Fig. 5).

By SEM, the surface SMC fibers gradually lost the typical uterine features as they approach the 2nd loop and, although keeping flattened, they became spindle-shaped. The fibers were articulated with each other on a single plane (layer) with their major axis oriented to form acute angles (Fig. 7). The SMC were closely packed and arranged into concentric, monolayered shells tied together, as in TUJ. A fine basked of collagen enveloping each SMC fiber was visible in partially digested specimens. As the most superficial levels SMC fibers surrounded the elbow-shaped folds of tubal loops thus originating ring-like systems (Fig. 8). The remaining portion of muscle coat showed the same plexiform structure found in TUJ.

Ampullary-isthmic junction

Heterologous musculature

Under the LM in transverse sections, muscle fibers were detected along different cutting planes within SCT.

By SEM, the heterologous musculature showed the same features as the isthmic segment.

Autologous musculature

By LM, in transverse sections, the most superficial portion of the myosalpinx appeared thickened along the inner edge of the 7th loop. The fibers in the thickened portion were irregularly oriented (Fig. 9).

Under the SEM, the musculature appeared noticeably thickened along the inner curve of the 7th loop. The fibers ran from the inner curve of the loop towards the two opposite arms of the loop itself, where they despersed obliquely (Fig. 10).

Ampulla/pre-ampulla

Heterologous musculature

By LM, in transverse sections, sectioned muscle fibers could be detected in different planes within the serous membrane (Fig. 11).

By SEM, the heterologous musculature showed the same features as the isthmic segment.

Autologous musculature

Under LM, in transverse sections, outer SMC fibers were circularly arranged while inner SMC fibers were longitudinally arranged. Therefore, two layers could be seen: an outer circular and an inner longitudinal layer (Fig. 11).

Under the SEM, SMC appeared rod-like and clavate at the surface level. They could reach even more than 100 μm in length (Fig. 12); therefore, they were longer than those observed in the other segments. Furthermore, they were rather uniformly arranged along a circular course with respect to the tubal axis. At a deeper level, SMC gradually modified their shape, becoming uneven and assuming both aspect and orientation as those of the two preceding segments (isthmus and TUJ) (Fig. 12).

Discussion

The results obtained from the 3-D observation by SEM settle the inconsistencies of literature on the architecture of the myosalpinx in the rat. The outer longitudinal layer reported by Nilsson and Reinius (1969) in the TUJ would be constituted by the oblique, coiled fibers of tubal heterologous musculature. Furthermore, the underlying coat «mostly of circularly arranged cells» described by these authors would be none other than the musculature typical to the tube

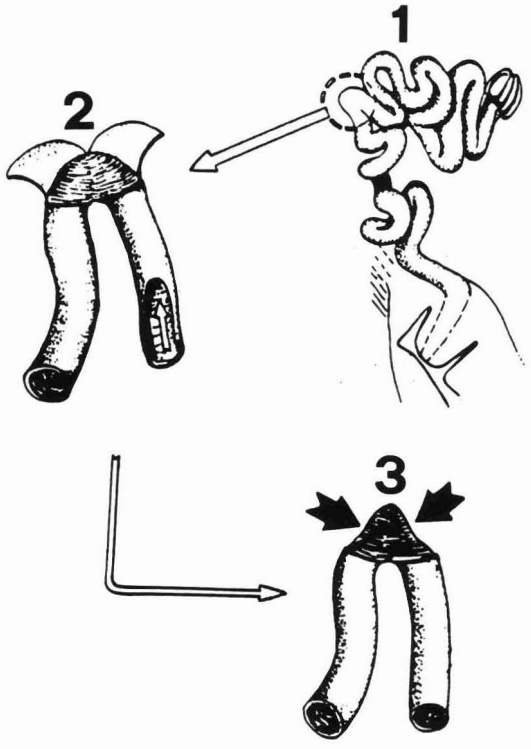

Diagram 2. The present drawing is based upon the present 3-D results and illustrates the tube (1) and the possible contraction role of the muscular ring at the level of the 7th loop of the isthmus - ampullary isthmic junction - (2). At this level of the loop, the SMC bundles bend towards the two opposite loop arms; the arrow in drawing 2 indicates the tubal lumen. The contraction of such bundles (black arrows in drawing 3) might act as a sphincter reducing both outer and inner tubal diameters.

(autologous musculature). Our observations allowed us to reveal a diversification within such coat. At the surface level, the SMC fibers followed an even circular course and closely resembled uterine fibers in shape, size and direction; where they ran deeper they gradually assumed the typical features of tubal musculature, in that they showed an irregular, elongated shape and a plexiform arrangement.

Beck and Boots (1974) reported two thin longitudinal layers in the isthmus outside and inside the so-called «tubal coat» described by Nilsson and Reinius (1969). Our findings demonstrate that the outer layer described by Beck and Boots (1974) corresponds to the heterologous musculature of the tube, which was discontinuous and irregular, while the inner layer corresponds to the plexiform component, which was present throughout the whole tube.

Also, the outer, thin, longitudinal layer observed by light microscopy in the ampulla and pre-ampulla by both Nilsson and Reinius (1969) and Beck and Boots (1974) would correspond to the tubal heterologous musculature. While Nilsson and Reinius (1969) reported that the deepest portion of the myosalpinx was constituted by longitudinal fibers, Beck and Boots (1974) described it as a circular structure. Our study, on the other hand, revealed an inner structure arranged in a plexus.

The above considerations suggest that the disagreement in the literature very likely arises from different interpretations of histological data on the rat myosalpinx. Observation based only on bidimensional sections by LM may be misleading when describing a 3-D plexiform architecture such as that of the myosalpinx. In fact, this may appear obliquely, longitudinally or unevenly circularly arranged according to the cutting plane, which never results perfectly transverse, specially in a tortuous salpinx such as that of the rat. Moreover, the ratio between percentages of bundles following different spatial directions which are found within a transverse section of a plexiform structure (myosalpinx) and which may appear either roughly longitudinal or circular, may vary greatly, further affecting the interpretation of data.

Although the rate of tubal transport is primarily controlled by the hormonal balance between oestrogens and progesterone, the structure of the tube plays a key role in this process (Hafez, 1973; Croxatto and Ortiz, 1975; Verdugo, 1986; Hunter, 1988). Tube locking, and the consequent retention of eggs and embryos at the AIJ during tubal transport, are an example of this crucial mechanism (Croxatto and Ortiz, 1975). Among the anatomical entities involved in tubal locking, the smooth musculature of the tube is likely to play an important role. In fact, observations of macerated specimens by means of SEM allowed us to demonstrate characteristic structures which might control the width of tubal lumen in the rat, particularly at the AIJ. In addition, where the SMC fibers were more regular in shape and, in turn, in their course (isthmus), they encircled the bend of loops forming true muscle rings. As a consequence, their contraction could narrow the bend itself as well as the tubal lumen (Diagram 2). Such a system of muscle rings was associated at the level of the 7th loop of isthmus (AIJ) with bundles running from the concave side of the loop bend towards the two opposite loop arms, where they dispersed obliquely. Contraction of such bundles could cause the local reduction of both outer and inner tubal diameters, thus enhancing the action played by muscle rings (Diagram 2).

Also on the basis of previous studies on different mammalian species (guinea pig, rat, rabbit, human) (Muglia et al., 1991a,b; Vizza et al., 1991) it is now rather logical to suggest that the regularity of myosalpinx architecture in various mammals is inversely related to the regularity of tubal course (Muglia et al., 1992). As the tube is tortuous in the rat, despite the rather regular myosalpinx architecture, the above mentioned muscular structures (rings around loops and bundles at the AIJ) might allow a more effective control on the width of tubal lumen and, as a consequence, on tube locking.

Our findings point out that the myosalpinx of rat consisted of a single muscular coat comprised of several concentric, monolayered, plexiform shells. These were composed in densely-packed SMC fibers whose shape eventually determined the architecture of myosalpinx. This is unlike what happens in he gut, whose musculature is geometrically arranged in regular layers which, by contracting, give rise to an even peristalsis (Gabella, 1981, 1987; Uehara et al., 1990). It is widely accepted that myosalpinx contractions propagate at random according to a backward-forward motion (Daniel et al., 1975; Talo and Hodgson, 1978) and are transmitted, usually for short distances, from different pace-maker sites (Talo and Pulkkinen, 1982). These data were also confirmed by recording the random myoelectrical activity of the tube (Daniel et al., 1975; Hodgson et al., 1977; Hodgson and Talo, 1978).

A plexiform structure of myosalpinx, such as that described in the present paper, does not seem to fit with a polar contraction scheme, owing to the uneven distribution of fibers. Such a structure, rather than narrowing the lumen or generating a series of regular contraction waves, is more likely to generate random contraction waves capable of stirring, and not of pushing, ampullar and tubal contents, including gametes and embryos.

Therefore, the myosalpinx architecture as revealed in this study provides an anatomical basis for an hypothesis of random tubal transport characterized by pendular «backward-forward» movements (Verdugo, 1986). Moreover, our results suggest that random propagation of muscular contraction may change the plexiform wall of the myosalpinx causing the stirring of tubal contents. This would make the contact between hormonal and nutritive substances occurring in the tubal fluids surrounding sperm, the surface of eggs and embryos more effective for a correct fertilization and early embryo development (Motta et al., 1994). Finally,

our observations showed circular and oblique fibers around loops and at the AIJ, whose main function is probably to regulate directly the width of the tubal lumen, thus being actively involved in the retention of the egg at the AIJ and tubal locking.

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