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Scanning Electron Microscopy of the Infundibulum, Ampulla, and Eggs of Mice

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The objective of the study reported here was to use scanning electron microscopy (SEM) to discover and describe the details of three-dimensional profiles and the natural (not surgically disturbed) topography/location of the infundibulum in the mouse. It will help new investigators to more quickly identify the infundibulum for successful transfer of microinjected eggs through a small opening into the oviduct/ampulla of pseudopregnant female mice for producing transgenic mice. Results of the study also illustrate the geographic orientation and natural topographic features of the ovary, infundibulum, ampulla, oviduct, and uterus. The presence of cilia on the surface of the crown foldings in the longitudinal section of the infundibular head stained with 1% toluidine blue provided direct evidence that evagination of the internal cilia of the infundibulum/oviduct results in formation of the infundibular crown. The new observation of the narrowing region of the infundibular head after surgical removal of the crown also suggests that formation of the infundibular crown may have resulted from the "evagination process" of internal cilia of the infundibulum/oviduct surface. The results also provide new evidence that the crown, terminal opening, and appearance of the left and the right infundibula of the same mouse differ.

Successful transfer of microinjected mouse eggs through the small opening of the infundibulum to the ampulla of the oviduct of a pseudopregnant (foster) mouse is a critical and difficult task in producing transgenic mice. This difficulty prompted development of an alternative method consisting of direct transfer of the microinjected eggs to the ampulla without going through the opening of the infundibulum (7). However, this direct ampulla-transfer method inevitably injured the ampulla and produced lower numbers (%) of foster mother pregnancies than did the infundibulum transfer method in our laboratory. Therefore, scanning electron microscopy (SEM) was conducted to illustrate how the three-dimensional topography of infundibulum, ampulla, and eggs facilitates quicker identification of these organs to achieve a more satisfactory transfer. A better geographic and topographic understanding of the infundibulum will reduce the searching effort, thus reducing the chance of damaging the infundibulum and oviduct, with less bleeding, to achieve an increased rate of successful transfer.

Transmission electron microscopic images of the ultrastructure of the muscular coat of the infundibulum and ampulla of the mouse oviduct during pregnancy and after delivery have been described (2), but to the authors' knowledge, SEM images of these organs of mice are unavailable. Each infundibulum of a mouse has a different appearance/ morphology. It also is a small organ embedded inside the bursa membrane. Once the bursa membrane is surgically opened to expose the infundibulum, bleeding occurs, which makes it more difficult to find the infundibulum in a short time for successful transfer. The natural geographic and topographic location of the infundibulum (Fig. 1) provides helpful orientation for searching for the opening of the

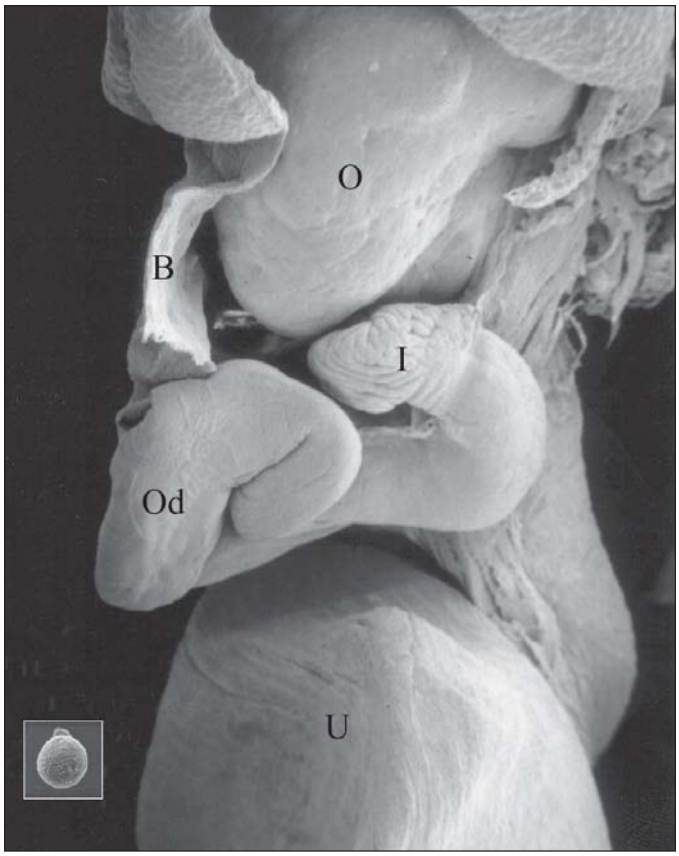

Figure 1. Scanning electron micrograph of the ventral view of the right infundibulum (I), ovary (O), oviduct (Od), and uterus (U) of a 30-day-old superovulated, 0.5-postcoital day (PCD) female CD-1 mouse after the bursa membrane (B) was opened to reveal the natural topography. Inset in the lower left corner shows the size of a fertilized egg proportional to that of the infundibulum for comparison. Magnification = 48×.

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infundibulum because the natural orientation between the infundibulum, ovary, and oviduct is carefully preserved and undisturbed in the SEM preparation. Because it represents the same orientation as that in live tissues, it should serve as a topographic map for other investigators to identify the most important anatomic landmarks.

Materials and Methods

Animals. Twelve adult CD-1 mice (*Mus musculus*) and FVB/N mice (*Mus musculus*), with body weight of approximately 25 to 30 g, were purchased from Charles River Laboratory (Wilmington, Mass.) and Jackson Laboratory (Bar Harbor, Maine), respectively. All animals were cared for and maintained humanely in our animal colony, under proper housing and husbandry conditions. All of the new and used cages, air filters, feeding bottles, and other items used to care for these animals were cleaned and autoclaved, as a pathogen-free environment is required for every animal in our colony. All animal care and handling procedures conformed to the US Public Health Service Guidelines for humane care and use of laboratory animals (5, 6). In addition, this study was part of our project entitled "Production and Breeding of APP and SCHAD Transgenic Mice," which was approved by the Animal Welfare Committee of our institute.

The mice were serologically tested (4) to confirm the absence of the following pathogens: Sendai virus, pneumonia virus of mice, mouse hepatitis virus, minute virus of mice, Theiler's virus, reovirus, *Mycoplasma pulmonis*, lymphocytic choriomeningitis virus, ectromelia virus, mouse pneumonitis virus, polyoma virus, mouse adenovirus FL/K87 1 & 2, epizootic diarrhea of infant mice virus, mouse cytomegalovirus, Hantaan virus, *Encephalitozoon cuniculi*, cilia-associated respiratory bacillus, mouse parvovirus, and mouse thymic virus. Mice were housed in a quarantine facility at our animal colony, with a 12:12-h light:dark cycle and 10 to 15 air changes/h. Mice were housed on sterilized corncob bedding (Harlan, Indianapolis, Ind.) in sterilized cages (Polysaufone Standard Mouse Cage, ACE, Allentown, Pa.) equipped with stainless steel wire bar tops and filtered cage tops. Mice were fed sterilized standard rodent chow (Purina Mills, St. Louis, Mo.), and hyperchlorinated water was available ad libitum in bottles. Cage litter was changed every seven days in a class-II biological safety cabinet in the animal colony room.

Superovulation in female mice around 28 days old was achieved by intraperitoneal (i.p.) administration of 0.15 to 0.2 ml of 50 IU of pregnant mare's serum /ml (Sigma-Aldrich, St. Louis, Mo.) at 47 h prior to i.p. administration of 0.15 to 0.2 ml of 50 IU of human chorionic gonadotropin /ml (Sigma-Aldrich). Each female mouse was then placed in a cage with a fertile stud male mouse at around 4 to 5 p.m. in the afternoon; the male and female mice were left together overnight. The presence of a copulation plug in the vagina the next morning indicated that the female mouse had been fertilized. All mice were sacrificed by i.p. administration of an overdose (> 0.017 ml/body weight in grams) of 2.5% Avertin anesthetic (Sigma-Aldrich).

Scanning electron microscopy of the reproductive system. The reproductive system—ovary, bursa membrane, infundibulum, ampulla, the remaining portion of the oviduct, and uterus—was removed and placed in 10% formalin. Under stereo-dissecting light microscopy, the bursa membrane was carefully opened to expose the ovary, infundibulum, ampulla, and oviduct. The crown of the infundibular head was surgically removed to expose its narrowing head terminal. These tissues were further fixed in 4% glutaraldehyde in 0.1*M* phosphate-buffered saline (PBS), pH 7.4, for 3 to 4 h, postfixed in 1% osmium tetroxide in PBS for 2 h, dehydrated in graded ethanols (70, 80, 95, 100, and 100%: 15 min for each step), and critical point dried in 100% ethanol in a Tousimis Samdri-790 critical point-drying apparatus (Tousimis Research Corporation, Rockville, Md.). Alternatively, the tissues in 100% ethanol were then immersed in a mixture of equal amounts of 100% ethanol and 100% propylene oxide (PO) for 15 min, followed by 100% PO for another 15 min.

The tissues were placed on the surface of a clean glass slide kept in a Petri dish. The top cover of the Petri dish was partially opened (approx. 25%) for 1 h until tissues were slowly and totally dried. Tissues from either critical point drying or this slow drying/evaporation of PO were coated with gold in the Polaron SEM Coating System for 150 sec. These tissues was examined by use of an ISI-SS40 SEM operated at 10 kV. Images were captured on Polaroid film and prints. Prints were made from either film or original Polaroid photos by use of computer photo-scanning.

Scanning electron microscopy of eggs. Fertilized eggs (embryos) were harvested from other anesthetized superovulated/pregnant female mice by tearing open the ampulla regions of the oviducts of the pregnant mice and placing the eggs in M-2 medium for mouse embryos (Sigma-Aldrich, St. Louis, Mo.). These eggs were placed in 1% hyaluronidase (10 mg of M-2 medium/ml; Sigma-Aldrich) to remove excess cumulus cells around the eggs, then placed in M-2 medium again, fixed in 4% glutaraldehyde, and finally coated with gold in preparation for SEM.

Results

Ovary, bursa membrane, infundibulum, oviduct, and uterus. Scanning electron microscopy of the ventral view of the right ovary and uterine horn (Fig. 1) revealed the ovary, bursa membrane, infundibulum, ampulla, winding oviduct, and uterus. For comparison between the infundibulum and the egg, the fertilized egg in the lower left corner of Fig. 1 is shown in proportional scale to the infundibulum, oviduct, and uterus. As seen by use of SEM (Fig. 2-6), the opening and appearance in the crown of the infundibular head of each mouse vary. The morphology of the left and the right infundibular crowns of the same mouse (Fig. 2-5) also differs from that of each other. The infundibulum is situated below the ovary, with the bursa membrane covering the ovary, infundibulum, and oviduct.

Many blood vessels are distributed in the bursa membrane of the naturally ovulating mouse. Massive blood vessels are present in the bursa membranes of superovulated mice, and these membranes appear to be deep red in color (data not shown). The head region of the infundibulum from an ovulating mouse generally appears to be swollen, with a central opening surrounded by many folds like a crown (Fig. 2, 3, 5-7). Longitudinal sections of the infundibulum indicated that the outer surface of the crown foldings and the internal linings of oviduct walls have similar cilia on their surfaces (Fig. 8-11). The cilia extended to the junction between crown foldings and the oviduct ampulla (Fig. 6 and 10). The schematic drawing (Fig. 11) summarizes and assembles the observations shown in Fig. 2, 3, and 5-10. The folding pattern of each crown differs from that of each other.

In ventral view, the opening of the right infundibulum was usually toward the left (Fig. 1), facing the center of the body,

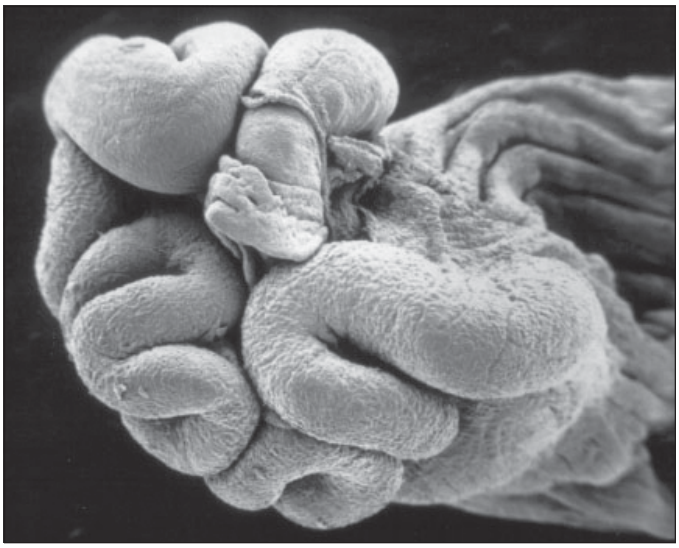

Figure 2. Right infundibulum and the coiling oviduct in their natural topography after removal of the ovary. Magnification = 52×.

Figure 3. Left infundibulum, ampulla, and coiling oviduct were separated. Magnification = 52×.

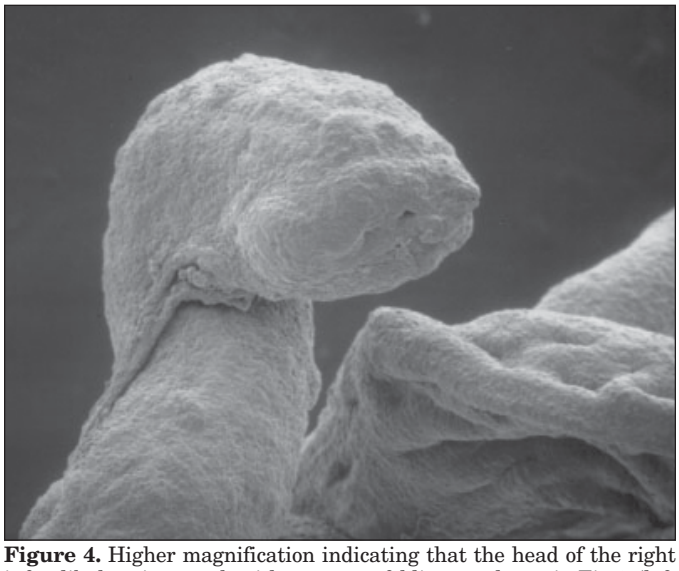

Figure 4. Higher magnification indicating that the head of the right infundibulum is smooth without crown folding, as shown in Fig. 5 (left infundibulum). Magnification = 93×.

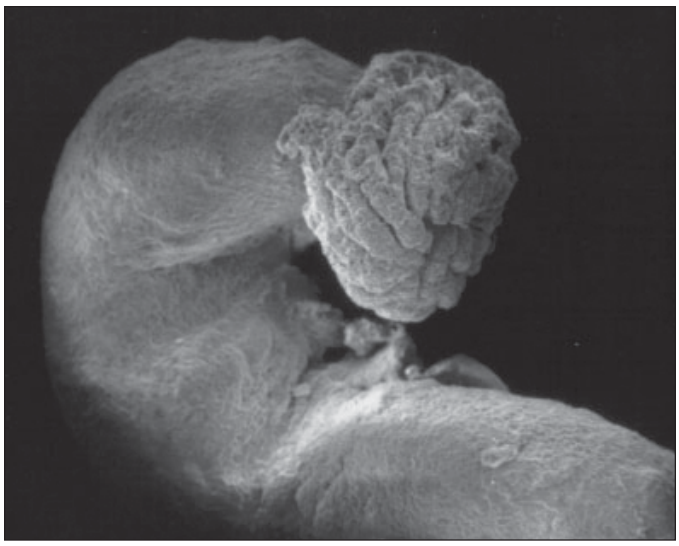

Figure 5. Higher magnification indicating that the head of the left infundibulum is surrounded by many folds like a crown. Magnification = 93×.

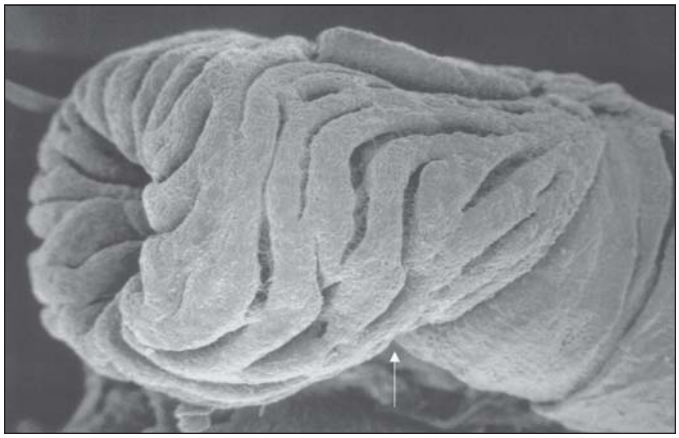

Figure 6. High magnification of scanning electron micrographs showing the crown, tip opening, and crown foldings of the right infundibulum from a 28-day-old superovulated, 0.5-PCD CD-1 mouse. Magnification = 145×. The hairy surface of the crown region represents the cilia as shown in Fig. 9 and 10. Arrow indicates the approximate location shown in Fig. 7 and 8.

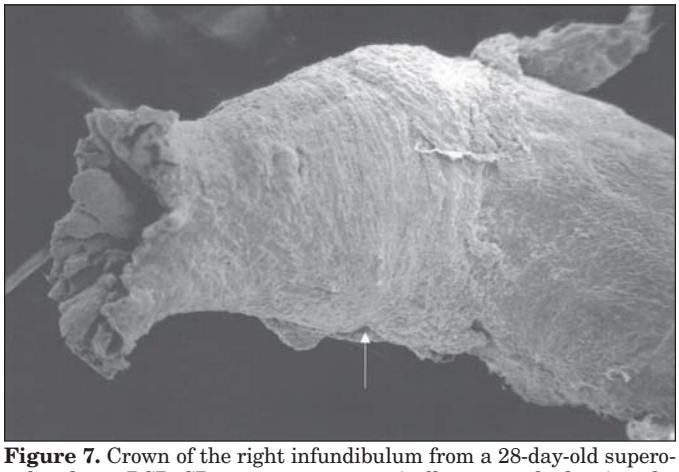

Figure 7. Crown of the right infundibulum from a 28-day-old superovulated, 0.5-PCD CD-1 mouse was surgically removed, showing the narrowing region of the infundibulum terminal. Magnification = 145×. Arrow indicates the approximate location shown in Fig. 6 and 8.

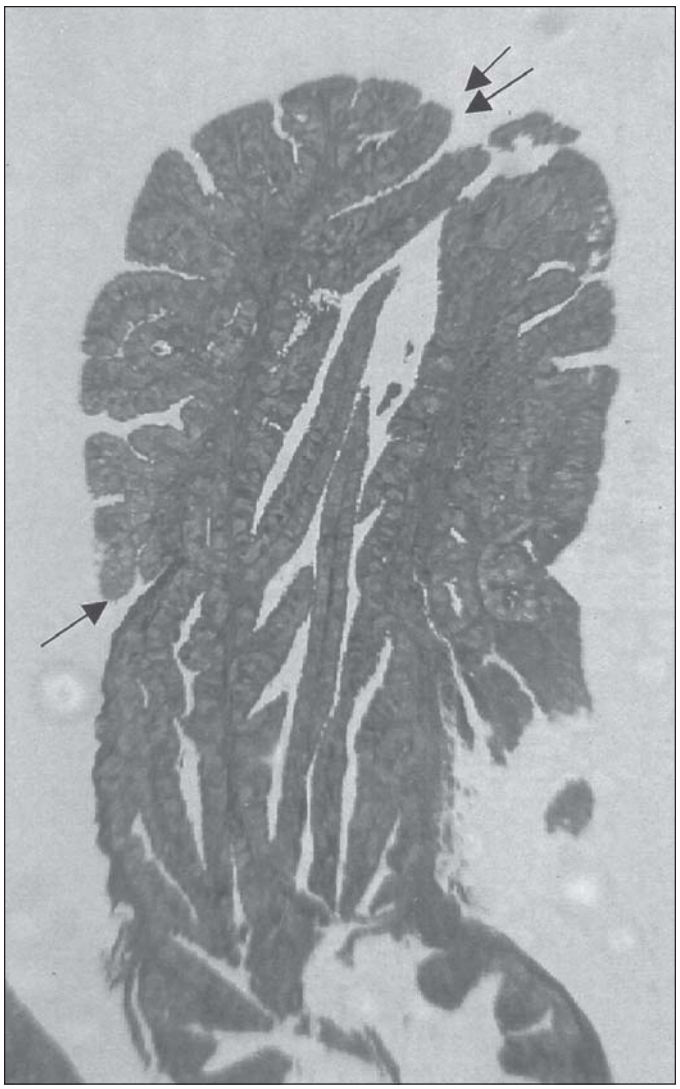

Figure 8. Longitudinal section of the infundibulum indicating that the outer surface the crown foldings and the internal linings of oviduct walls have similar cilia on their surfaces. Arrow is pointing the cilia extending to the junction between crown foldings and the oviduct ampulla. Double arrows are pointing the cilia present in the crown opening of infundibulum. Magnification = 210×.

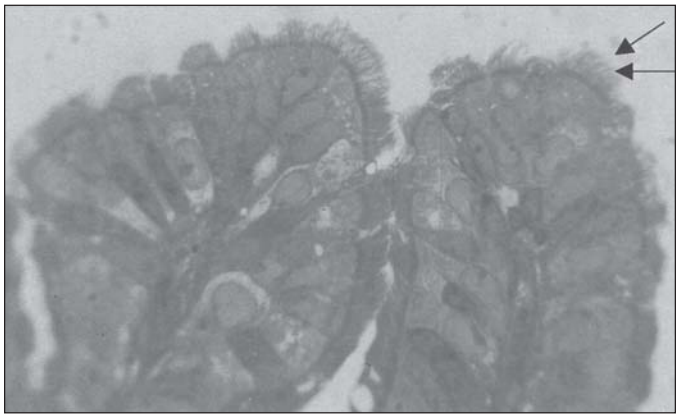

Figure 9. High magnification of cilia (double arrows) around the crown opening of infundibulum as shown in the Fig. 8 with double arrows. Magnification = 1,570×.

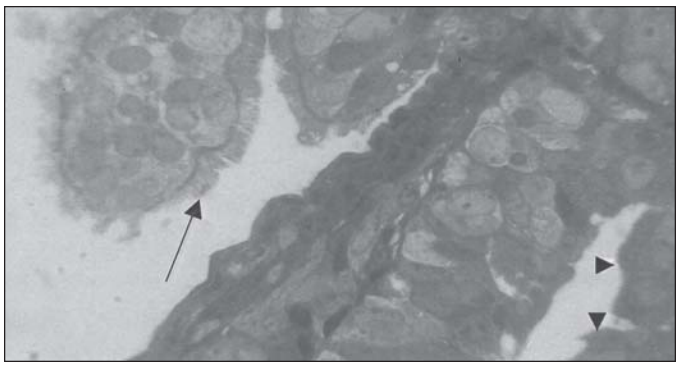

Figure 10. High magnification of cilia (arrow) in the junction between crown foldings and the oviduct ampulla. The double arrow-heads pointing the internal linings of cilia in the oviduct wall. Magnification = 1,130×.

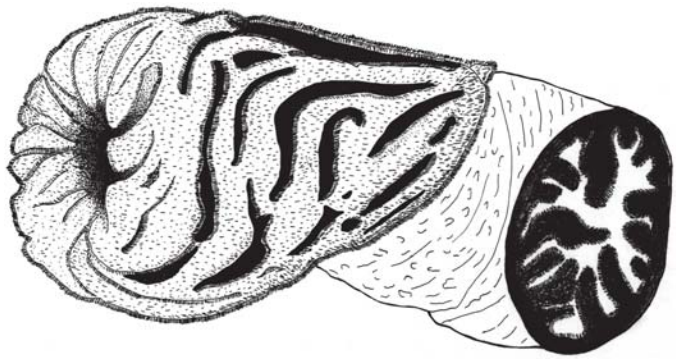

Figure 11. Schematic drawing of the right infundibulum and oviduct cavity of a superovulated mouse showing the funnel-shaped opening and the head crown foldings with cilia on their surfaces and on the surfaces of the oviduct cavity.

whereas the opening of the left infundibulum was generally toward the right, also facing the center of the body. The ampulla was a swollen portion of the oviduct (Fig. 2, 3, and 5) in an ovulating mouse at 0.5 postcoital day (PCD). Many fertilized eggs accumulated in this swollen ampulla region of the oviduct. The thickness of the oviduct wall was approximately 18 to 25 μm, whereas the uterus wall was about 190 to 380 μm thick.

Fertilized egg. Generally, a fertilized egg (embryo) at the one-cell stage is spherical (Fig. 12 and 13). Strictly, the shape of each egg ranged from spherical to oval. The surface of the fertilized egg had a rougher appearance (Fig. 12 and 13) than that of the non-fertilized egg. Each egg was enclosed in a mucoprotein envelope, the zona pellucida, with thickness of 2.5 to 3.0 mm (Fig. 13). In addition, there were one or two polar bodies from which one or two projections were protruding (Fig. 12 and 13).

Discussion

In this SEM study of the reproductive system of mice, the bursa membrane covering the ovary and oviduct was carefully and surgically opened to expose the natural location of the infundibulum between the ovary and oviduct. Each infundibulum of a mouse has a different appearance/morphology, as indicated by our results. It is a small organ embedded inside the bursa membrane. Once the bursa membrane is surgically opened, bleeding will occur, making it even more difficult to visualize the infundibulum in a short period for successful transfer. Scanning

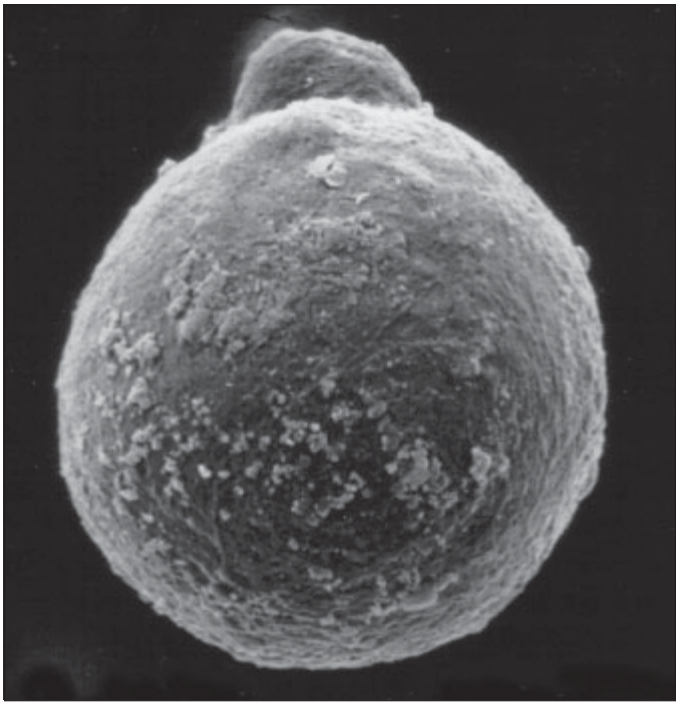

Figure 12. Scanning electron micrograph of a fertilized egg from a 30-day-old superovulated, 0.5-PCD FVB/N mouse showing the rough surface and protrusion of polar body. Magnification = 1,790×.

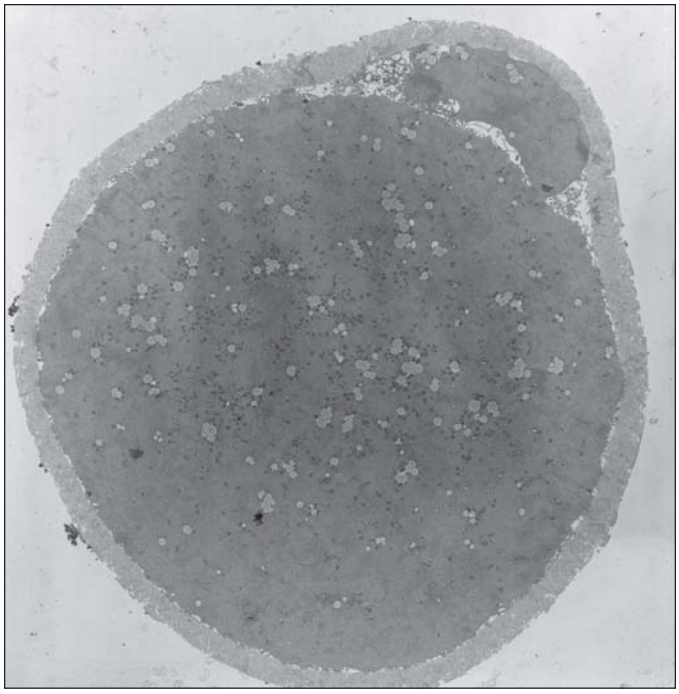

Figure 13. Transmission electron micrograph of a fertilized egg from a 30-day-old superovulated, 0.5-PCD FVB/N mouse showing the rough surface of the zona pellucida and the dividing polar bodies (magnification = 2,010×).

electron microscopy of the same infundibulum was not available at the moment of transfer, but the SEM preparation still preserved the similar or same appearance and the natural/geographic/topographic location of the infundibulum in relation to the ovary and oviduct. However, the appearance and natural habitat (geographic and topographic understanding) of the infundibulum as seen by use of SEM will help less-experienced investigators find it quickly and be able to transfer more microinjected mouse eggs into the infundibulum in a shorter period. Quicker identification of the infundibulum also reduces the searching effort, with less bleeding of the bursa membranes, which should lead to a higher percentage of pups being born.

Observation of the same or similar type of cilia on the surface of the crown folds of the infundibular head as those on the surface of the internal linings of the infundibulum/oviduct walls provides direct evidence that the crown folds of the infundibular head are the evagination extension of the internal surface of the infundibulum/oviduct walls. The new observation of the narrowing region of the crown in the infundibular head after surgical removal of the crown further supports the theory of the so-called evagination process of the internal cilia of the infundibulum/oviduct wall that forms the crown of infundibular head (Fig. 7).

The gross morphology/outline of the crowns of the infundibular heads (Fig. 6 and 7) can be seen under the binocular dissecting light microscope. For more detailed morphology, these tissues were processed using SEM protocols. These crowns of the infundibular heads had similar outline gross morphology/outline profile under dissecting light microscopy and SEM. With our experience in SEM for 27 years, it is certain that the "evagination process" is not the result of preparation artifact. By use of SEM, however, the detailed appearance of each crown of the infundibular head differed. It may be safe to state that the detailed morphology and folding appearance of each crown of the infundibular head represents its own unique profile, like a fingerprint.

Intestinal cilia and microvilli are believed to be the site of important chemical events in digestion and transport (1). The exact function of cilia in crown folds of the infundibulum is unknown at present, but they may play a role in attracting newly released eggs (ova) from the ovaries so that these eggs can find a way to enter the small opening of the infundibulum of oviduct. The funnel-shaped opening of the infundibulum may further facilitate eggs reaching and entering the infundibulum. The movement of cilia may further facilitate the inward movement of eggs from infundibulum to ampulla to oviduct to uterus. It is believed that the ciliated epithelia of the infundibulum beat rapidly and form a current in the basic (pH 8.05) oviductal fluid that draws liberated ova into it and thence to the bulbous, thin-walled ampulla (8). During artificial insemination of mice, the movement of the cilia in the infundibulum may also facilitate the entrance of intrabursal transfer of spermatozoa to fertilize these eggs at the ampulla/oviduct (9).

The overall diameter of the mouse egg at the one-celled stage is approximately 80 to 120 μm (6, 8). The size of eggs from the CD-1 and FVB/N mouse strains was usually > 100 μm in diameter, and they were easy to microinject. The zona pellucida is composed of three major acidic sulfated glycoproteins—Zp1, Mr 200,000; ZP2, Mr 120,000; ZP3, Mr 80,000 (3). It has been reported that ZP3 functions as a spermatozoa receptor and initiates the acrosome reaction, which must occur if a spermatozoa is to fertilize the egg (3). This may explain why the fertilized egg has a rough surface. Eggs collected from a superovulated mouse are not all fertilized and may not be suitable for microinjection. The rough surface of the fertilized egg is an important sign to observe before selecting an egg for microinjection.

Acknowledgments

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