ULTRASTRUCTURAL ANALYSES OF UTEROVAGINAL SPERM STORAGE GLANDS OF TURKEY BREEDER HENS

by

George T. Schuppin

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APPROVED:

M. R. Bakst

D. M. Denbow

R. G. Saacke

J. H. Wolford

H. P. Van Krey, Chairman

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Blacksburg, Virginia

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INTRODUCTION

Currently, maximum fecundity by turkey breeder flocks is affected adversely by so-called seasonal declines in fertility. Attempts to associate seasonal declines in fertility with disease organisms have, in general, been unsuccessful. Although considerable research regarding the effects of semen volume, insemination frequency, depth of insemination, time of insemination, as well as the effects of the immune system on fertility has been conducted, the cause of seasonal declines in fertility remains an enigma.

The ability of turkey hens to retain viable spermatozoa within the oviductal storage sites has been implicated in the problem. It has long been known that hens exhibiting seasonal declines in fertility do not retain adequate numbers of spermatozoa within the oviduct, explaining the reduced fertility. More recently it was reported that the ability of the uterovaginal sperm glands of turkey hens to hold spermatozoa decreased as the egg production season advanced. The progressive diminution in the ability of the glands to hold spermatozoa suggested that glandular changes were occurring over time. Thus, the intent of this study was to characterize the ultrastructural morphology of the uterovaginal sperm glands of turkey hens at varying ages. Comparative studies between glands from fertile and infertile hens and between glands collected throughout the

egg laying season were also to be undertaken. Possible changes in ultrastructure were then to be correlated with time and/or fecundity.

REVIEW OF LITERATURE

One of the more intriguing facts regarding the domestic hen is an ability to produce fertile eggs for a prolonged period of time following a mating. The mechanism by which birds are able to maintain such a prolonged fertility period is perhaps amongst the oldest mysteries concerning birds. It has not been until comparatively recent times that some aspects of the mechanism in question have begun to be understood.

The key to the extended fertility period in the hen is an ability to store and maintain the viability of spermatozoa following insemination into the oviduct. Spermatozoa are known to be stored in at least two regions of the oviduct. They were first observed in the caudal infundibulum by Tauber (1875) and later rediscovered in this region by Van Drimmelen (1946). More recently another region of the oviduct at the junction of the uterus and vagina was found to be a site of sperm storage (Bobr et al., 1962; Fujii and Tamura, 1963; Bobr et al., 1964a and b).

Infundibular Sperm Nests

The morphology of the infundibular sperm nests has been studied in a cursory sense only, and such studies have been restricted to the chicken. The sperm nests are generally found along the last centimeter of the infundibulum, immediately adjacent to the magnum (Aitken and Johnston,

1963; Blom, 1973). These sperm nests differ from the tubular glands of the magnum both in type and degree of secretory activity. Cells comprising the magnal glands exhibit a high degree of secretory activity which results in the distribution of relatively large vacuoles throughout the cell. Vacuolization occurs in magnal gland cells to such an extent that the nucleus is generally compressed.

Cells from infundibular sperm nests, by comparison, contain smaller vacuoles which are confined to the apical region, and the nuclei of these cells have a regular ovoid shape. In addition, the secretory material of infundibular sperm nests stains more intensely with PAS, but less intensely with Heidenhain's haematoxylin and amido black than does the secretory material of the magnal cells. Furthermore, the infundibular sperm nests stained slightly, or not at all, with alcian blue, and stained orthochromatically with toluidine blue (Aitken and Johnston, 1963).

Aitken and Johnston (1963) also characterized the fine structure of the infundibular sperm nests. They identified two cell types in the infundibular sperm nests. The first type, termed Type I cells, were found on an occasional basis only. These cells contained an abundant layered endoplasmic reticulum (ER) in the basal region. In conjunction with the ER, active golgi apparati were also observed. Secretory

vacuoles, composed of homogenous election dense granules, were concentrated apically. Type I cells also contained a single large vacuole which had electron lucent contents and was associated with a golgi apparatus. Numerous mitochondria with closely packed cristae were located throughout the cells. Lumenal borders of Type I cells were made up of dense microvilli.

In contrast to Type I cells, the more common Type II cells appeared to be less active in a secretory sense. Type II cells did not contain an extensive ER, and they contained fewer mitochondria. Type II cells did contain electron dense secretory vessicles, which were located apically and had a "granule studded flocculent" appearance. Microvilli were sparsely located on the lumenal surface of a Type II cell.

Aitken and Johston (1963) also observed secretory material present in the lumena of infundibular sperm nests on an occasional basis. The material was uniformly distributed and had a "floculent or finely granular appearance". Schindler et al. (1967), studying infundibular glands containing spermatozoa, described the presence of a low density secretory material. The material they observed, however, was not uniformly distributed.

The relationship between infundibular sperm nests and spermatozoa stored within them has been described by Van

Drimmelen (1946), Fujii and Tamura (1963), Bobr et al. (1964), Van Krey et al. (1967) and Schindler et al. (1967). In all cases spermatozoa were found to be arranged in bundles oriented with their heads directed toward the blind (i.e. distal) end and their tails directed toward the proximal end of the gland. Spermatozoa were also found to be in close association with cells of the infundibular sperm nests. They were often observed embedded in lumenal microvilli.

Morphology of Uterovaginal Sperm Glands

More extensive research has been conducted to characterize the morphology of uterovaginal sperm host glands (UV glands). Initial studies utilizing light microscopy did much to elucidate the general structure of UV glands. Fujii (1963) found that the UV glands were located on the vaginal side of the junction between the uterus and vagina in a 1 cm wide band of oviduct. He noted that the glandular openings were located apically and laterally on vaginal folds, and that the UV glands extended into the lamina propria of the oviduct. On the basis of shape he classified the UV glands as simple tubular. Finally, he observed that the UV glands were composed of a single layer of tall, 25 µm columnar cells which gave the appearance of being a continuation ofthe oviduct lumenal mucosa. The lumen of a gland was found to be 14 µm in diameter. Verma

and Cherms (1964) confirmed that the basic findings of Fujii (1963) concerning the chicken applied to the turkey as well.

A later study by Mero and Ogasawara (1970) identified two types of UV glands. One type had a large lumenal area and the other type had a small lumenal area. The UV glands with large lumenal area had an average diameter of 42.8 µm. The UV glands with small lumenal area had an average diameter of 11.2 µm. Both types of UV glands had an average length of 500 µm.

Past studies have also revealed information regarding the tissue surrounding UV glands. Fujii (1963) identified the surrounding tissue as being dense connective tissue. Van Krey et al. (1967) found the connective tissue to be collagenous in nature, while Tingari and Lake (1973) found the connective tissue to be composed of scattered collagenous fibers and tropocollagen. It was also noted by Van Krey et al. (1967) and in subsequent studies by Gilbert et al. (1968b) and Tingari and Lake (1973) that no smooth muscle or myoepithelial cells were associated with the uterovaginal sperm storage glands. Gilbert et al. (1968b) found that there were no nerves associated with UV glands, but that an extensive blood capillary system was associated with the glands.

The three dimensional topography of the uterovaginal region has been described in studies utilizing scanning

electron microscopy. Ogasawara and Marquez (1974) noted that cilia in the uterovaginal region formed a "dense mat". The study of Fujii (1975) confirmed that the uterovaginal region was composed predominately of ciliated cells, but non-ciliated secretory cells were also present in the region. Fujii (1975) went on to describe sperm gland openings as "hollow crypts among the cilia".

A number of transmission electron microscopy studies have been conducted on the ultrastructure of UV glands from the fowl (Van Krey et al. 1967; Schindler et al., 1967; Burke et al., 1972; and Tingari and Lake, 1973). The ultrastructure of the UV glands in Japanese quail was studied by Friess in 1978. To date, no studies on the ultrastructure of UV glands in the turkey are known to have been conducted.

The following is a summary of the transmission electron microscopy studies cited above. Since few structural differences exist in the UV glands of quail and fowl, observations from the quail, unless otherwise specified, will be treated synonymously with those from the fowl.

Uterovaginal sperm storage glands were composed of two cell types. Proximal cells, located in the neck region of the sperm storage glands, were ciliated. Distal cells, located in the pouch region of the glands, were non-ciliated (op. cit.).

Proximal cells were a continuation of the oviductal mucosa. They formed a single layer of columnar cells with cilia located on the apical or lumenal surface of cells. The cilia were composed of the typical 9 + 2 axial filament complex. Associated basal bodies were observed at the apical surface. Long microvilli were interspersed, on an occasional basis, between cilia. The lateral walls displayed little or no interdigitation. Lateral walls of adjacent cells were joined by apically located junctional complexes. No infolding of the basal lamina was observed (op. cit.).

Intracellular aspects of proximal cells suggested a low level of metabolic activity. Nuclei were centrally located and were generally oval in shape with some surface irregularity. Small clumps of heterochromatin were found within the nucleus, and occasionally nucleoli were visible within the nucleus. Present in the cytoplasma were golgi apparati with an associated smooth endoplasmic reticulum. A sparse rough endoplasmic reticulum was also present. Small membrane bound electron dense particles were located perpherally in the cytoplasm, while large lipid droplets were located in apical and basal portions of the cytoplasm. Lysosomes were generally located supranuclearly (op. cit.). Mitochondria were dispersed throughout the proximal neck cells, but were concentrated basally in quail (Friess,

1978), and apically in fowl (Tingari and Lake, 1973).

Friess (1978) observed a second type of cell in the proximal region of the UV glands of the quail, which he termed basal cells. The basal cells were located adjacent to the basal lamina and did not project to the glandular lumen. The cells were irregular in shape, and contained few cytoplamic organelles.

The distal pouch region of the UV glands was composed of a single layer of non-ciliated columnar cells. The apical surface of the distal cells was characterized by dense microvilli. Unlike proximal cells, the lateral walls displayed a high degree of infolding or interdigitation basally. Lateral walls of adjacent cells were bound together by junctional complexes apically and desmosomes basally. As with proximal cells, no infolding of the basal lamina was observed in the distal cells.

Distal pouch cells of the UV glands appeared to be secretory in nature. They were occasionally observed to be engaged in an apocrine type secretion which manifested itself as a blebbing off at microvilli (Burke et al., 1972). Free glycogen particles and masses of cytoplasm were also observed in the lumen. Tingari and Lake (1973) observed some micropinocytotic vesicles and suggested that besides secretion, absorption of luminal contents might occur.

Intracellular aspects of distal UV gland cells

suggested a high level of metabolic activity. Nuclei were basally located, were spherical in shape and were composed primarily of euchromatin with little or no heterochromatin. At least one and sometimes two nucleoli were frequently observed within the nucleus. Golgi apparati observed supernuclearly were associated with smooth endoplasmic reticulum. Sparse rough endoplasmic reticulum was located throughout the cytoplasm. Large lipid droplets were observed primarily supranuclearly, but occasionally infranuclearly. Mitochondria were observed to be distributed throughout the cytoplasm. Lysosomes were apically located (op. cit.).

Electron dense granules were observed in the cytosol of the distal cells. The latter were identified as glycogen particles by Tingari and Lake (1973) and as free ribosomes by Friess (1978).

Burke et al. (1972) and Tingari and Lake (1973) noted the presence of fine filaments oriented with the long axis of UV cells in fowl. Friess (1978) was unable to detect such filaments in the UV glands of quail. Friess did, however, detect in the quail an apical tubular system that had not been described in the fowl.

Histochemistry of Uterovaginal Sperm Glands

In his original characterization of the UV glands of the chicken, Fujii (1963) found that cells of the glands

gave negative results when tested for polysaccharides with the periodic acid - Schiff (PAS) reagent. In a later study, Gilbert et al. (1968a) observed a positive PAS reaction. These latter researchers also found the PAS reaction was not stable to treatment with amylase, indicating that the polysaccharide was glycogen. The presence of glycogen was confirmed by positive reactions with Best's carmine and with alcoholic iodine. The glycogen was located primarily intracellularly and only once was it detected in a glandular lumen indicating limited secretion. They also noted that the UV storage glands were the only oviductal tissue in that general area to contain glycogen suggesting a possible role in sperm storage.

Renden (1981) obtained a negative PAS reaction in the UV glands of the quail. This observation for the quail is in agreement with the earlier observation of Friess (1978) that the UV sperm storage glands of the quail did not stain with phosphotungstic acid-chromic acid, indicating an absence of glycogen. Both Fujii (1963) and Gilbert et al. (1968) using a variety of tests obtained negative reactions for the presence of mucopolysaccharides.

Only one study (Gilbert et al., 1968) examined the UV glands for protein content. Reactions for protein were very weak indicating a low protein content. Based on test results, the authors concluded that proteins were not

secreted.

There is a general consensus in past literature that lipids are present in cells of the UV glands. Fujii (1963) obtained positive results when the cells were stained with Sudan Black B, a general stain for lipids. Gilbert et al. (1968) in addition to obtaining positive results with Sudan Black B, also obtained positive results with oil red O, another general stain for lipids. Renden (1981) confirmed that the results obtained with chickens also apply to quail. He found that quail sperm glands stained positively with oil red O. Both Fujii (1963) and Gilbert et al. (1968) observed that the positive staining material was located perinuclearly within the cells, and tended to be most concentrated supranuclearly. Renden (1981) found the positive staining material was located apically and basally within glandular cells of the quail.

Whether or not the lipid material is secreted into the lumen is unclear. Fujii (1963) and Renden (1981) observed positive staining lipid material in the lumen. In addition, they noted that the distribution of lipid material within glandular cells and its presence in the lumen was influenced by the passage of a hard shelled egg during oviposition. In the chicken Fujii (1963) found that lipid was present in the glandular lumen and that the amount present in the glandular cells had decreased following oviposition. The distribution

within glandular cells had also changed. Instead of being located exclusively perinuclearly, lipid material was detected throughout the cytoplasm following oviposition. The situation in the quail differs from that observed in the chicken. In the quail, the largest concentrations of positive staining lipid material were found following oviposition as opposed to the larger preoviposition levels observed in the chicken (Renden, 1981). As a further point of interest, Renden found that the presence of spermatozoa did not influence the distribution nor the concentration of lipid material within the glandular cells. Gilbert et al. (1968) were unable to detect the presence of positive staining lipid material in the lumen.

Attempts to specify the type of lipid present have also produced conflicting results. Fujii (1963) concluded that the material was not phospholipid since it gave negative results when stained with Baker's hematein, Menshik's Nile Blue and Ciaccio's Sudan III. He further concluded that the material was not glycolipid because it did not stain positively with PAS. Fujii identified the material as being a cholesterin ester since positive results were obtained using Schultz's test for cholesterin and Okamoto's method for ester forming cholesterin.

The findings of Gilbert et al. (1968) contradict the earlier findings of Fujii (1963). They obtained positive

results for phospholipid when using Mallories Nile Blue A, Baker's acid haemetin and Feyrter's metachromatic method. They atttributed the differences between their results and those of Fujii to the latter using acetone and/or heat in the staining process which led to erroneous results. Gilbert et al. (1968) accumulated strong evidence that the lipid material was phospholipid, but were unable to positively identify the material as phospholipid. The lipid material displayed solubility characteristics in different solvents which were uncharacteristic of phospholipids. Furthermore, when the Menschik Nile Blue A method for phospholipids was used, spermatozoa in the lumen stained positively but the glandular cells did not stain at all. This finding indicated that the lipid material in the glandular cells differed from the phospholipid component of spermatozoa.

One additional point of contention exists between the work of Fujii (1963) and that of Gilbert et al. (1968). Whereas Fujii obtained positive reactions when testing for cholesterol, Gilbert et al. was unable to confirm the presence of cholesterol using Adam's method, Schultz's method or Okamoto's method.

Information pertaining to enzymes present in cells of the UV glands has been obtained using histochemical techniques. Acid phosphatase has been observed in the cells

of the UV glands of chickens (Fujii, 1963; Gilbert et al., 1968; Aire and Steinbach, 1976). It has also been observed in quail (Renden et al., 1981). In the case of the chicken, Gilbert et al. (1968) found that acid phosphatase was located apically in UV gland cells, and was presumably secreted since it was observed in glandular lumina. In addition, Aire and Steinbach (1976) observed that acid phosphatase was only present in mature oviducts and could not be detected in immature oviducts. Some differences were noted by Renden et al. (1981) in the case of the quail. Like the chicken acid phosphatase was located in the apical portion of storage gland cells. However, no acid phosphatase was detected in the lumen suggesting that it was not secreted.

Conflicting reports exist concerning the presence or absence of alkaline phosphatase. Fujii (1963), using Gomori's calcium phosphate method, was unable to detect the presence of alkaline phosphatase in the UV glands of chickens. However, Aire and Steinbach (1976), using Gomori's calcium phosphatase method and Burstone's method, did detect alkaline phosphatase. The later authors found alkaline phosphatase activity in grooves and pits throughout oviducts of immature hens. In mature hens alkaline phosphatase activity was found only in the uterovaginal area of the oviduct. The enzyme was located in the apical

portion of storage gland cells and also in glandular lumina suggesting that it was secreted. Sinowatz et al. (1976) observed alkaline phosphatase activity in the uterovaginal region of the quail oviduct, but only when an egg had passed through the region. Sinowatz et al. (1976) also found a prevalence of enzymes involved in oxidative pathways and evidence for strong steroid dehydrogenase activity.

Spermatozoal-sperm Gland Interrelationships

It is conceivable that absorption of lumenal contents by cells of UV glands occurs. Micropinocytotic vescicles were observed by Tingari and Lake (1973). The absorptive nature of UV glands was studied by Friess et al. (1978). The inability of UV gland cells to take up horse radish peroxidase, lantham hydroxide, ruthenium red or phosphotungstic acid-chromic acid led the researchers to conclude that absorption of lumenal contents did not occur.

In their initial study Bobr et al. (1964a) found that spermatozoa in a storage gland lumen were arranged in parallel bundles with their heads directed toward the pouch region of the gland and their tails directed toward the oviductal lumen. The ultrastructural studies of Schindler et al. (1967), Burke et al. (1968), Tingari and Lake (1973), and Friess et al. (1978) revealed that spermatozoa were not closely associated with cells of UV glands. In fact, only occasionally were spermatozoa found to be in

contact with microvilli of glandular cells.

Fujii and Tamura (1963) observed that membranes surrounding sperm heads tend to be intimately associated, resulting in a head to head agglutination within the glandular lumen. The study of Schindler et al. (1967), however, found that spermatozoa were separated from each other within a sperm gland. The discrepancy may be due to the fact that Schindler et al. (1967) inseminated washed spermatozoa. Van Krey et al. (1981) observed that there was a natural tendency for fowl spermatozoa to agglutinate head to head in vitro. They hypothesized that agglutination at the level of sperm gland was a prerequisite for sperm storage.

The fate of dead spermatozoa within the storage glands has been a point of interest among researchers. As pointed out earlier, Fujii and Tamura (1963) observed the spermatozoa tended to agglutinate in clumps within uterovaginal sperm storage glands. They further pointed out that dead spermatozoa did not seem to be able to participate in this clumping phenomena. In addition, they found that 12 to 20 days post-insemination, mass disintegration of spermatozoal clumps was observed to occur. In a more recent study, Van Krey et al. (1981) studied the fate of aging spermatozoa labeled with tritium. They were unable to detect labeled spermatozoa within the glands 10 days post-

insemination. This observation indicated that UV glands did not retain dead spermatozoa. In addition, they were unable to detect the presence of the radio tracer within cells of UV glands indicating that phagocytosis of dead spermatozoa by glandular cells did not occur.

Koyanagi and Nishiyama (1981a) studied the fate of dead spermatozoa in the infundibular region of the oviduct. They observed that spermatozoal degeneration within the sperm nests was associated with secretion of an electron dense substance into the lumen of the nests beginning about 15 days post-insemination. In this study, as in the study of Van Krey et al. (1981), no phagocytosis of spermatozoa by glandular cells was observed. In addition, no leukocytes or macrophages were observed in the lumina of sperm nests. However, macrophages were observed within the intercellar spaces of the mucosal epithelium and also in the outer layer of the vitelline membrane. The macrophages had increased in number by 7 days post-insemination, and macrophages in both locations were commonly observed phagocytizing spermatozoa (Koyanagi and Nishiyama, 1981b).

Transport of Spermatozoa to the Uterovaginal Sperm Host Glands

Much work has also been done to determine the overall fate of spermatozoa in the oviduct following insemination. Lorenz (1966) reported that following natural mating or

artificial insemination (AI) spermatozoa were deposited mid-vaginally. Bobr et al. (1964a) reported that following natural mating, spermatozoa could be found in the UV glands within 10 minutes. Takeda (1974) found spermatozoa in the uterus within 90 minutes following AI into the distal vagina and within 40 minutes following AI into the proximal vagina. Verma and Cherms (1965) reported that spermatozoa reached the UV glands of the turkey within 90 minutes following AI.

It is generally thought that spermatozoal movement within the vagina to the UV glands requires active motion by the spermatozoa. This hypothesis stems from the observation that following placement of methylene blue within the vagina, the dye particles were not transported, but remained localized (Mimura, 1939). It has also been found that dead spermatozoa, incapable of active motion, when inseminated into the vagina were not transported beyond the point of placement (Mimura, 1939; Takeda, 1974).

Evidence exists which suggests that the oviduct distal to the uterovaginal junction (UVJ), and perhaps the junction itself, function as a selective barrier to spermatozoal transport. Grigg (1964) reported that when spermatozoa were inseminated beyond the UVJ into the uterus more spermatozoa were observed in the infundibulum, as opposed to intravaginal AI where most spermatozoa were found in the UV glands. This observation was confirmed by Takeda (1966;

1967). Ogasawara et al. (1966) obtained higher fertility levels with poor quality semen by inseminating into the uterus. They observed that following intravaginal AI, the degree to which the UV glands were occupied by spermatozoa was greatly reduced. In a similar experiment, Kempenich-Pinto et al. (1970) obtained higher fertility when inseminating turkey semen into chicken hens by depositing semen beyond the UVJ, either in the uterus or the magnum. Schindler et al. (1970) found turkey spermatozoa in the UV glands of chicken hens following intravaginal AI, suggesting that the UVJ itself was the barrier. Takeda (1974) also examined the fate of heterologous spermatozoa from rabbits, rams and bulls in the reproductive tract of the chicken. Following AI into the posterior vagina, heterologous spermatozoa were not transported, whereas when inseminated into the anterior vagina, heterologous spermatozoa were transported to the uterus only occasionally. Intrauterine AI of heterologous spermatozoa did result in transport to the infundibulum. In addition, as pointed out earlier, dead sperm were not found beyond the UVJ (Takeda, 1974).

Certain observations regarding the physical nature of the UVJ support its purported barrier function. Bobr et al. (1965) reported that the UVJ sphincter was always firmly closed. Fujii (1975) reported that sperm transport was retarded by ciliary motion in the region of the UVJ. Chen

and Hawes (1970) and Verma and Hawes (1971) reported that the region of the UVJ was characterized by relatively weak irregular contractions compared to the relatively high contractile activity of other oviduct regions.

The work of Howarth (1971) is not consistent with the concept that the UVJ acts as a barrier. He observed that following AI, both live and dead spermatozoa reached the infundibulum in 15 minutes. The exact location of semen deposition was not indicated. It is possible that the semen was placed in the anterior vagina or even beyond the UVJ which would help to explain the discrepancy.

Allen and Grigg (1957) and Howarth (1971) observed that the majority of spermatozoa were excreted following AI. Howarth (1971)also found that a higher proportion of dead spermatozoa were excreted. These observations agree with those of Takeda (1974) who was unable to detect heterogenous or dead spermatozoa in the oviduct 30 minutes post AI.

Filling of the Uterovaginal Sperm Glands

Much attention has also been focused on the manner in which spermatozoa fill the UV glands. Schindler et al. (1967) observed that 24 hours after intravaginal AI spermatozoa were located in mucosal grooves of the UVJ and in the UV glands. Within 2-3 days after AI, spermatozoa could no longer be found in the grooves, being located exclusively in the UV glands. They also noted that the

accumulation of spermatozoa within the glands peaked 24 hours after AI and then declined with time.

Van Krey et al. (1971) made the surprising observation that 18 hours after insemination of 0.05 cc semen only 27% of the UV glands contained spermatozoa. The remaining 73% of the UV glands were empty. Subsequently, they observed that the same type of situation existed in turkeys (Van Krey et al., 1974). It was found that 24 hours following insemination of 0.05 cc semen, only 40% of turkey UV glands contained spermatozoa. In yet another study, Compton and Van Krey (1979) established that the UV glands are capable of trapping only a critical number of spermatozoa. Doubling the dose of semen did not change the trapping rate.

Verma and Cherms (1965) postulated that the UV glands became filled in a progressive, posterior to anterior, fashion within the oviduct. The actual filling of individual UV glands occurred in such a manner so as to culminate in a stratification of spermatozoa within the glands (Compton et al., 1978). A filling scheme resulting in stratification offers a ready explanation for the observed phenomena whereby the second of two consecutive inseminations gives rise to a majority of subsequent offspring (Warren and Gish, 1943; Smyth, 1963; Atkinson et al., 1966).

It was suggested that the lower UVJ contractile

activity observed by Chen and Hawes (1970) and Verma and Hawes (1971) might aid in sperm entry of the UV glands. In fact, Verma and Hawes (1971) observed that the presence of semen resulted in further decrease in contractile activity of UVJ.

Several factors have been observed to affect UV gland filling. Gilbert et al. (1968) observed that stitching a surgical thread into the uterine wall resulted in an accumulation of fewer spermatozoa within the UV glands following an AI. Burke et al. (1969) treated hens with pregnant mares serum to suppress ovulation and oviposition, and noted a higher percentage of UV glands contained spermatozoa. The studies of both Fujii and Tamura (1963) and Takeda (1964) showed that dead spermatozoa were capable of entering the UV glands of nonlaying hens in which the oviducts were partially regressed.

Emptying of the Uterovaginal Sperm Glands

Much research has been aimed at elucidating the mechanism by which spermatozoa are released from the UV glands. Bobr et al. (1964b) were able to recover spermatozoa from the oviduct only around the time of ovulation (OV) and oviposition (OP). They, therefore, concluded that OV and/or OP were directly involved in spermatozoal release. Likewise, in the case of the turkey, Verma and Cherms (1966) were able to recover more

spermatozoa from the oviduct lumen following a recent OV. However, Burke and Ogasawara (1969) found that spermatozoa were present in the UVJ lumen before and after OP, and that they were also present during pauses in egg production. Burke et al. (1969) were also able to recover spermatozoa from the lumen of the uterus and infundibulum in hens treated with pregnant mare's serum to prevent OV and OP. More recently Bakst (1981) found spermatozoa in washings from all oviductal segments, regardless of time after OP. Compton and Van Krey (1979) prevented OV and OP by treating hens with pregnant mare's serum, and found no difference in the rate of UV gland emptying between treated hens and controls.

Takeda (1967) observed that when an egg was forced through the UVJ spermatozoa could be recovered from oviduct lumina previously devoid of spermatozoa. This same phenomena had earlier been observed to occur in the infundibulum by Grigg (1957) who forced an artificial ovum through the glandular region. Bakst (1978), using scanning electron microscopy, noted that an ovum in the distal infundibulum exposed openings of the sperm storage sites to the oviductal lumen. Verma and Hawes (1971) found a decrease in uterine contractile activity following OP which they suggested may aid in spermatozoal release.

Fertility of hens inseminated at various times relative

to OV and OP has been studied to determine the effects of these two events on sperm release. Burke et al. (1969) noted low levels of fertility when hens were midvaginally inseminated immediately after OP. Fertility level was improved by placing semen in the anterior vagina. They also observed that fertility of the first egg in a clutch was unaffected when hens were inseminated following the last egg in the previous clutch. Since the first egg in a clutch is not preceded by an OP, these results indicate that OP is not necessary for sperm release. In a similar experiment, Benoff and Renden (1978) compared the fertility of the first and second eggs in a clutch, and found no difference in fertility. Again, if OP were required for sperm release it would be expected that fertility of the first egg in a clutch would be low since it was not preceded by an OP. Benoff and Renden also noted there was no difference in fertility between two eggs laid on the same day. If OP were involved in sperm release and subsequent fertility it would be expected that fertility of the second egg laid on the same day would be poor since it had migrated past the site of fertilization before the OP of the first egg had occurred.

Verma and Cherms (1965) described the emptying of UV glands in the turkey as occurring in such a way that there was a decrease in the number of spermatozoa per gland with

time. Compton et al. (1978), trying to explain the predominance of offspring from the second insemination of a sequence concluded that sperm gland emptying proceded as a slow leakage of spermatozoa into the lumen. Burke et al. (1969) had suggested such an emptying process earlier. Van Krey et al. (1981) found that spermatozoa from a second sequential AI did not displace spermatozoa from the first AI within the UV glands.

Transport of Spermatozoa to the Site of Fertilization

Following release from the UV glands, it appears that spermatozoa are passively carried to the site of fertilization in the infundibulum. Following intramagnal AI, spermatozoa are primarily transported anteriorly to the infundibulum, and are only rarely found in the UV glands (Van Krey et al., 1966; Schindler et al., 1967; Schindler et al., 1970; Bakst, 1981; 1983). Takeda (1966; 1967) found a similar distribution of spermatozoa following intra-infundibular AI. Following intrauterine AI more extensive filling of the UV glands occurred (Fujii and Tamura, 1963; Schindler et al., 1970 and Donovan et al., 1969). Thus, it appears that sperm are capable of backward motion in the uterus. However, the close proximity of the UV glands to the site of AI should be considered. Nevertheless, variation in degree of backward motion may be a function of the activity gradient described by Chen and Hawes (1970).

They observed that oviductal contractile activity increased proximally and decreased distally. Thus, contractile activity was greater in the infundibulum and magnum than in the uterus.

Whether sperm transport is affected by the presence of a hard shelled egg in the oviduct appears to be a moot question. Bakst (1978) and Howarth (1971) both found that a hard shelled egg in the uterus was not an obstacle to sperm transport. Bakst (1978), utilizing scanning electron microscopy, found only a slight alteration of uterine mucosal folds when an egg was present. These findings are at odds with those of Ogasawara and Marquez (1974) who found that an egg tended to obscure mucosal folds to such an extent that it blocked sperm transport.

There is agreement within the literature that an ovum in the magnum (Bobr et al., 1964 a,b; Howarth, 1971; Bakst, 1978) or in the isthmus (Bakst, 1978) will essentially block sperm transport. Bobr et al. (1964a) were able to detect spermatozoa in the albumin surrounding an ovum and in the vitelline membrane and yolk of fertile eggs. This is consistent with the observation of Bakst (1981) who found reduced numbers of spermatozoa in oviduct flushings when an ovum was present.

Relationship Between Infundibular Sperm Nests and Uterovaginal Sperm Glands

The exact relationship between UV sperm glands and infundibular sperm nests is as yet unclear. Following a natural mating or an insemination into the vagina, greater numbers of spermatozoa are stored in the UV glands for longer periods of time than are stored in infundibular sperm nests (Fujii and Tamura, 1963; Bobr et al., 1964a; Takeda, 1964). Takeda (1964) found that spermatozoa were present in the infundibular sperm nests only during the fertile period following insemination, but were present in the UV sperm glands nine days after the last fertile egg had been laid. The above observations suggest that the UV sperm glands are the primary site of sperm storage. However, in the case of the chicken, at least, it appears that the infundibular sperm nests have a greater capacity to store spermatozoa. Following intramagnal or intra-infundibular AI great numbers of spermatozoa were found in the infundibular sperm nests. A concommittant increase in the level and duration of fertility was also observed (Van Krey et al., 1966; Takeda, 1966 and 1967; and Lorenz and Ogasawara, 1968). One effect noted in the studies of Van Krey et al. (1966) and Lorenz and Ogasawara (1968) was that the incidence of preovipositał mortality was increased.

The findings of Takeda (1965) also indicate that infundibular sperm nests have a greater capacity to store spermatozoa. He ligated the infundibulum and UVJ and he

inseminated spermatozoa into the ligated regions. He was able to recover motile spermatozoa from the infundibulum up to 21 days after insemination.

The turkey appears to differ from the chicken with respect to infundibular storage capacity. Ogasawara and Fuqua (1972) compared fertility following intramagnal and intravaginal AI. They found that fertility levels were lower and the duration was shorter following intramagnal AI. In addition, they found that preoviposital mortality did not increase. Unfortunately, these authors did not determine whether or not higher than normal numbers of spermatozoa had entered the infundibular sperm nests following intramagnal AI, as had occurred in chickens.

Spermatozoal Survival in the Oviduct

Oviduct chemistry is of importance in that it has the potential to influence sperm survival and activity. Studies have been conducted to determine the composition of uterine fluid in the chicken (El Jack and Lake, 1967) and the turkey (Rieser et al., 1972). In both studies it was observed that the composition of uterine fluid changed relative to the stage of uterine egg production. El Jack and Lake (1967) noted a large decrease in Na^{+} and a large increase K^{+}, Ca^{++} and Mg^{++} in uterine fluid at the time of oviposition in chickens. A small decrease in chloride and small increase in CO_2 were also noted. Reiser et al. (1972) noted a large

decrease in Na^+ and an increase in K^+ and Mg^{++} in the uterine fluid of the turkey at the time of oviposition. A two fold increase in Ca^{++} occurred. Rieser et al. (1972) also noted that the concentration of K^+ in the uterine fluid was many times greater than its concentration in blood serum.

The hydrogen ion concentration (pH) has been measured in the chicken oviduct (Ogasawara et al., 1964; Bakst, 1980) and in the turkey oviduct (Bakst, 1980). In all cases the pH was found to vary throughout the oviduct, being highest or most basic in the infundibulum and UVJ. It is interesting to note that Ogasawara et al. (1964) found pH to be quite variable even within a particular region of the oviduct except in the area of the UVJ which had a relatively constant pH. Bakst (1980), on the other hand, found that the infundibular, UVJ and vaginal pH varied relative to time of OP in the chicken, as did the isthmal, uterine, UVJ and vaginal pH in the turkey. Ogasawara et al. (1964) found the overall oviduct pH to range from 7.17 to 7.87 in the chicken. Bakst found pH to range from 6.8 to 7.8 in the chicken and 6.0 to 7.3 in the turkey.

Van der Horst and Litgen (1969) found that the turkey oviduct contained only a small amount of glucose, but relatively high concentrations of inositol were detected in the uterus and UVJ. In vitro studies by these authors

revealed that inositol resulted in increased preservation of sperm motility.

Harrison et al. (1966) and Harrison and Heald (1966) isolated a protein, poly α-L-glutamic acid, which could only be found in the regions of the infundibulum, upper magnum and UVJ, i.e., the regions of sperm storage. They observed that in vitro poly α-L-glutamic acid was able to preserve sperm motility for several hours. However, in a subsequent study (Heald et al., 1967) found that inducing an absence of poly αL-glutamic acid by immunizing birds against it did not affect fertility.

The effects of various regions of the oviduct on spermatozoa have been studied in some detail. Several studies have determined the effects of incubating spermatozoa in ligated sections of the oviduct. Hamner and Williams (1963) found that when spermatozoa were incubated in vivo in the ligated magnum the respiratory rate of the sperm cells increased. As pointed out earlier, Takeda (1965) determined the length of time spermatozoa remain motile in ligated sections of the oviduct. Motile spermatozoa were found in the infundibulum 21 days post-insemination, in the UVJ 14 days post-insemination, and in the vagina, isthmus and magnum for only two days post-insemination. It is interesting to note that even though motile spermatozoa were found for only 14 days in the UVJ,

nonmotile spermatozoa were found until the 21st day post insemination. Schindler and Hurwitz (1966) incubated spermatozoa *in vivo* in different ligated segments of the oviduct for two and 24 hours. Following incubation, they found that all oviduct segments preserved motility longer than a semen diluent did. Maximum preservation of motility was obtained in the lower isthmus and lower magnum. The UVJ preserved motility for a relatively short period of time with very few motile spermatozoa being observed after 24 hours of incubation. Buckland (1970) incubated spermatozoa for two hours in different oviduct segments *in vitro*. He studied sperm enzyme activity representative of the citric acid cycle, glycolytic cycle, glutamate metabolism, ATP formation, sperm motility and proteolytic activity. Overall, no increase in enzyme activity was observed, and in many cases enzyme activity decreased. He also observed that semen pH, spermatozoal motility and fertilizing capacity of spermatozoa all decreased. He concluded that decline in enzymatic activity lead to a decline in fertility.

One study, Harrison and Heald (1966), examined the effect of washings from different segments of the oviduct on sperm motility. They found that only washings from the infundibulum, upper magnum and UVJ facilitated extended preservation of sperm motility.

Several researchers have studied the effects of oviduct

extracts on sperm activity. Ogasawara and Lorenz (1964) found that extracts from the infundibulum and magnum caused an increase in respiratory activity of spermatozoa. They determined that the active substance was of a high molecular weight, organic in nature, and most probably a protein.

Lehrer and Schindler (1969) studied the effects of homgenates from the infundibulum, magnum, uterus and UVJ on spermatozoal motility and respiration. All homogenates facilitated preservation of sperm motility at normal body temperature. Homogenates from the infundibulum and UVJ increased respiratory activity of the spermatozoa. Magnum homogenates were variable in their ability to affect sperm respiration, being dependent on the stage of egg production. Sperm respiratory activity was increased when an ovum was in the magnum. The authors also noted that spermatozoa incubated four hr in oviduct homogenates were capable of producing fertile eggs, whereas spermatozoa were infertile following incubation for four hr in a phosphate buffer.

Van Krey et al. (1970) studied the effects of magnal and UVJ homogenates on sperm respiration. They found that both homogenates increased respiratory activity, but that UVJ homogenates were more stimulating than magnal homogenates.

Howarth and Huston (1974) examined the effect of oviduct homogenates on sperm glucose utilization, and found

infundibular extracts to be significantly more stimulating than other regions of the oviduct. A difference in sperm glucose utilization was noted when homogenates were obtained from hens maintained at two different temperatures. Homogenates obtained from hens maintained at 31°C stimulated greater glucose utilization than did homogenates obtained from hens maintained at 19°C.

Most recently Bakst and Pierson (1981) studied the effects of incubating spermatozoa in homogenates from different oviduct segments collected at various times after oviposition/ovulation on sperm respiration, sperm motility and percentage dead spermatozoa. All homogenates were found to stimulate respiration and in general decreased motility and increased the percentage dead spermatozoa. Neither the stage of egg production nor the particular segment from which homogenates were obtained caused a significant change in behavior.

The effects of oviductal cell cultures on sperm activity have also been examined. Ashizawa (1976) found that when spermatozoa were incubated with cell cultures of infundibular uterine and UVJ tissue, sperm motility was preserved for 5-6 days and fertilizing capacity was preserved for four days. They observed that at 41°C (normal body temperature) spermatozoa remained immobile and in close proximity to cultured oviduct cells. When the temperature

was changed to 23°C spermatozoa became very motile. In a latter study, Ashizawa and Nishiyama (1977) found that cell cultures derived from kidney, UVJ, striated muscle or chick embryo were all capable of preserving motility 7-8 days and fertility for four days. This finding indicates that cultured somatic cells are equally as capable of preserving sperm activity as are cultured oviductal cells.

Howarth (1980) found that the lipid content of spermatozoa declined following 24 hr of incubation in a chick embryo cell culture. Lipids, other than phsopholipids, were responsible for the decline. Fujihara and Howarth (1981) found that actual contact with cultured cells was not necessary to preserve motility and fertility of spermatozoa. They incubated spermatozoa in fluid derived from cultured uterine cells, and were able to preserve motility 4-5 days. Spermatozoa that had been incubated 48 hours in culture fluid were able to fertilize eggs for one week following intramagnal AI.

Artificial Insemination Parameters Affecting Fertility

To remain economically viable, turkey breeder farmers must strive to produce a maximum number of fertile eggs at minimal cost. Quite obviously, any change which increases the number of fertile eggs is of financial benefit to the turkey breeder farmer. Not only is profit enhanced by increasing the number of poults, but losses incurred from

incubating infertile eggs are minimized.

As indicated, one area which has real potential for improvement is the fecundity of turkey breeder hens. In addition to relatively poor peak fertility levels (ca 90%), turkey hens become progressively less fertile as egg production continues. Sudden precipitous declines in fertility can also occur.

Fertility following artificial insemination is influenced by a number of factors. Although there is disagreement within the literature regarding the proper depth of insemination, it has been found that the site at which semen is deposited within the oviduct does affect fertility. Most data suggest that relatively deep inseminations result in higher levels of fertility (Biellier, et al., 1961; Ogasawara and Rooney, 1966; Holleman and Biellier, 1970; Holleman and Biellier, 1976). However, data also exists which indicates that shallow inseminations results in optimum fertility (Paton et al., 1971; Besulin and Sakhatsky, 1974; Wentworth, 1975). This discrepancy may be the result of semen placement beyond the UVJ with deep AI. Fujii and Tamura (1963) found that when semen was placed in the uterus beyond the UVJ, the number of spermatozoa entering UV sperm glands was considerably less than following an intravaginal AI. Conceivably this would result in lower fertility rates.

Frequency of artificial insemination has also been shown to affect fertility. It is generally accepted that biweekly artificial inseminations result in higher fertility than triweekly inseminations (Brown, 1974; Holleman and Biellier, 1976). There is some question, however, concerning the relative effects of fertility of weekly verses biweekly artificial insemination. Brown (1974) observed no significant difference between hens inseminated weekly or biweekly. However, Bajpai and Brown (1964) and Christensen (1981) found that weekly artificial insemination resulted in higher fertility than did biweekly inseminations. It has also been observed that switching from biweekly to weekly inseminations during the later stages of the egg laying cycle can result in slight increases in fertility (Ogasawara and Rooney, 1966; Nestor and Brown, 1968). Others found that such a switch had no effect on fertility (Harper and Arscott, 1969; Sexton, 1977).

Time of day when hens are artificially inseminated can also affect fertility. It has been found that turkey hens inseminated in the evening and at night have higher fertility than those inseminated in the morning or the afternoon. The relatively poorer fertility associated with morning and afternoon inseminations is thought to be the result of a hen being inseminated within a critical time

interval prior to oviposition and ovulation (Christensen and Johnston, 1975, 1977; Giesen et al., 1980). Christensen and Johnston (1975) also noted that the time of peak flock oviposition shifted from early afternoon to late afternoon toward the end of the egg laying season. They hypothesized that the shift may account for some of the decline in fertility observed during the later stages of egg production.

Artificial insemination when a hard shelled egg is in the uterus is thought by some to result in depressed fertility levels. Poor fertility associated with the presence of a hard shelled egg at the time of AI may result from poor semen placement (Lake, 1967; Ogasawara and Marquez, 1974) or interference with spermatozoa entering the UV sperm glands (Ogasawara and Marquez, 1974).

Hughes and Parker (1970 and 1972) observed that low levels of fertility were also obtained when AI was performed within an hour after oviposition. They found that injecting oxytocin or vasopressin immediately after AI resulted in depressed fertility. They also found that oxytocin was capable of reducing sperm motility in vitro. They concluded that levels of these hormones associated with oviposition had a detrimental effect on fertility.

Another factor affecting turkey fertility is the number of spermatozoa artificially inseminated. When an inadequate

number of spermatozoa are inseminated, fertility declines relatively rapidly (Ogasawara and Rooney, 1966). There is some question as to what constitutes an adequate number of spermatozoa. Takeda (1966) and Sexton (1977) found that 100 million spermatozoa were necessary to maintain optimum fertility throughout the laying season. Studies on diluted semen indicate that smaller numbers of sperm are required to maintain optimum fertility. One study by van Tienhoven and Steel (1957) found that 12-15 million spermatozoa produced maximum fertility, while Sexton (1976) found that 25 million spermatozoa yielded maximum fertility. Lake (1975) observed an individual hen variation regarding the minimum number of spermatozoa necessary for optimum fertility. When birds are inseminated with an inadequate number of spermatozoa, some hens were infertile while others did lay some fertile eggs. Sexton (1977) was unable to improve fertility late in the season by increasing the number of spermatozoa inseminated.

Semen quality also affects fertility. Brown and Graham (1971) found that toms with low semen quality, as assessed by visual observations and motility rating, were unable to yield optimum levels of fertility. However, it has been shown that utilizing semen from young toms late in the egg production season does not improve fertility (Lorenz, 1970).

In isolated cases it has been possible to improve fertility by changing the depth of semen placement during

insemination, the volume of semen inseminated, the frequency of insemination, the time of insemination or the semen source. However, to date, it has not been possible to curb the progessive decline in fertility nor to prevent the sudden precipitous declines in fertility that can occur in turkey hens by manipulation of the factors cited above.

Infertility Syndrome

As indicated previously,a flock of turkey hens will occasionally undergo sudden precipitous declines in fertility. Characteristically, fertility will go from relatively high levels to exceptionally low levels of fertility in a very short period of time. This form of fertility decline has been termed the "Infertility Syndrome". Because it occurs only infrequently, it has been studied on a limited basis. After a transient short-term interruption in egg production, the affected hens show a rapid decline in fertility. The problem is also characterized by a decline in egg shell quality. Nevertheless, affected hens continue to lay normal numbers of eggs (Ball et al., 1969; Yu and Burke, 1979a). The decline in flock fertility is not due to a universal decline in fertility. Instead, the fertility of individual hens approaches zero percent. The continued decline in flock fertility is a reflection of the increasing number of hens becoming essentially totally infertile (Yu and Burke,

1979a).

Ball et al. (1969) explored the possibility that the Infertility Syndrome might be due to microorganisms. In fact, they were able to transmit the malady to healthy hens by using a common inseminating syringe. However, after assaying for Newcastle, fowl pox, CELO, avian infectious bronchitis, avian encephalomyelitis, ornithosis viruses, bacterial and mycotic mycoplasms, they were unable to identify a possible pathogen. They did, however, find increased numbers of lymphoid foci in the infundibular and isthmal regions of the oviduct. In association with the lymphoid foci, they observed increased plasma cell infiltration.

Yu and Burke (1979a) found that the oviducts of infertile hens appeared normal with no excess lymphoid activity. However, in an earlier flock with Infertility Syndrome, Yu and Burke (1979a) noted disruption of the oviduct mucosa, an accumulation of debris on the mucosal surface, and an increased lymphocytic accumulation in the area of the isthmus.

It is possible to restore high levels of fertility in flocks that have exhibited precipitous fertility declines symbolic of Infertility Syndrome. Following forced molting and a period of sexual rest previously infertile hens resumed laying normal numbers of fertile eggs (Moyer et al.,

1966). However, these flocks again underwent premature fertility declines characteristic of the Infertility Syndrome (Moyer et al., 1966; Leighton et al., 1971; Yu and Burke, 1979a). Yu and Burke (1979a) were also able to restore high levels of fertility to a limited number of hens by inseminating the hens intramagnally.

Yu and Burke (1979b) went on to study the effect of incubating spermatozoa prior to insemination in blood sera from high and low fertility hens afflicted with the Infertility Syndrome. They found that incubation of spermatozoa in serum from low fertility hens resulted in lower fertility than did incubation of spermatozoa in serum from hens with high fertility.

Seasonal Declines in Fertility

Seasonal declines in fertility (SD) are the predominant type of fertility declines being observed in virtually all turkey flocks. Seasonal declines in fertility are characterized by a gradual drop in fertility of 20% or more over the course of an egg laying season. Like the Infertility Syndrome, the drop in flock fertility is not due to a slight decrease in the fertility of all hens in the flock, but rather is due to an increase in the number of essentially totally infertile hens within a flock (Almquist, 1964; Lorenz, 1970). In a flock, some hens maintain relatively high levels of fertility over the entire course

of an egg laying season, but as the egg laying season progresses, more and more hens go from high levels of fertility to being essentially infertile, thus, accounting for the observed drop in flock fertility. The fact that some hens maintain high levels of fertility over an entire laying season indicates that it is potentially possible for an entire flock of hens to maintain high levels of fertility.

The seasonal fertility decline problem is compounded by the fact that infertile hens exhibit no evidence of the affliction. They appear healthy and continue to lay normal numbers of settable eggs (Almquist, 1964). Graham et al. (1971) were unable to find any differences in the mineral content, enzyme activity or reducing substance content of various oviductal segments when comparing high and low fertility turkeys.

A difference between fertile and infertile turkey hens was detected at the level of the UV glands. Following AI, the UV glands of infertile hens were found to be essentially devoid of spermatozoa (Van Krey et al., 1967; Yu and Burke, 1979). One possible explanation is that the spermatozoa were unable to reach the uterovaginal storage glands.. In a subsequent study, Van Krey and Leighton (1970) showed that the sperm glands of fertile turkeys became progressively more filled with spermatozoa over a 24 hour period, whereas

the sperm glands of infertile hens remained essentially devoid of spermatozoa.

Recently, Christensen (1981) compared sperm gland filling and emptying early and late in the laying season. Hens were initially given three tandem inseminations with semen of one phenotype to ensure that the glands were filled to capacity. Hens were then given inseminations with semen of another phenotype at weekly or biweekly intervals. The percentage of progeny resulting from the initial tandem inseminations and the percentage of progeny resulting from subsequent insemination were then monitored to evaluate sperm gland filling and emptying. The experiment was repeated once in the beginning of the laying season and once half way through the laying season to compare filling and emptying of UV glands early and late in the laying season. It was found that the initial tandem inseminations accounted for a higher percentage of progeny early in the season than they did later in the season. He concluded that late in the season UV glands are not able to retain as many spermatozoa. It was hypothesized that the observed late season decline in fertility was the result of fewer UV glands being filled with spermatozoa.

The importance of the UV glands to fertility is further demonstrated by the work of Lake and Gilbert (1962, 1964a) and Gilbert et al. (1968). They found that when a

surigical thread was placed in the uterus of chickens the rate of OP was affected and that when eggs were laid, oviposition occurred prematurely resulting in soft shelled or shell-less eggs. More importantly, they found that the eggs were always infertile. Evidently, sperm transport was adversely affected since less sperm were found in the vitelline membrane of eggs 2-6 days post AI. In addition, sperm storage in the UV glands was also adversely affected since it was observed that fewer glands contained spermatozoa. Interestingly, the UV glands appeared normal histochemically, and spermatozoa were found in the infundibular glands. It is thought that an inflammatory reaction to the thread may have been the cause of observed infertility.

The effects of the hen's immune system on fertility have also been investigated. Wentworth and Mellen (1964) found that chicken hens exposed to semen by natural mating, or by intraperitoneal, intravaginal and intrauterine artificial insemination developed significantly higher antisperm antibody titers than did virgin control hens. In the case of artificial insemination, it was observed that antibody titer increased as the number of inseminations increased. A negative correlation was found to exist between antibody titer and duration of fertility as antibody titer increased, duration of fertility decreased.

Antisperm antibodies seem to have a greater effect on the fertility of turkeys. Burke et al. (1971) immunized hens against semen by repeatedly injecting semen and adjuvent intraperitoneally. When the fertility of immunized hens was compared to the control unimmunized hens, it was found that immunized hens had lower fertility. However, they were unable to detect antisperm antibodies by complement fixation, ouchterlony double diffusion, sperm agglutination or sperm immobilization methods (Burke and Rieser, 1972; Burke and Yu, 1979).

In another experiment Burke and Rieser (1972) incubated spermatozoa in blood serum, uterine fluids and a commercial diluent for 15 to 30 minutes prior to insemination into healthy turkey hens. Fertility levels were not affected when spermatozoa were incubated in the diluent or in serum from virgin hens or hens that had been inseminated triweekly. However, when hens were inseminated with spermatozoa that had been incubated in serum from hens previously immunized against semen, significantly lower levels of fertility were obtained. It was also found that within the group of sera from hens immunized against semen, lowest fertility resulted when spermatozoa were incubated in serum obtained from hens which had themselves displayed low fertility.

Burke and Riser (1972) also incubated spermatozoa in

the uterine fluids of high and low fertility hens which had previously been immunized against semen. Even though fertility levels obtained after spermatozoa had been incubated in uterine fluids were considerably higher than those obtained following incubation in blood serum from the same group of hens, the uterine fluid from low fertility hens depressed fertility when compared to the commercial diluent. Incubation of spermatozoa in uterine fluids from low fertility hens prior to AI produced fertility levels 9 percent lower than when incubated in uterine fluids from high fertility hens. This difference, however, was not statistically significant. In an effort to confirm that any decrease in fertility was due to an adverse immunological interaction, fractions of the uterine fluids from high and low fertility hens were heat treated to destroy possible antibody activity. Heat treatment did not result in higher fertility.

Burke and Yu (1979) examined the effects on fertility of incubating spermatozoa in the gamma globulin fraction of serum from hens previously immunized against semen. They found that the gamma globulin fractions of hens immunized against semen reduced fertility compared to the commercial diluent or gamma globulin fractions from nonimmunized hens. The latter two were not statistically different. Furthermore, the reduction in fertility resulting from

incubation in gamma globulins from hens immunized against sperm was dependent on the concentration of gamma globulins. Higher concentrations of gamma globulins induced a greater reduction in fertility. A solution of 4.5% gamma globulins was required to reduce fertility to the same extent that whole serum from the same birds did.

MATERIALS AND METHODS

Hens utilized in this study were randomly selected from a commercial flock of large white turkey breeder hens. For the study, 220 hens were isolated in a pen separate from the main flock, but had been reared and were being maintained under the same management scheme as the remainder of the flock. The hens were exposed to a restricted lighting program when 18 weeks of age and to a stimulatory lighting program at 31 weeks of age. The test hens were isolated from the main flock at the time of stimulatory lighting.

For the purposes of monitoring individual hen fertility, birds were fitted with a numbered saddle and were trapnested two days per week. All identified eggs were incubated for 24 hours, and fertility was assessed on the basis of blastodisc development following macroscopic examination.

Hens were inseminated twice prior to lay (33 weeks of age), and were inseminated weekly thereafter. Hens were inseminated with .025 cc of pooled undiluted semen. Semen for AI was from the same semen pool as that utilized to inseminate the main flock. All inseminations were made by a commercial crew.

Uterovaginal sperm gland tissue samples were collected monthly from hens exhibiting tendencies toward high and low fertility. Tissues were collected through 24 weeks of egg

production. Fertility classification was based on a minimum of two AI intervals. This was necessary to insure that a faulty AI was not the cause of the decline in fertility. When possible, samples from two fertile and two relatively infertile hens were obtained. Tissues were collected from a total of 25 hens over the course of the egg production season. Of this total, four hens were necropsied immediately prior to, or at the onset of egg production, eleven hens were highly fertile, and ten hens ranged from being relatively infertile midway through the egg laying season to being totally infertile by the end of the season.

Immediately prior to tissue collection, hens were anesthetized with sodium pentabarbital. Following laporatomy, a segment of the distal oviduct, which included the uterus and vagina, was rapidly removed. The lumenal mucosa was then immediately bathed in fixative in a petri dish. The fixative consisted of 2 percent glutaraldehyde in 0.1 M sodium cacodylate buffer adjusted to pH 7.4. Very quickly, small tissue samples were removed from the lumenal muscosa using iris scissors. Tissue samples were removed to fresh fixative and examined under a stereoscope to ascertain the presence of sperm glands. Tissue samples containing sperm glands were trimmed to a size of approximately one millimeter square with a scalpel.

Tissue samples were fixed in the glutaraldehyde for two

hours. Following initial fixation, the tissues were washed twice for 10 minutes each in fresh 0.1 M sodium cacodylate buffer. When necessary, tissue was stored at this point under refrigeration until further processing could be carried out. Tissues were postfixed with one percent osmium tetroxide in collidine buffer for two hours. Tissues were then dehydrated using a graded series of ethanol. Following dehydration, tissues were infiltrated with Spurr's embedding medium. Finally, the tissues were embedded using Beem capsules at a polymerization temperature of 60°C for 48 hours.

Sectioning was carried out on a Reichert ultramicrotome, using either glass or diamond knives. Silver and gold sections, approximately 70 to 90 nanometers thick, respectively, were collected on copper grids.

Sections were stained for electron microscopy with one percent uranyl acetate in 50 percent alcohol for 25 minutes. Sections were then washed and stained again for 8 minutes in a lead citrate solution. Sections were examined using a JEOL 100C transmission electron microscope.

RESULTS AND DISCUSSION

Micrographs depicting typical uterovaginal sperm glands taken from high fertility hens in the middle of the laying season are presented in Plates 1 through 4. Distal cells of sperm storage glands were simple columnar in type. They were separated from connective tissue by a basal lamina (Plate 5). The complex was bilayered, being composed of the basal cell membrane and a basal lamina bordering the connective tissue. The basal lamina had a smooth contour. There was no evidence of infolding or other surface modifications to facilitate the uptake of precursor substrates. A lack of such modifications suggests a moderate demand for raw material by cells of UV glands.

The apical, or luminal, surfaces of cells lining the distal UV sperm storage glands contained numerous microvilli (Plate 6). No true cilia were present on the apical surfaces of the distal cells. The microvilli were relatively uniform in length and diameter, and were densely distributed across the lumenal surface of a cell. The microvillar contents were of the same electron density as the cytoplasm proper, and appeared tc be contiguous with the latter. Filaments were observed lying parallel with the longitudinal axis of the microvilli (Plate 6). These filaments appeared to extend into a terminal web of filaments lying just beneath the base of the microvilli.

The area of the terminal web was slightly more electron dense in appearance than the surrounding cytoplasm.

Evidence of micropinocytotic activity was observed to occur in the pit between adjacent microvilli (Plates 6 and 7). The micropinocytotic vesicles extended into the terminal web of filaments. The membrane surrounding the vesicles appeared to be thickened, a condition sometimes associated with absorptive micropinocytosis (Fawcett, 1979). The contents of the micropinocytotic vesicles were translucent suggesting that the material within was discrete in nature.

Junctional complexes were observed apically between the lateral walls of adjacent cells (Plate 7). Desmosomes were observed scattered along the entire border between adjacent cells (Plate 7). Lateral wall interdigitation was frequently observed to occur along the entire border between adjacent cells (Plate 8). Such an association is thought to facilitate the exchange of substances between adjacent cells (Blom and Fawcett, 1976). A modification such as interdigitation of the lateral wall would lead to increased surface area over which fluid and chemical exchange could occur. The presence of such a modification suggests that a high degree of chemical exchange, i.e. intercellular communication, occurs between cells of the UV sperm storage glands.

The cytoplasmic ground substance of sperm storage gland cells was relatively electron dense, giving the cells a darker appearance than the surrounding connective tissue. At higher magnifications, the heterogeneous nature of the ground substance became evident (Plates 8, 9, 12). It was made up of dark staining particles within a relatively light colored matrix, giving the cytoplasm a granular appearance. The particles were uniformly dispersed throughout the cytoplasm. They were never observed to become aggregated or to loose their particulate appearance. The particles were presumed to be a combination of free ribosomes along with individual beta glycogen granules. Because of the close similarity between the particles and ribosomes on the rough endoplasmic reticulum, it was assumed that the majority of the particles were free ribosomes.

Rough endoplasmic reticulum was found on a limited basis (Plate 9). Short segments consisting of single cisternae were observed to be randomly dispersed throughout a cell. The lack of a well developed rough endoplasmic reticulum suggested that protein secretion occurred at low levels.

Aggregates of membrane bound vesicles were observed apically beneath the terminal web of filaments (Plate 10). These vesicles were generally small, but showed variation in both size and shape. The appearance of such vesicles is

generally indicative of a tubular system cut in cross section. Most probably, these vesicles were components of smooth endoplasmic reticulum, which would be consistent with the known tubular nature of that organelle (Borysenko et al., 1979). The presence of smooth endoplasmic reticulum is suggestive of carbohydrate and lipid metabolic activity.

Well developed golgi apparati were observed in the supranuclear region (Plate 11). A lamellar type of structure consisting of several flattened curved cisternae was common. Small transitional vesicles were found to be associated with the convex lamellar surface (forming face), while presumed secretory vacuoles were associated with the concave lamellar surface (maturing face). Golgi apparati function in concentrating and packaging products synthesized by the cellular endoplasmic reticulum. Secretory activity is normally associated with well developed golgi apparati.

Mitochondria were located throughout the cells. However, a difference in the number, size and shape of mitochondria was observed with respect to location within a cell. Mitochondria located apically were relatively sparse (Plate 12). In addition, apical mitochondria tended to be elongated and sinuous. Basally located mitochondria, on the other hand, were congregated into compact aggregates, and tended to be short and round.

Nuclei of sperm gland cells were generally situated

basally within a cell (Plate 13), and were either rounded or ovoid in shape. Most frequently nuclear membranes had a smooth contour, but on occasion the nuclear membranes were irregular. Nuclear material was predominantly euchromatin, which is normally seen during active genetic transcription. Heterochromatin appeared in the form of a thin ring adjacent to the nuclear membrane and as small isolated patches within the nucleus.

Usually only one, but sometimes two nucleoli were observed within a nucleus (Plate 13, 14). When a nucleolus was centrally sectioned, the pars amorpha, in the form of light staining rounded mass, was discernable. The darker staining nucleolonema was also observed around the pars amorpha (Plate 14). The presence of distinct nucleoli indicated active nuclear transcription which was consistent with the relatively high ratio of euchromatin to heterochromatin.

Aggregates of lysosomes were observed apically within sperm gland cells (Plate 15). Primary, predigestive lysosomes had a homogeneous electron dense appearance. Secondary lysosomes actively engaged in digestion were relatively heterogeneous in appearance. The latter were electron dense, but did contain electron lucent spots. Residual bodies were also present which were primarily electron lucent, but they did contain some debris (Plate

15).

The residual bodies frequently contained microbodies (Plate 16). The lysosomes did not appear to be engaged in the breakdown of extraneous material, since phagocytosis of extracellular substance was not observed. Therefore, it is probable that the lysosomes were involved in autophagic internal cell maintenance.

Large electron lucent droplets were a dominant feature associated with the UV sperm gland cells (Plate 17). Such droplets were located predominantly supranuclearly, but occasionally they were located infranuclearly. The droplets were generally irregular in shape, contained a mixture of filamentous and flocculent appearing material, and did not appear to be enveloped by a membrane. The electron lucent droplets tended to be of considerable size and were in such close proximity to the nucleus that they frequently caused a depression in the nuclear membrane. Apically, the droplets were in very close proximity to aggregated golgi apparati. Based on appearance and location, these droplets were assumed to contain lipoidal material.

On occasion, no large electron lucent droplets could be detected in any of the cells of a particular section (Plate 18). When this occurred, smaller apically displaced electron lucent droplets were found. In addition, in such cells, nuclei were observed to be located more basally

within the cell, in very close proximity to basal laminae.

Microvilli were frequently observed to be engaged in an apocrine type of secretion into the glandular lumen (Plate 19). Fixation artifact was not ruled out as a very logical explanation for the observed microvillar blebing. Nevertheless, when such phenomena was observed, the substance being extruded was typically homogeneous in nature and of a moderate electron density. Debri, which was presumed to be a form of secretory product, was found within glandular lumina on an occasional basis (Plate 19). Such debri tended to be discrete, having the appearance of empty vesicles or amorphous flocculent material.

Spermatozoa present in the glandular lumena, for the most part, were not in contact with microvilli of sperm gland cells (Plates 20, 21 and 22). Only when a glandular lumen was packed with spermatozoa did contact occur between the tips of microvilli and spermatozoa (Plate 20). In no case were spermatozoa observed to be deeply embedded amongst microvilli or to make contact with the underlying membranes of lumenal cells. Spermatozoa appeared to be stratified within the glands, since cross sections of sperm glands tended to bisect spermatozoa at comparable levels.

No particular type of sperm-to-sperm relationship predominated. When relatively few spermatozoa were present in a gland, they were usually separated (Plate 21).

Spermatozoa were also observed to make intimate contact with each other, appearing to be agglutinated in an interrupted (i.e., periodic) fashion (Plates 20, 21 and 22). Intimate contact between cell membranes did not occur over long distances (Plate 20). Furthermore, the interrupted agglutination was found to be head-to-head, head-to-tail or tail-to-tail. While the elongated nature of avian spermatozoa may have dictated the form of agglutination, the effects of a presumed relatively slow intraglandular fixation were not established in this study.

Generally, spermatozoa present in a glandular lumen displayed a high degree of structural integrety, an integrety characteristic of viable spermatozoa. Only rarely were spermatozoa observed to be in a degenerative state within a sperm gland lumen (Plate 23). Spermatozoal degeneration was characterized by the presence of dispersed chromatin, ruptured membranes and residual filaments. Phagocytosis of spermatozoa by glandular cells was not observed.

Lymphocytes were frequently observed to be in close association with sperm gland cells. Such lymphocytes were generally in the connective tissue surrounding the sperm glands (Plate 24). On other occasions the lymphocytes appeared to infiltrate sperm glands by migrating across the basal lamina and down between lateral walls of adjacent

sperm gland cells (Plate 25). Lymphocytic migration, however, was restricted to the basal aspects of the sperm gland cells. Migration apically to the junctional complex of a cell or into a glandular lumen was not observed (Plates 3 and 4).

The lymphocytes were characterized by having a high nucleus to cytoplasm ratio, with very little cytoplasm being apparent. Nuclei contained a large amount of dark staining heterochromatin which usually was peripherally located.

Plasma cells were found in the connective tissue surrounding sperm glands (Plate 26). As with the lymphocytes, plasma cells also had a high nucleus to cytoplasm ratio, with very little cytoplasm being apparent. The cytoplasmic space of the plasma cells contained primarily rough endoplasmic reticulum which was arranged in an orderly lamellar form. The nucleus contained a large amount of dark staining heterochromatin which was distributed along the inside of the nuclear membrane.

The ultrastructure of distal cells of the UV sperm storage glands did not appear to change over the course of the egg production season. Tissue taken from hens at the beginning (Plates 27, 28 and 29) and at the end of the laying season (Plates 30 and 31) possessed all of the characteristics found in a typical sperm gland from a fertile hen collected at mid-season. Cell membranes

displayed the same modifications, i.e. a smooth basal lamina, long microvilli in close aposition, lateral wall interdigitation with junctional complexes and desmosomes. Furthermore, the characteristic types and displacement of cellular organelles observed in a typical mid-season sperm gland were observed in glands at the beginning and end of the laying season. Discrete bits of rough endoplasmic reticulum were found throughout the cell. Aggregates of smooth endoplasmic reticulum were observed apically. Concentrations of small round mitochondria occurred basally while limited numbers of elongated mitochondria were found throughout the apical cytoplasm. Nuclei contained primarily euchromatin and had distinct nucleoli. Large lipid droplets were found in a very close association with nuclei and aggregates of well developed golgi apparati. Apically there was an abundance of primary and secondary lysosomes along with an abundance of residual bodies. Spermatozoa were found with regularity in the sperm glands of both groups of birds. Finally, lymphocytic infiltration of sperm gland cells was found to occur during all stages of the egg production season.

Sperm glands taken from infertile hens (Plates 32, 33, 34) were ultrastructurally indistinct from those of fertile hens. No characteristic changes were detected. Although it was difficult to isolate a sperm-containing gland from an

infertile hen, when isolated, the resident spermatozoa were intact and normal in appearance. Spermatozoal-glandular interrelationships also appeared regular. The extent of lymphocytic infiltration into glands from infertile hens was similar to that seen in glands from typical high fertility hens.

GENERAL DISCUSSION

In this study the ultrastructure of cells comprising the distal portion of the uterovaginal sperm storage glands in the oviduct of the turkey hen was examined. These cells are thought to be of vital importance to the overall fertility of the hen; it is in this location that spermatozoa are stored for the duration of the fertile period. The results of this study with turkeys were consistent with earlier ultrastructural studies of sperm glands of chickens (Van Krey et al., 1967; Schindler et al., 1967; Burke et al., 1972 and Tingari and Lake, 1973) and of quail (Friess et al., 1978).

It is curious that sperm gland cells which are presumed to be integrally involved in the maintenance of sperm survival appear to exhibit only a moderate degree of secretory activity. The fact that the rough endoplasmic reticulum was not extensively developed suggests that protein synthesis for subsequent secretion was minimal. The lack of a protein secretory product was consistent with the histochemical analysis of the chicken sperm gland cells conducted by Gilbert et al. (1968) in which only low levels of protein were detected.

The abundance of assumed free ribosomes in the cytoplasm is suggestive of a high intracellular protein demand by the sperm storage glands. It is possible that the

free ribosomes were engaged in the synthesis of enzymes necessary for the large number of lysosomes observed within the cells. In view of the extensive lumenal and lateral wall cell surfaces, it is also possible that the free ribosomes were involved in the synthesis of cell membrane receptor(s) and transfer proteins.

In contrast to the rough endoplasmic reticulum, the tubular smooth endoplasmic reticulum was relatively well developed. The apical cytoplasm adjacent to the terminal webs was primarily occupied by the tubular system of smooth endoplasmic reticulum. The ample distribution of smooth endoplasmic reticulum connotes more active levels of carbohydrate and lipid metabolism.

Past histochemical studies provided conflicting evidence regarding the presence of glycogen in the cytoplasm of sperm gland cells. Fujii (1963) was unable to detect polysaccharides in the UV glands of chickens, while Gilbert et al. (1968) not only detected polysaccharides, but identified them as glycogen. In studies with UV glands from quail, Renden (1981) was unable to establish the presence of polysaccharides while Friess et al. (1978) was unable to detect glycogen directly. In the current study, it is probable that some of the particles observed distributed throughout the cytoplasm were individual individual β glycogen particles. Based on our ultrastructural studies,

it seems likely that in some of the earlier studies the concentration of glycogen may have been too low, and the individual particles too dispersed to be readily detected histochemically. The presence of glycogen in small concentrations and the even dispersion throughout the cytoplasm suggests that the glycogen present served as a stored source of energy for normal cell functions, and probably was not secreted into the glandular lumen. If glycogen were to be secreted, it would probably have been aggregated apically near the lumenal border, and this was not seen to occur. Ultrastructurally, indications were that the levels of protein and carbohydrate metabolism were so low as to be involved only in cellular maintenance.

The only metabolic activity that UV glandular cells appeared to be engaged in at a relatively elevated level was the production and aggregation of lipids. The latter were readily apparent as large perinuclear droplets. Histochemical studies have confirmed the presence of lipid in the sperm glands of chickens (Fujii 1963 and Gilbert et al., 1968) and quail (Renden, 1981).

Organelles within the UV glandular cells seemed to be organized to facilitate an efficient production of lipids. Based on the ample apical distribution of smooth endoplasmic reticulum, lipid production probably occurred in the apical portions of the sperm gland cells. Smooth endoplasmic

reticulum is often thought to be involved in lipid synthesis (Fawcett, 1979). It was also in the apical portion of a cell that the elongate sinuous mitochondria were found. Presumably these mitochondria provided the energy necessary for lipid synthesis and transport.

Following synthesis, the lipid appeared to be concentrated and aggregated into droplets by the golgi apparati. In the absence of preformed lipid droplets, the golgi apparati were most often circularly arranged and discharged electron lucent vacuoles centrally (Plate 11). Presumably these vacuoles contained lipid which coalesced to form large lipid droplets. Even when large lipid vacuoles were present, golgi apparati were often seen on the periphery (Plate 17).

The production of lipid did not appear to be excessive. Even though lipid droplets were occasionally of sufficient size to cause an indentation of the nuclear membrane, they did not cause a noticeable basal displacement of nuclei. A wide margin generally existed between nuclei and basal laminae. In addition, shortening of the microvilli or bulging of the lumenal border were not observed to occur in cells of sperm storage glands. In contrast, Aitken and Johnston (1963) observed that the mucous secreting cells of the infundibulum and the albumen secreting cells of the magnum were so active, that the accumulated secretory

vacuoles crowded the nuclei against the basal cell membranes, and caused lumenal cell surfaces to bulge to such an extent that microvilli appeared shortened and sparse.

Following the aggregation of lipids into large droplets, it is unclear what happened to them. Fujii (1963) reported that in the chicken lipids become dispersed apically following the passage of an egg at oviposition. Conversely, Renden (1981) reported that in the quail, lipid droplets become aggregated following oviposition. During the course of our study, in only one hen were lipid droplets observed to be dispersed on a consistent basis (Plate 18). In tissue sections from this hen, cells did not contain a large supranuclear lipid droplet. Instead, small droplets were distributed throughout the apical cytoplasm. It is interesting to note that the nuclei present in cells from this hen were situated more basally, in close juxtaposition to the basal laminae. Also, the cells themselves seemed to have become more shortened, taking on a pyramidal shape.

In other respects, this hen was typical in that she was fertile and had been laying for three months. The hen in question did have a hard shelled egg in the uterus at the time of tissue collection, but other hens utilized in the study also had a hard shelled egg in the uterus at the time of tissue collection, and they did not display the lipid dispersion phenomenon. It is possible that during tissue

collection from the hen under consideration that the egg had exerted enough pressure in the uterovaginal area to simulate an oviposition. If true, it would appear that the passage of an egg results in the dispersal of lipid droplets. Nevertheless, the effects of oviposition on intracellular lipid distribution were not formally established in our study.

It is unclear whether or not lipids were secreted into the glandular lumen. Microvilli were observed to be engaged in a secretory type of activity very infrequently. When observed, it consisted of an apocrine type blebing of microvillar tips, or else a more pronouced swelling of a whole microvillus. On rare occasions when the more wholistic swelling of microvillar tips occurred, electron lucent areas were observed which may have been lipid droplets (Plate 19). Both of the secretory type phenomena described could have been artifacts, the results of differential fixation or could have been due to osmotic gradients. In any event, microvilli did not normally contain lipid droplets. They appeared to be of the same ground substance that was observed in the cellular cytoplasm. Thus, if lipids were secreted routinely by the UV sperm gland cells, the secretory products passed from the cell in a very discrete manner.

The presence of such well developed and abundant

microvilli on UV sperm gland cells was puzzling. Microvilli are usually thought to be an adaptation to increase absorptive surface area. Microvilli have been observed on secretory cells such as infundibular mucous cells and magnal albumen secreting cells; however, microvilli on such secretory cells are usually relatively short and widely separated.

In the past it has been generally assumed that cells of the sperm storage glands secreted a metabolizable substrate(s) into glandular lumina which enabled resident spermatozoa to survive in vivo for prolonged periods of time. However, the ultrastructure of turkey sperm gland cells was not consistent with the concept of secretion as a primary role. Alternatively, it seems quite likely that sperm gland cells may be more actively engaged in absorption of substances from the lumen. It is not inconceivable that long-term sperm storage may be achieved by removing substrates which enhance spermatozoal activity. This concept is consistent with what is known about spermatozoa. Spermatozoa do not store large amounts of energy as substrate, but obtain it from the surrounding medium (Wishart, 1982). Thus, it is plausible that by removing an exogenous energy substrate(s), spermatozoa could be rendered quiescent.

If true, a question remains regarding the fate of the

absorbed energy substrates. Within cells of the sperm storage glands the greatest accumulations of smooth endoplasmic reticulum (Plate 10) were located just beyond the terminal webs of microvilli. The close positioning of the smooth endoplasmic reticulum to the microvilli would allow for easy acquisition by the endoplasmic reticulum of both discretely absorbed substances and/or substances taken in by micropinocytosis. Furthermore, these substances could then very readily be converted to lipid if they were not already lipidoidal. Following the conversion, the lipid was probably packaged into large supranuclear droplets as described earlier. The final fate of the lipid appeared to be injestion and assimilation by the abundant supply of lysosomes located apically. Secondary lysosomes were often observed to contain round electron lucent areas (Plate 15) assumed to be lipid undergoing digestion.

As pointed out earlier, aggregates of mitochondria were observed basally in close juxtaposition to the basal cell membrane. The close positioning of mitochondria to the membrane implies that they were involved in some type of energy dependent transport across the cell membrane. It was not possible to ascertain whether such a system was primarily involved in the transport of materials into or out of the cell. However, it is interesting to speculate that such a transport system, if it in fact, did exist, might

have functioned in the final removal from the cell of discrete substances degraded by lysosomal action. The relatively short rounded morphology of basal mitochondria indicates that they may also play an anticedent role in replacing worn apical mitochondria.

It is currently considered unlikely that cells of the sperm storage glands are involved in the uptake of dead sperm cells. For absorption of dead spermatozoa to occur via the microvilli, the sperm cells would first have to undergo considerable degradation. Only rarely were dead spermatozoa even observed in the lumen of a sperm gland. Typically, the sperm gland lumena were observed to be clear and free of debris. Direct phagocytosis of spermatozoa by cells of the sperm storage glands was never found to occur. In fact, spermatozoa were rarely observed to make contact with microvilli. Only when a lumen was packed with spermatozoa did contact with microvilli occur, and then it was just the microvillar tips that touched the spermatozoa. Never were spermatozoa observed to be embedded between microvilli as was observed in the infundibular sperm nests of the chicken (Van Krey et al., 1967; Schindler et al., 1967).

The fate of dead intraglandular spermatozoa is still vague. It was noted by Koyanagi and Nishiyama (1981b) that cells of the infundibular sperm nests in the chicken

facilitated-sperm cell degradation by secreting electron dense substances into the lumen. Such a phenomenon was not found to occur in the uterovaginal sperm glands of the turkey. As stated earlier, the cells lining the sperm glands in turkeys did not appear to be secretory in nature. In addition, no electron dense secretory granules, such as those reported by Koyanagi and Nishiyama (1981a), were observed in the cytoplasm of turkey UV sperm glands.

It is interesting to note that when spermatozoa were present in a glandular lumen, there was a tendency for adjacent spermatozoa to congregate or agglutinate. In cross sections it appeared that sperm cell membranes were adhering to each other, suggestive of agglutination. Spermatozoal agglutination was observed so commonly that it appeared to be conducive to, if not necessary for, prolonged sperm storage. The observed agglutination, along with the limited incidence of dead sperm cells in glandular lumena, and the absence of electron dense secretory granules in sperm gland cells, all suggested that once the sperm cells lost cellular integrity (Senger and Saache, 1976) they disengaged from an agglutinated group and drifted out of the sperm gland and into the oviduct lumen as suggested by (Van Krey et al., 1981).

The effect that lymphocytes have on long term sperm storage is difficult to rationalize, for lymphocytes have

the potential to directly influence the functions of sperm gland cells. In the current study, lymphocytes were observed to infiltrate sperm glands by migrating across the basal lamina and between the lateral walls of adjacent cells. Adjacent cell wall junctions are thought to to serve as a medium across which intercellular communication occurs (Bloom and Fawcett, 1975). Junctions between cells lining sperm storage glands have undergone adaptation in the form of lateral wall interdigitation to increase the junctional surface area and thereby facilitate intercellular exchange. By situating themselves within cellular junctions, lymphocytes have direct access to any exchange of chemicals which might be occurring between adjacent cells.

It is also possible that lymphocytes play a role in sperm-antigen recognition, although it is unlikely that the lymphocytes were directly involved in antigen recognition. They were not found to migrate beyond the level of the nucleus. Lymphocytes were always observed to be situated basally, in close juxtaposition to the basal cell membrane. It is possible that lymphocytes could be exposed to antigens present in the glandular lumen, if such antigens migrated to the lymphocytes. However, the antigens would have had to travel from the lumen along the gap between adjacent cells to the basally located lymphocytes. Passage of antigens from lumen to the intercellular space would have involved

movement through the junctional complexes. Movement through junctional complexes is unlikely, however, since junctional complexes are known to close the gap between adjacent cells at the level of the zona occludens. In the study under consideration, gaps were not observed at the junctional complexes (Plate 7). Friess (1978) observed that when quail sperm gland tissue was fixed along with the tracers lanthanum and ruthenium red, neither tracer entered the junctional complex. This finding indicates that the junctional complex offers a complete block between the lumen and the intercellular space.

A role by lymphocytes in the recognition of sperm-antigens would also require the presence of sperm-antigens. The presence of antigens in the lumen on a regular basis is unlikely since spermatozoal degeneration within a lumen was seen to occur only rarely. It also seems likely that if lymphocytes were involved in sperm-antigen recognition, they would have migrated further along the gap between adjacent cells assuming a more apical posture. Apical migration would bring the lymphocytes closer to the sperm gland lumen thereby facilitating sperm-antigen recognition.

It would seem that lymphocytes were primarily involved in monitoring or controlling cell function by influencing the chemical exchange which may be occurring between adjacent cells. However, some type of antigen recognition,

be it spermatozoal or some other antigen, seemed to be occurring. This was suggested by the common appearance of plasma cells in the connective tissue surrounding sperm glands.

The role of the immune system in fertility is difficult to estimate. The immune system has the potential to influence the sperm storage phenomena in view of the intimate association between lymphocytes and cells of the sperm storage glands. In addition, it is possible that an alloimmune reaction could be mounted directly against spermatozoa. The past work of Wentworth and Mellen (1964), Burke et al. (1971), Burke and Rieser (1972) and Burke and Yu (1979) all suggest that in the chicken some type of humoral immune reaction against spermatozoa does occur, and that such an immune reaction may adversely affect fertility.

During the course of this study there was no apparent evidence that the immune system caused infertility. The level of immunological activity in the UV gland area was the same in fertile and infertile hens. No proliferation of immunological cells as occurs during an immune reaction was observed in infertile hens. There was no increase in the number of lymphocytes infiltrated between sperm gland cells or in the surrounding connective tissue of infertile hens. Likewise, the number of plasma cells in infertile hens did not appear to be greater than in fertile hens. Spermatozoal

degradation by macrophages in the region of the infundibular sperm nests of the chicken (Koyanagi and Nishiyama, 1981b) was not observed to occur in the uterovaginal sperm glands of the turkey. Thus, any influence the immune system of turkey hens has on fertility was subtle. Whether or not lymphocytes caused infertility by exerting a detrimental influence on sperm storage via the intercellular junctional system is at present unknown.

The results of the current study indicate that the amount of time a hen had been in egg production did not affect sperm gland morphology. No detectable differences existed in the ultrastructure of uterovaginal sperm glands collected from the beginning of the egg laying season to the end of the laying season. Sperm glands collected at the onset of egg production appeared to be fully developed and were capable of sperm storage. Hens inseminated immediately prior to the initiation of egg production, showed numerous sperm-containing glands. Apparently glands attained full development before the hen started to lay.

Sperm glands collected at the end of the laying season were also comprised of cells with a normal healthy appearance. No noticeable degeneration of sperm gland cells had occurred by the end of the laying season. Sperm glands appeared to remain functional for at least as long as the hen was laying, and possibly longer. Fujii and Tamura

(1963) reported that even though hens had stopped laying and the oviduct had regressed, spermatozoa were still capable of entering the UV glands of domestic fowl. Interestingly, they found that these spermatozoa did not agglutinate once inside the glands.

The relationship between UV glands and fertility in the turkey hen remains largely an enigma. Even though the sperm glands from infertile hens were generally devoid of sperm, no detectable changes in sperm gland morphology were observed. The ultrastructure of sperm gland cells from infertile hens suggested that the glands were functioning normally at the time of tissue collection. The cells retained an appearance characteristic of sperm gland cells from highly fertile hens.

Not only did the UV glands from infertile turkey hens appear normal, but no extraneous aberrant matter was observed to be immediately associated with the UV glands. Ball et al. (1969) suggested that the infertility syndrome, at least, might be caused by a transmissible disease organism. However, they were unable to isolate any responsible organisms. Consistent with these earlier results, no readily apparent disease organisms were detected in sperm glands during the course of our study. Furthermore, detrimental effects which should presumably have been manifest following a glandular infection by a

disease organism, were not observed. It might also be expected that following infection of the sperm glands with a disease organism, an immune reaction would be mounted. No such reaction was observed at the level of the sperm glands. Thus, it would seem likely that seasonal declines in fertility are not due to an infection by a disease organism.

One very consistent observation made in this study was that the lumina of UV glands from infertile hens were essentially devoid of spermatozoa. It was very difficult to isolate UV glands containing spermatozoa from infertile turkey hens. This observation agrees with earlier histological observations (Van Krey et al., 1967; Yu and Burke, 1979). There is no evident explanation for the lack of sperm storage, for the UV glands appeared physically capable of receiving spermatozoa, and the glandular lumena were clear. As indicated, there was an absence of possible interfacing factors such as lumenal debris, microorganisms or cells of the immune system.

It is conceivable that spermatozoa never reached the UV sperm glands. A hostile environment at the level of the vagina may have accounted for the lack of spermatozoa in the UV glands. Following insemination of hens which were out of egg production, Fujii and Tamura (1963) found that many spermatozoa became embedded in the thick mucus of the vagina and underwent degenerative changes.

Although not readily apparent, the possibility remains that a change(s) has occurred at the level of the UV sperm storage glands. If, as has been postulated, the cells of sperm glands function to remove energy substrates from the environment, it is likely that a malfunction in the absorption process could render the glands incapable of retaining spermatozoa. If energy substrates were not removed from a glandular lumen, spermatozoa entering such a gland might exhibit levels of motility incompatible with long term storage. Because of excessive motility,the spermatozoa would only be retained temporarily. Once free of a gland, such sperm cells would become trapped and removed from the oviduct by developing eggs (Bobr et al., 1964b; Koyanagi and Nishiyama, 1981b).

A malfunction in glandular absorption would be difficult to detect immediately. If energy substrates were converted to lipid droplets as suggested, then only over time would a visible change in lipid droplets occur. Because the droplets would be digested by lysosomes on a continuing basis, and would not be renewed in the absence of absorption from a lumen, it is only after a prolonged period of infertility that detectable changes in lipid droplet density would occur. Unfortunately, tissues utilized in this study were collected at or near the onset of infertility, thereby making it impossible to determine if

there was, in fact, an absorptive malfunction.

In summary, it was found that the uterovaginal sperm storage gland cells of the turkey exhibit only a moderate level of cellular activity. They appear to be engaged primarily in absorption of lumenal contents, as opposed to secretion of cell products into the lumen. No morphological changes at the level of the UV glands were observed to occur over the course of an egg laying season. Furthermore, microbial infections were not detected. Aside from the fact that the UV glands of infertile hens rarely contained spermatozoa, such glands appeared normal in all respects.

SUMMARY AND CONCLUSIONS

The ultrastructure of the uterovaginal sperm storage glands of turkey hens was characterized. The ultrastructural morphology of these cells was monitored over an entire egg laying season. In addition, comparisons were made between glands from fertile and infertile hens.

Based on ultrastructural observations, it was determined that cells of the uterovaginal sperm storage glands exhibit only a moderate level of metabolic activity. The limited presence of intracellular secretory products, along with an infrequently observed cellular release of secretory material, suggested that the effect of glandular secretion on spermatozoal survival was minimal. The density and degree of development of the apical microvilli, however, suggested that a primary function of glandular cells may be absorption of lumenal contents.

The length of time that a hen has been producing eggs did not influence the ultrastructure of storage gland cells. The ultrastructural morphology of glands from hens collected immediately prior to commencement of egg production was similar to that of glands taken from hens that had completed an entire season of egg production. Thus, the uterovaginal sperm glands were fully developed when hens started to lay eggs and remained fully developed for as long as the hens continued to lay.

Aside from the observation that very few spermatozoa could be found in the uterovaginal sperm glands of infertile hens, no detectable ultrastructural differences were observed between glands from fertile and infertile hens. The uterovaginal sperm glands from infertile hens were observed to maintain the same degree of structural integrity and level of functionality as glands from fertile hens. Furthermore, there was no evidence of glandular microbial infections, nor was there evidence of an adverse immunological response at the level of the sperm glands.

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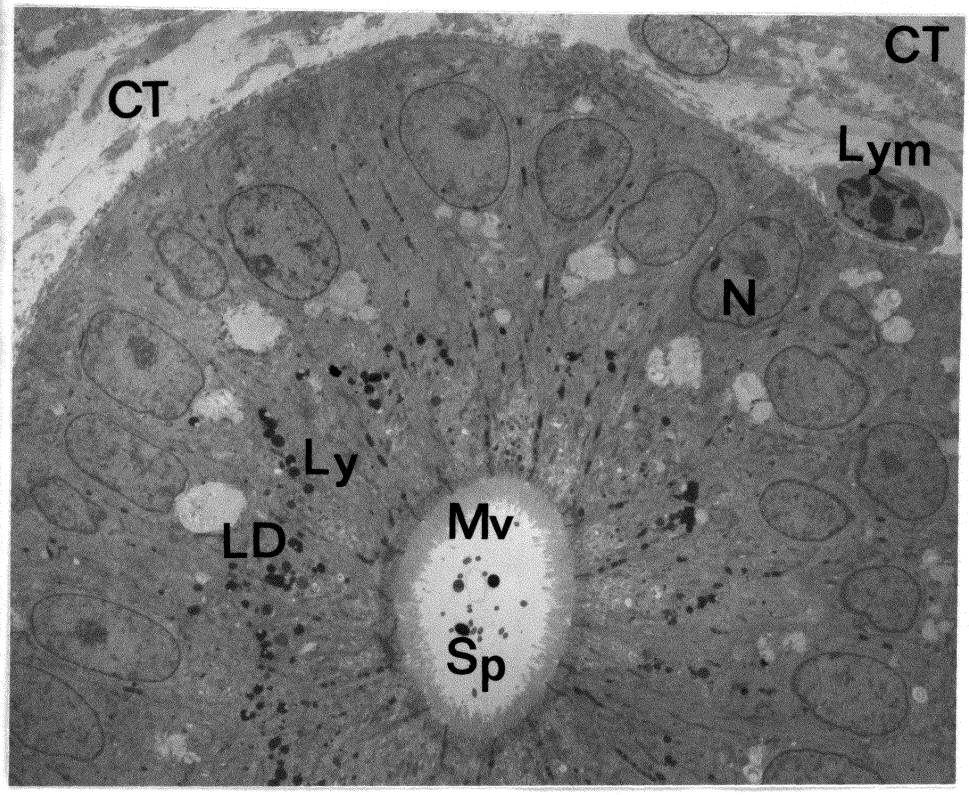
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PLATES

Plate 1
UV Sperm Gland - Fertile Hen

Fig. 1. Cross section of a typical UV sperm gland from a highly fertile hen in the middle of the egg laying season. Note the basally located nuclei (N) and large supranuclear lipid droplets (LD) A dense array of microvilli (Mv) project into the lumen of the gland. Spermatozoa (Sp) are located in the lumen. A lymphocyte (Lym) present in the connective tissue (CT) is seen immediately adjacent to a cell of the UV gland. (1300 X)

Fig. 2. Saggital section of cells comprising a typical UV gland with basally located nuclei (N) and lipid droplets (LD) located both supranuclearly and infranuclearly. Basally the cells are separated from connective tissue by a basal lamina (BL) while apically microvilli (Mv) project intothe lumen. Concentrations of smooth endoplasmic reticulum (SER) were observed apically. (2600 X)


CT
CT
Lym
N
Ly
LD
Mv
Sp

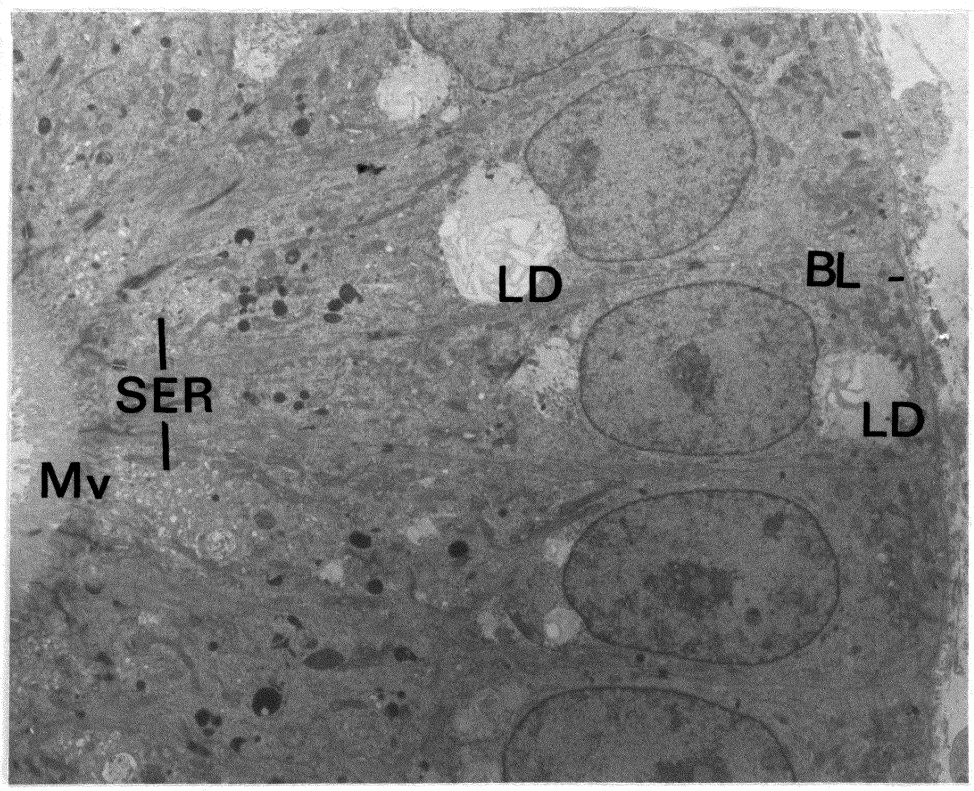
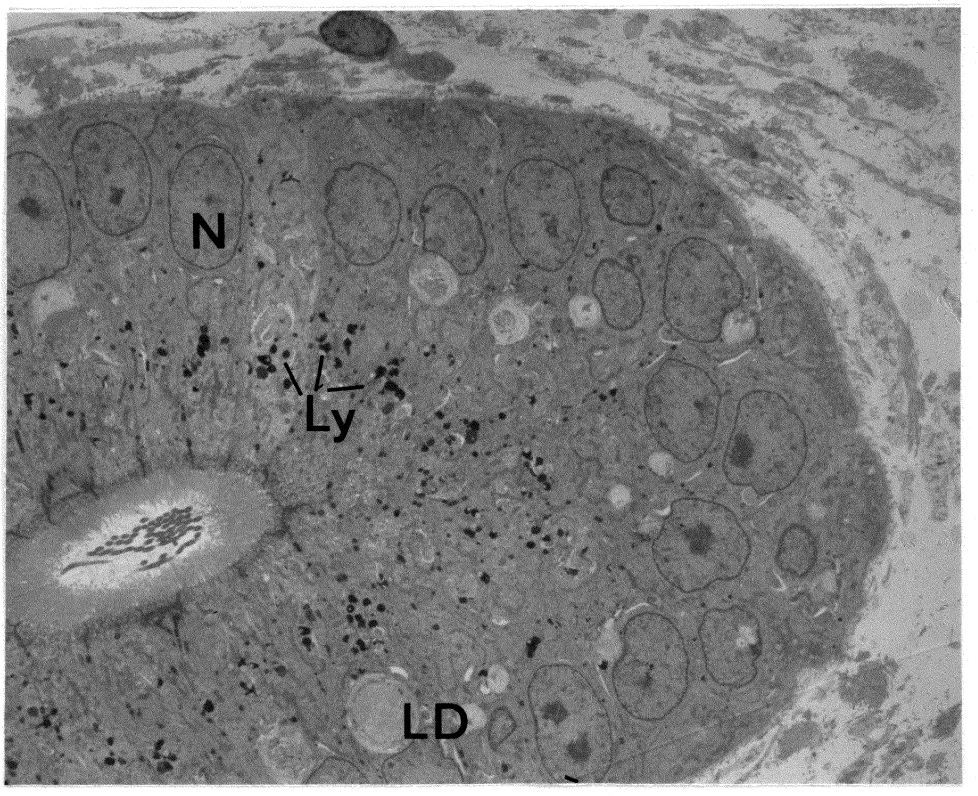

LD
BL
SER
LD
Mv

Plate 2
UV Sperm Gland - Fertile Hen

Fig. 1. Cross section of a sperm gland from a highly fertile hen collected at the middle of the egg laying season. Nuclei (N) are basally located and large supranuclear lipid droplets (LD) are present. Numerous lysosomes (Ly) are located apically. (1300 X)

Fig. 2. Saggital section of UV gland cells. Concentrations of mitochondria (Mi) were localized basally. A lymphocyte (Lym) is present in the surrounding connective tissue. Lysosomes (Ly) are locatedin the apical cytoplasm. (2600 X)


N
Ly
LD

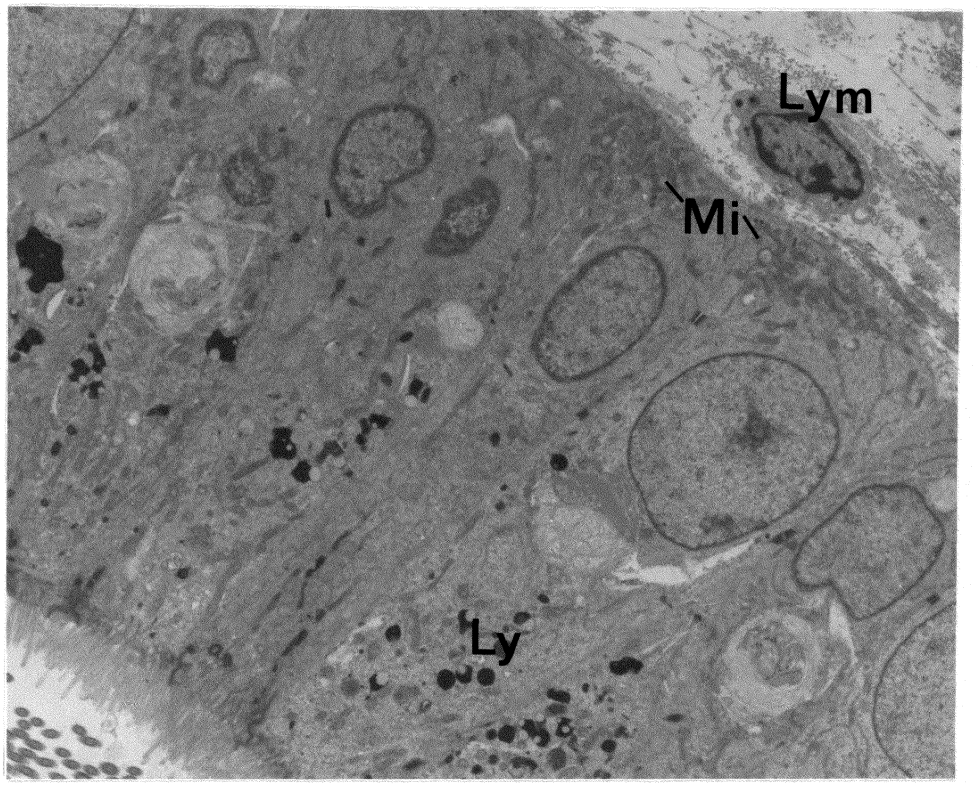
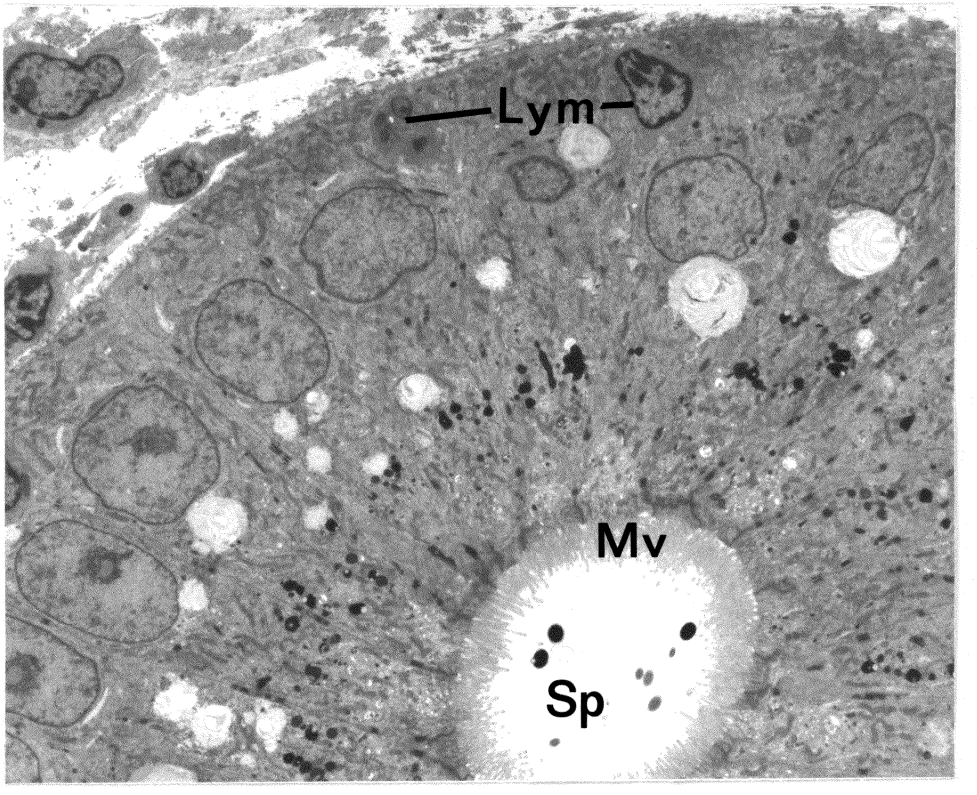

Lym
Mi
Ly

Plate 3
UV Sperm Gland - Fertile Hen

Fig. 1. Saggital section of a UV gland collected at mid-season. Note the dense array of microvilli (MV) protruding into the lumen which contains spermatozoa (Sp) cut in cross section. Lymphocytes (Lym) have infiltrated the gland. (1600 X)

Fig. 2. Saggital section of cells observed in Fig. 1. Aggregates of mitochondria (Mi) are observed basally. A lymphocyte (Lym) is seen basally between UV gland cells. (2600 X)


Lym
Mv
Sp

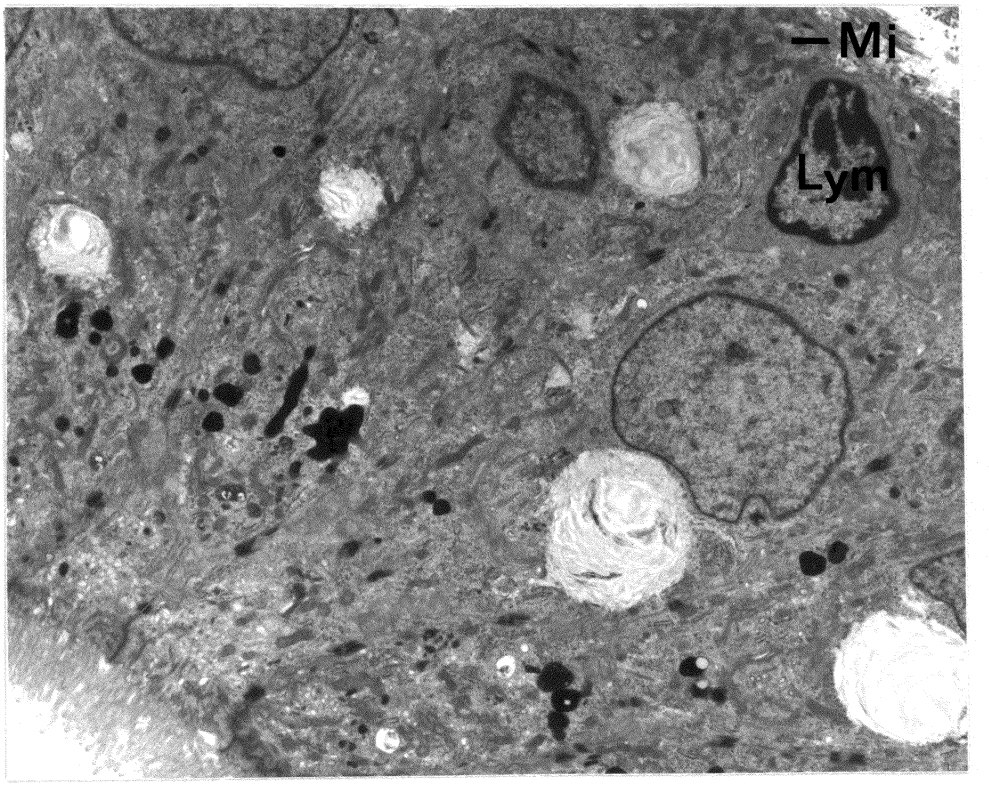

Mi
Lym

Plate 4
UV Sperm Gland - Fertile Hen

Oblique section of a UV gland taken from a highly fertile hen in the middle of the laying season. Very commonly, an abundance of spermatozoa (Sp) are contained within the lumena of such glands. Lymphocytes (Lym) are present among UV gland cells. (3200 X)

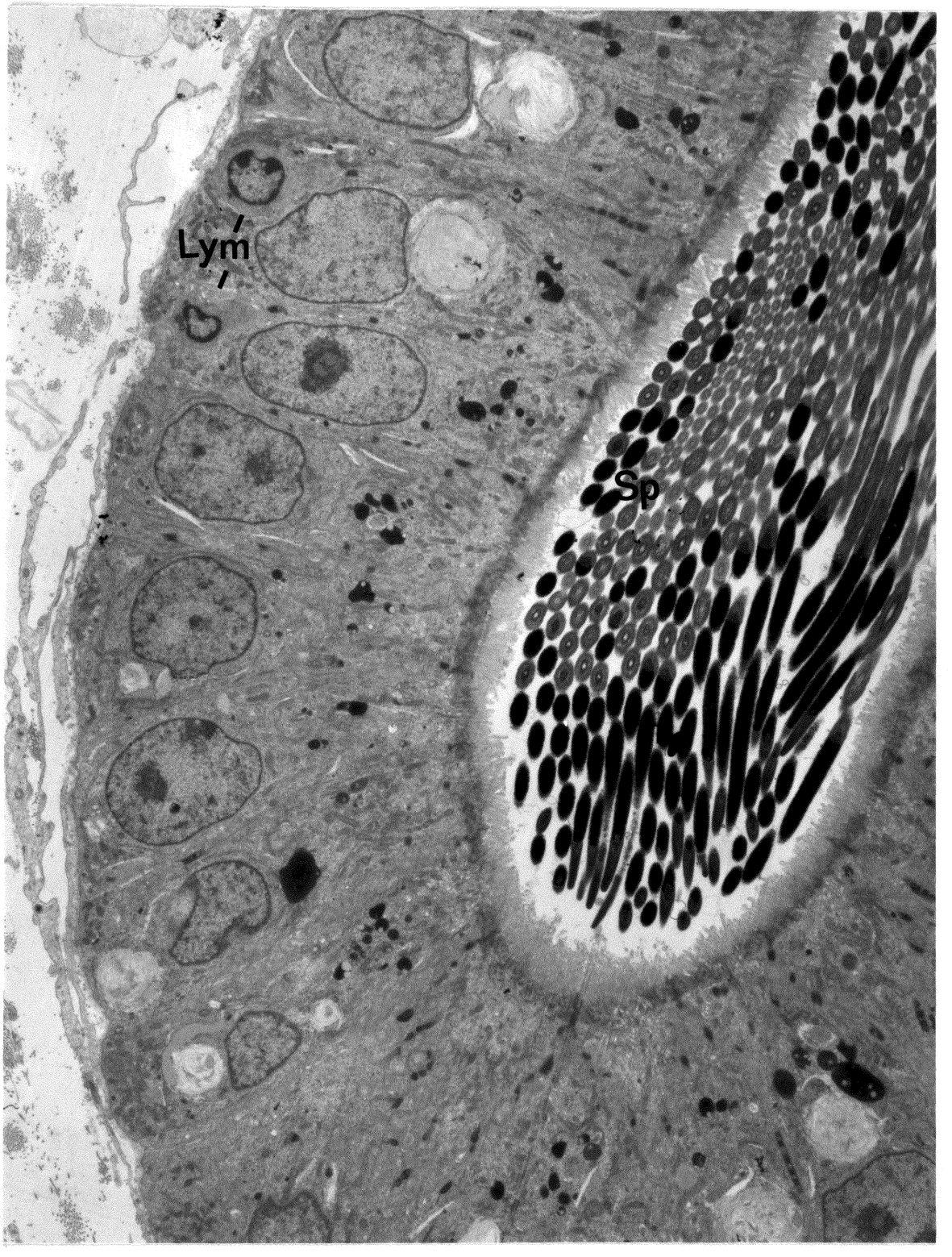

Lym
Sp

Plate 5
UV Sperm Gland - Fertile Hen

The basal border was composed of a basal cell membrane (Cm) and basal lamina (BL) separating UV gland cellsfrom connective tissue (CT). Numerous rounded mitochondria (Mi) are localized basally.

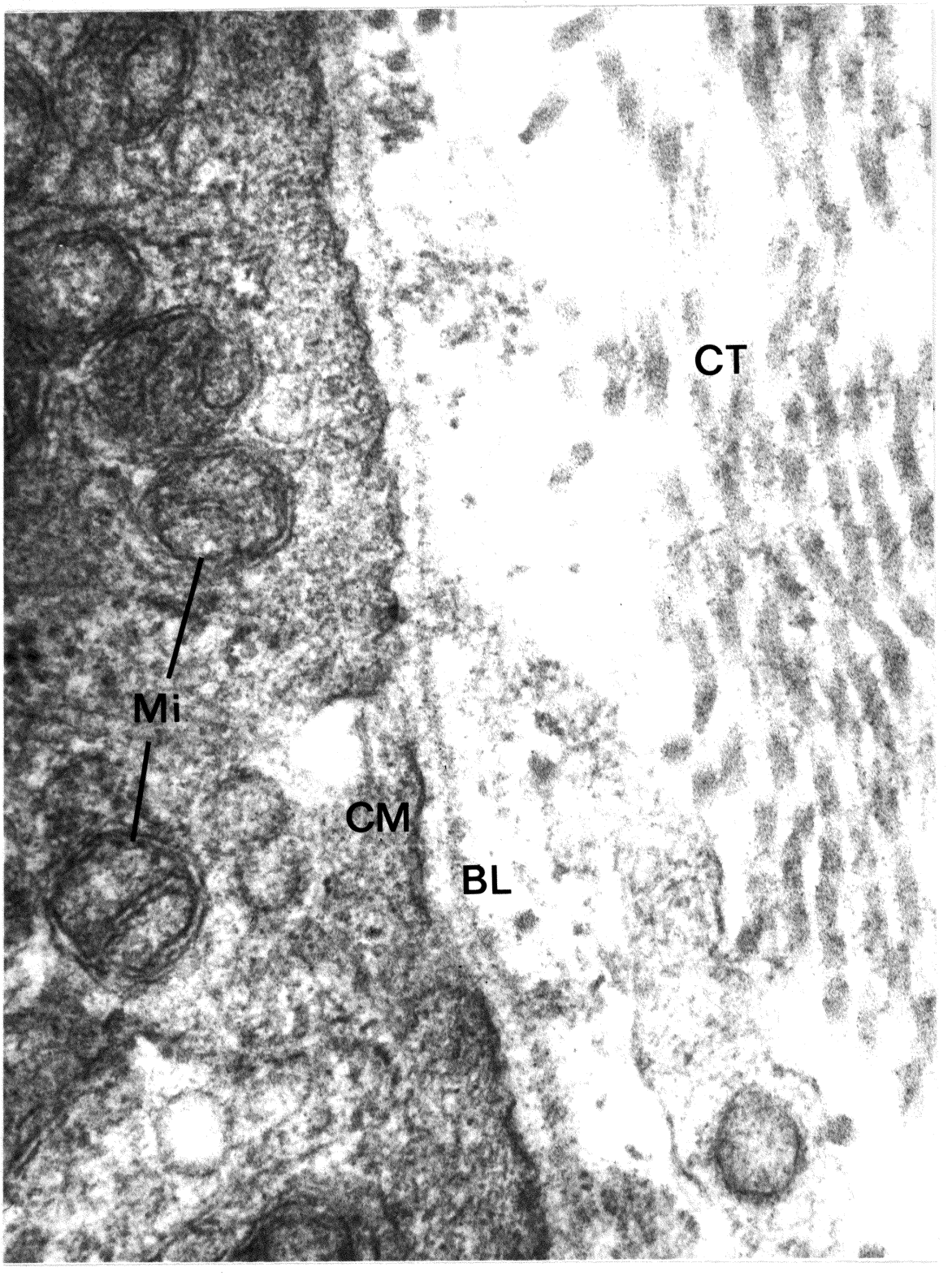
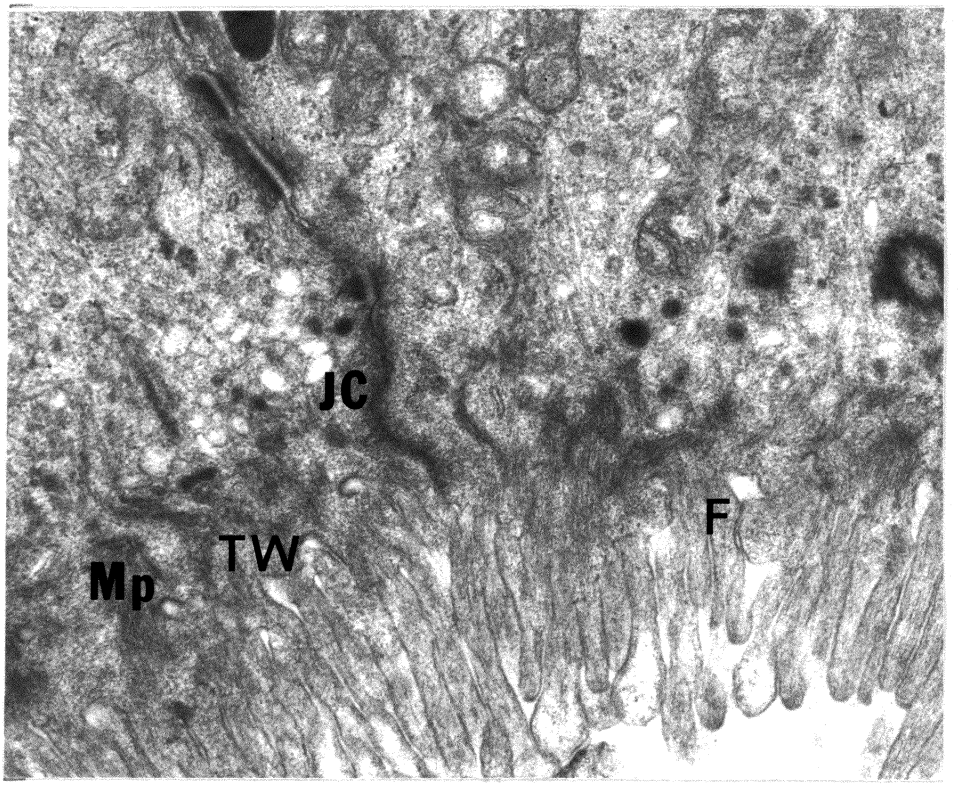

CT
Mi
CM
BL

Plate 6
UV Sperm Gland - Fertile Hen

Fig. 1. Microvilli (Mv) located on the lumenal border of UV gland cells. Filaments (F) originating in the terminal web (TW) extend into microvilli. A junctional complex (JC) is visible. Micropinocytotic (Mp) vesicles are seen at the base of microvilli. (16,000 X)

Fig. 2. Microvilli (MV) extending into a glandular lumen. Filaments (F) extending from the terminal web (TW) extend into microvilli. (26,000 X)


JC
F
TW
Mp

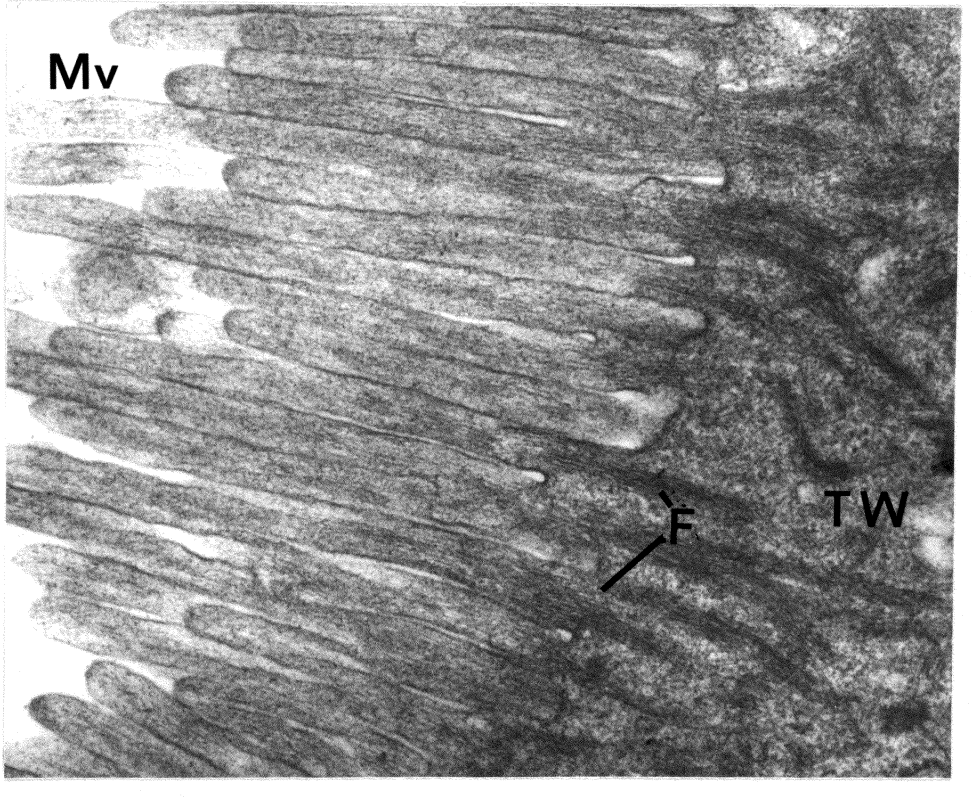
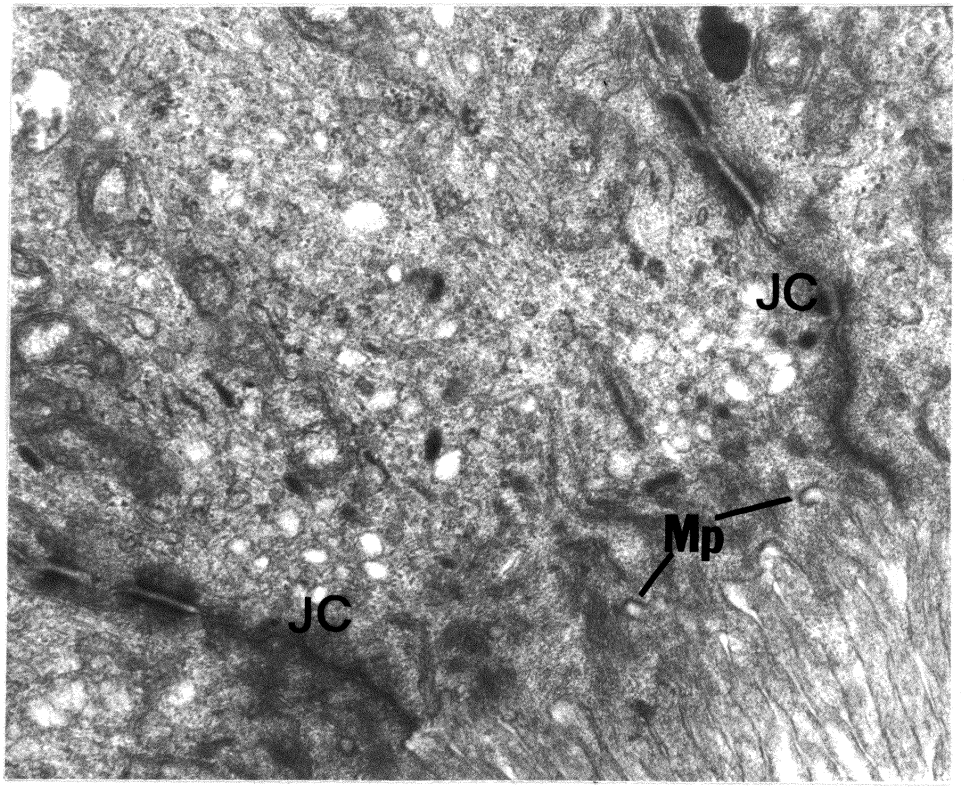

Mv
F
TW

Plate 7
UV Sperm Gland - Fertile

Fig. 1. Junctional complexes (JC) separating adjacent cells at the lumenal border. Micropinocytotic (Mp) vesicles are extending into the terminal web (TW). (16,000 X)

Fig. 2. Apical cytoplasm of UV gland cells with junctional complexes (JC) located at the lumenal border. Desmosomes (D) are observed in the more basal regions of cell junctions. Aggregates of smooth endoplasmic reticulum (SER) are found beneath the terminal web (TW). Cells are also engaged in micropinocytosis (Mp). (5,000 X)


JC
Mp
JC

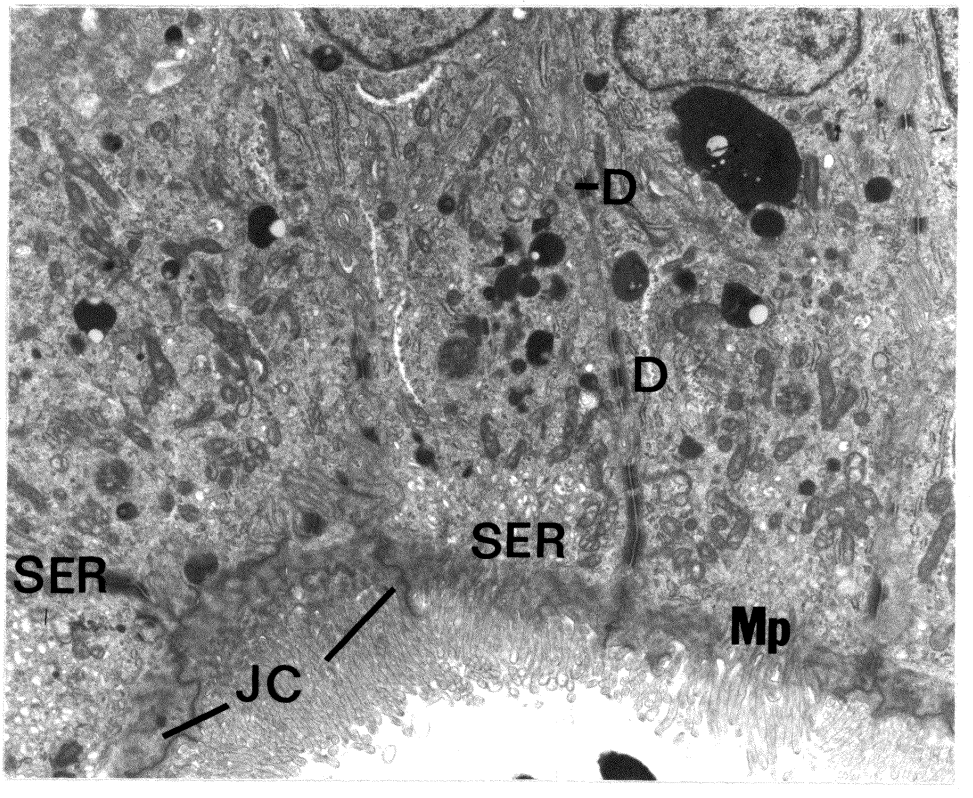
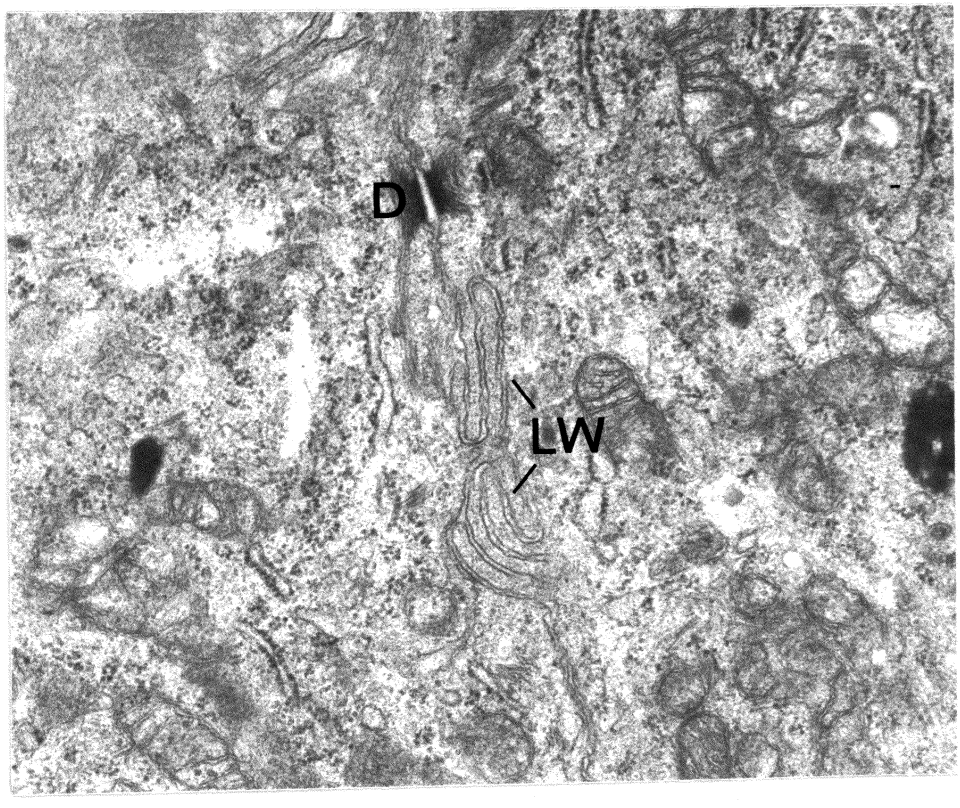

D
D
SER
SER
Mp
JC

Plate 8.
UV Sperm Gland - Fertile Hen

Fig. 1. Junction of lateral cell walls (LW) displaying moderate interdigitation. A desmosome (D) is seen apically. (16,000 X)

Fig. 2. Junction of lateral cell walls (LW) displaying extensive interdigitation. Desmosomes (D) are situated throughout the junction. (13,000 X)


D
LW

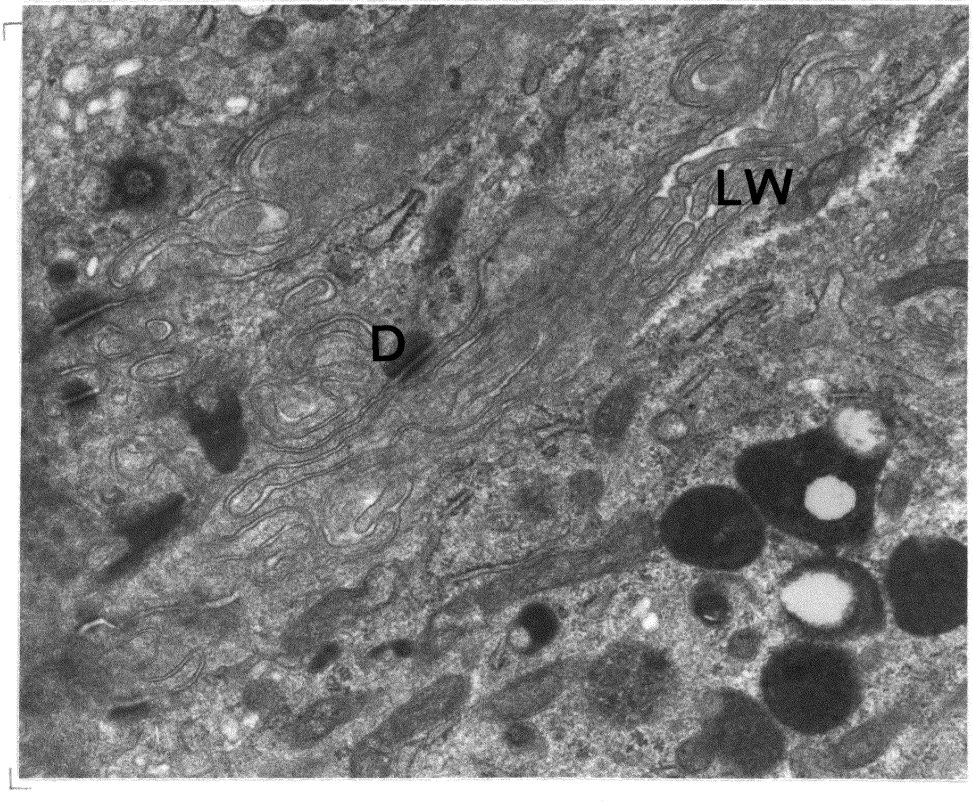

LW
D

Plate 9
UV Sperm Gland - Fertile Hen

Cytoplasm of cells with a lateral wall (LW) and associated desmosomes (D) of two adjacent cells. Scattered pieces of rough endoplasmic reticulum (RER) are seen throughout the cytoplasm as are free ribosomes (R). (52,000 X)

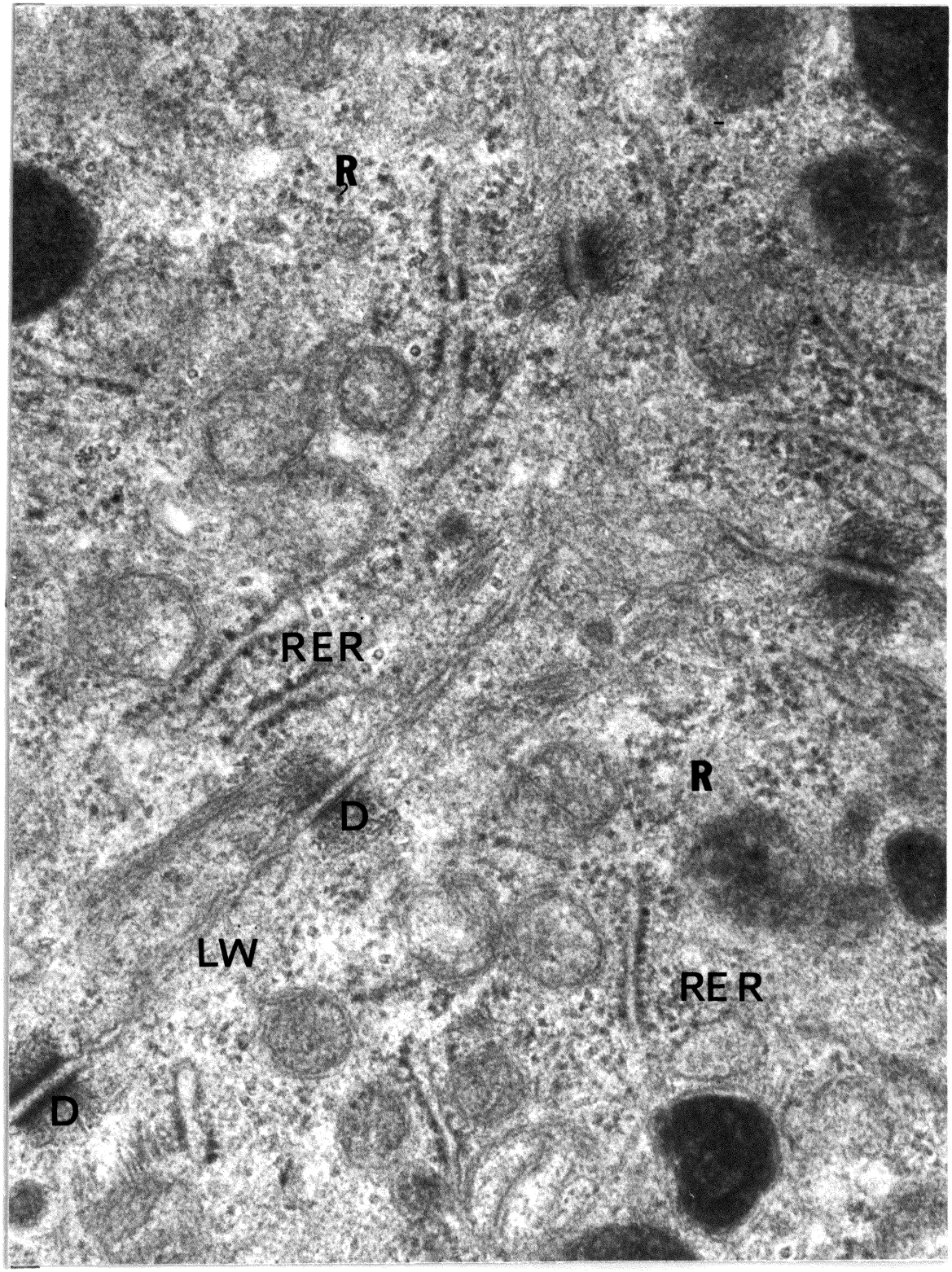

R
RER
D
R
LW
RER
D

Plate 10
UV Sperm Gland - Fertile Hen

Lumenal border of glandular cells with microvilli (MV) extending from the terminal web (TW). A tubular system of smooth endoplasmic reticulum (SER) lies beneath the termina web. A pair of centrioles (C) can also be seen beneath the terminal web.

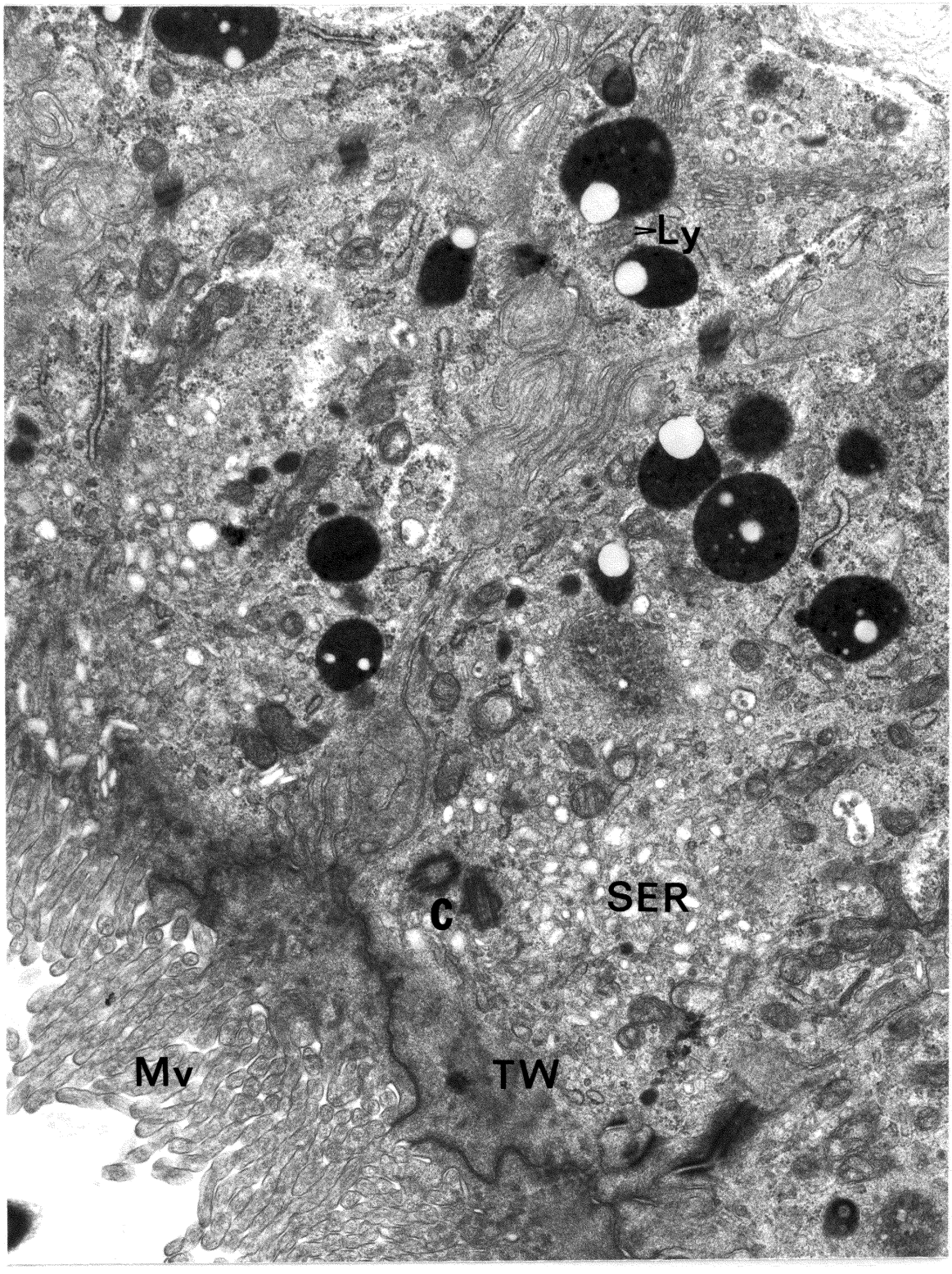

Ly
C
SER
Mv
TW

Plate 11
UV Sperm Gland - Fertile Hen

Golgi complex (Go) and associated vacuoles located perinuclearly. The surrounding cytoplasm contains scattered rough endoplasmic reticulum (RER) and free ribosomes (R). (32,000 X)

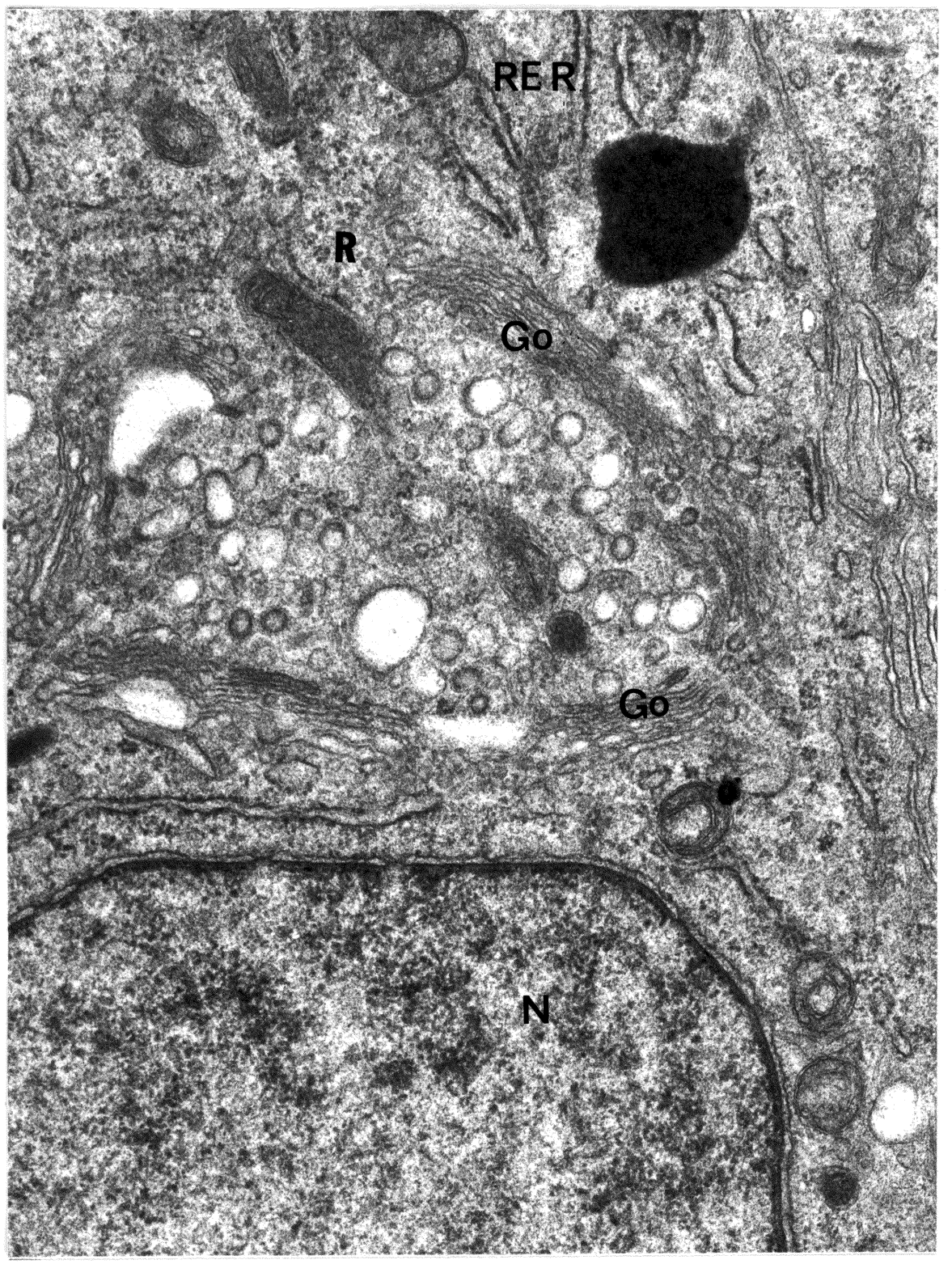
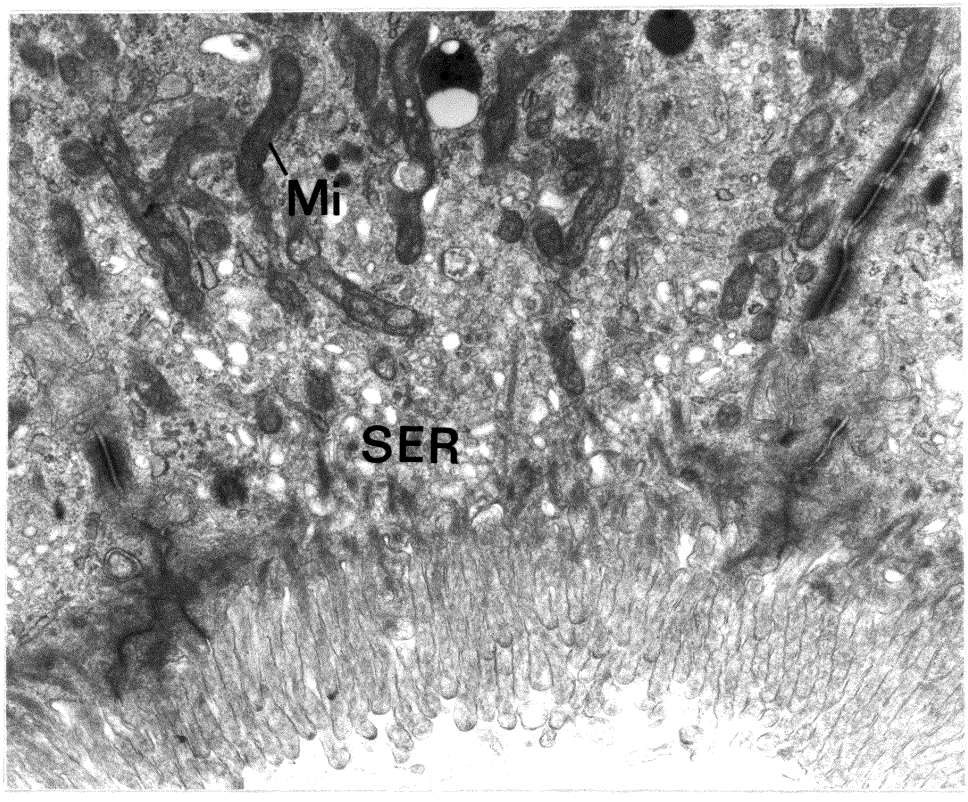

RER
R
Go
Go
N

Plate 12
UV Sperm Gland - Fertile Hen

Fig. 1. Apical cytoplasm containing smooth endoplasmic reticulum (SER) and numerous elongate mitochondria (Mi). (10,000 X)

Fig. 2. Basal cytoplasm containing an abundance of short-rounded mitochondria (Mi) and free ribosomes (R). The basal lamina (BL) separates thecell from connective tissue. (20,000 X)


Mi
SER

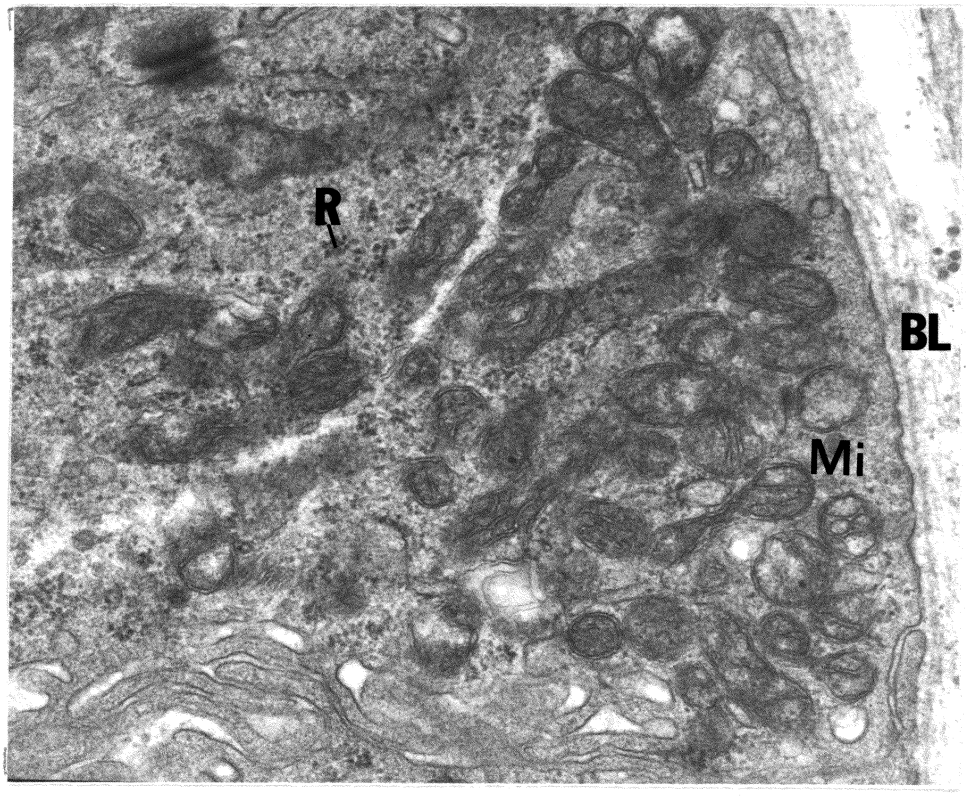

R
BL
Mi

Plate 13
UV Sperm Gland - Fertile Hen

A nucleus containing euchromatin (Eu) heterochromatin (He) and a nucleolus (Nu) within the nuclear membrane (NuM). (20,000 X)

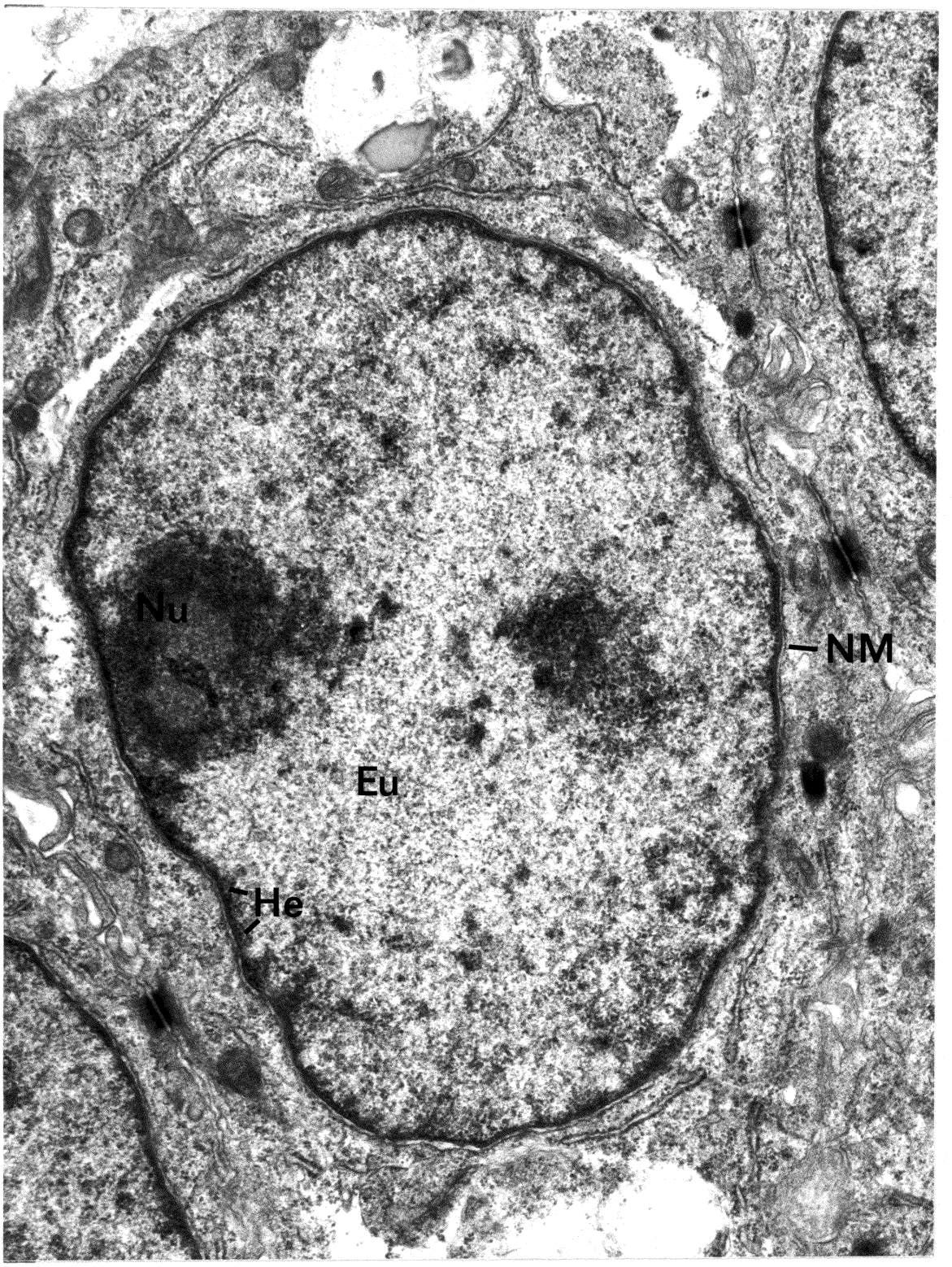

Nu
NM
Eu
He

Plate 14
UV Sperm Gland - Fertile Hen

A nucleolus made up of pars amorpha (PA) and nucleolonema (Ne) and surrounded by heterochromatin. (33,000 X)

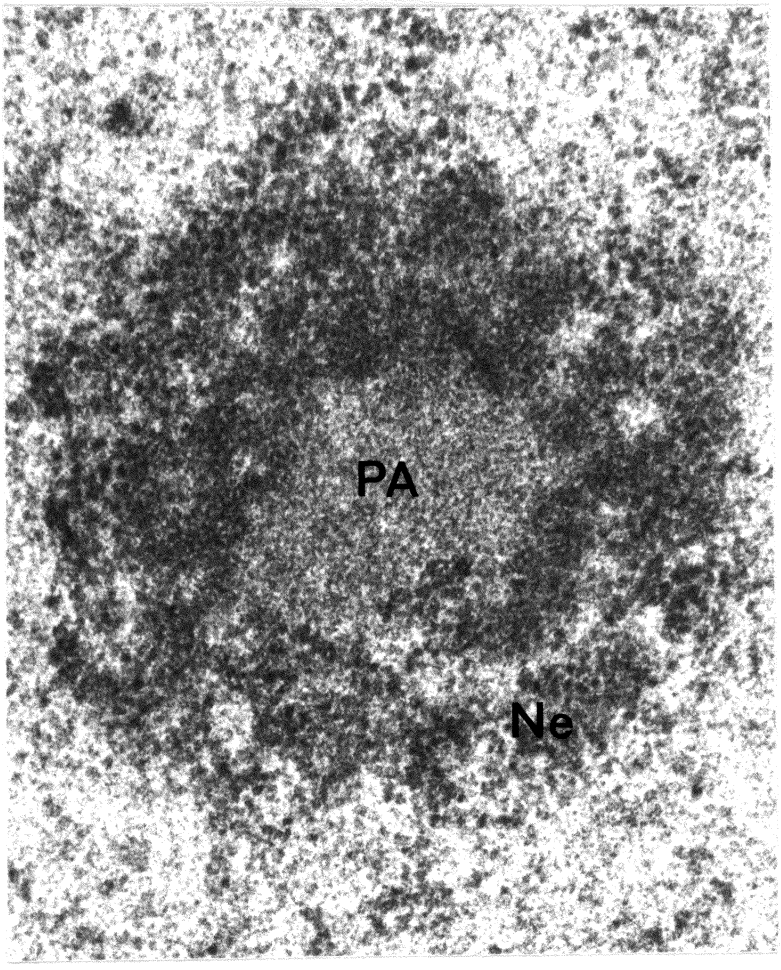
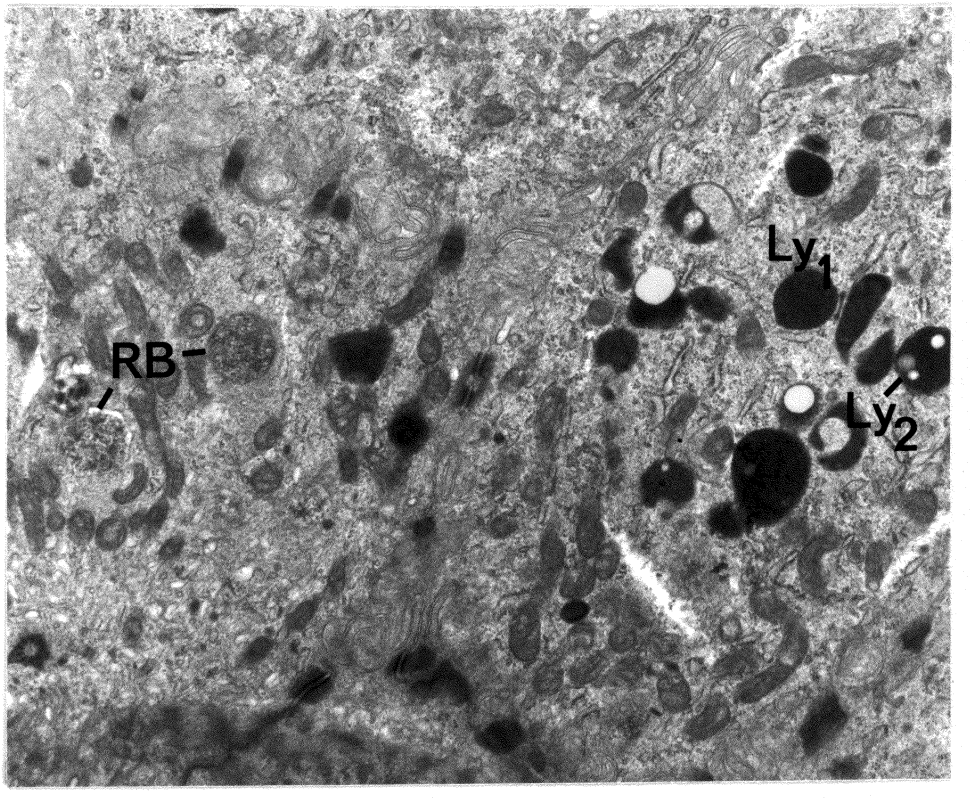

PA
Ne

Plate 15
UV Sperm Gland - Fertile Hen

Fig. 1. Apical cytoplasm containing elongate mitochondria (Mi), primary lysosomes (Ly1), secondary lysosomes (Ly2) and residual bodies (RB). (8,300 X)

Fig. 2. Apical cytoplasm containing primary lysosomes (Ly1), secondary lysosomes (Ly2) and residual bodies (RB). (16,000 X)


RB
Ly1
Ly2

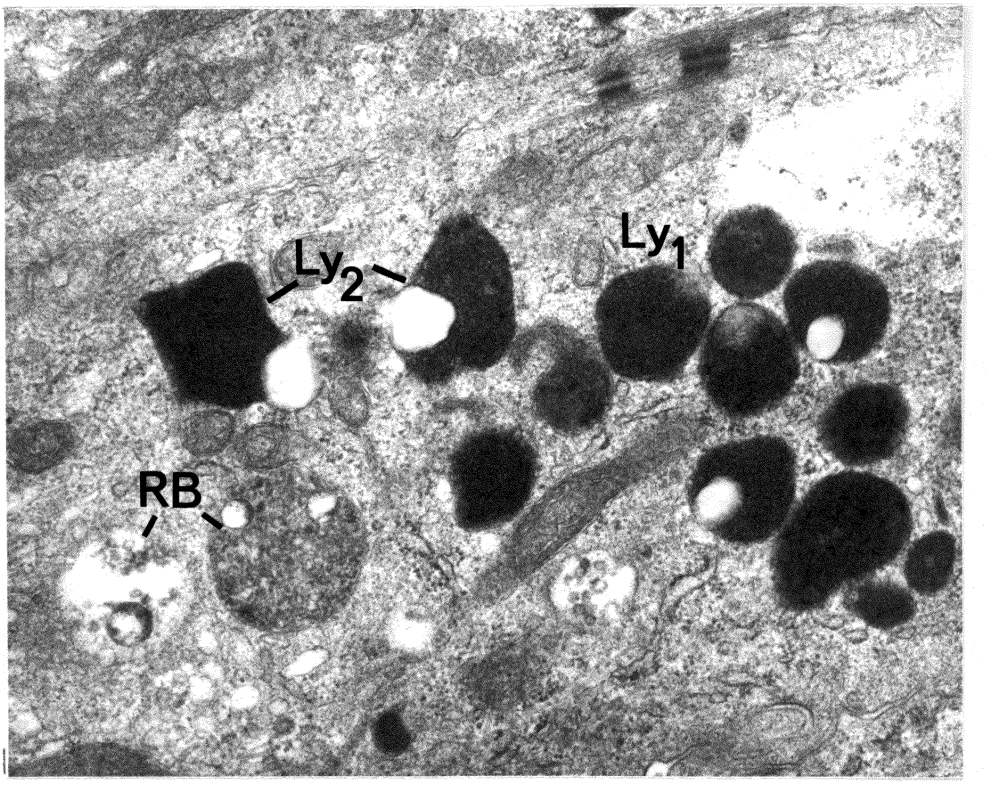

Ly2
Ly1
RB

Plate 16
UV Sperm Gland - Fertile Hen

Residual bodies (RB) with microbodies (Mic) within a sperm gland cell. (33,000 X)

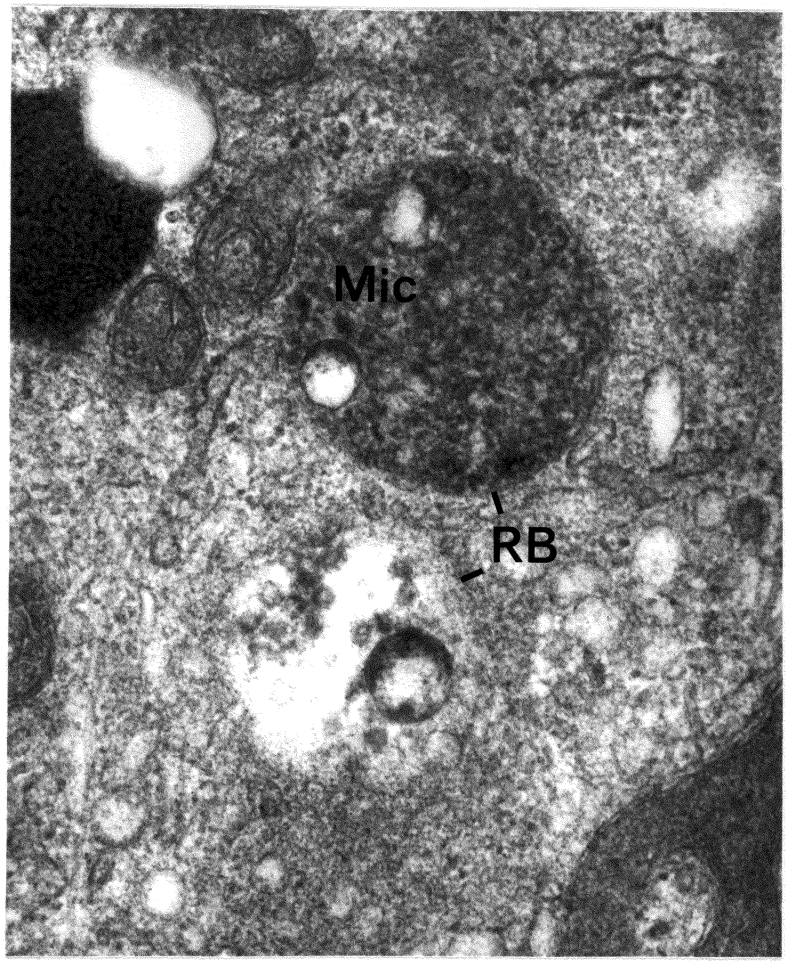
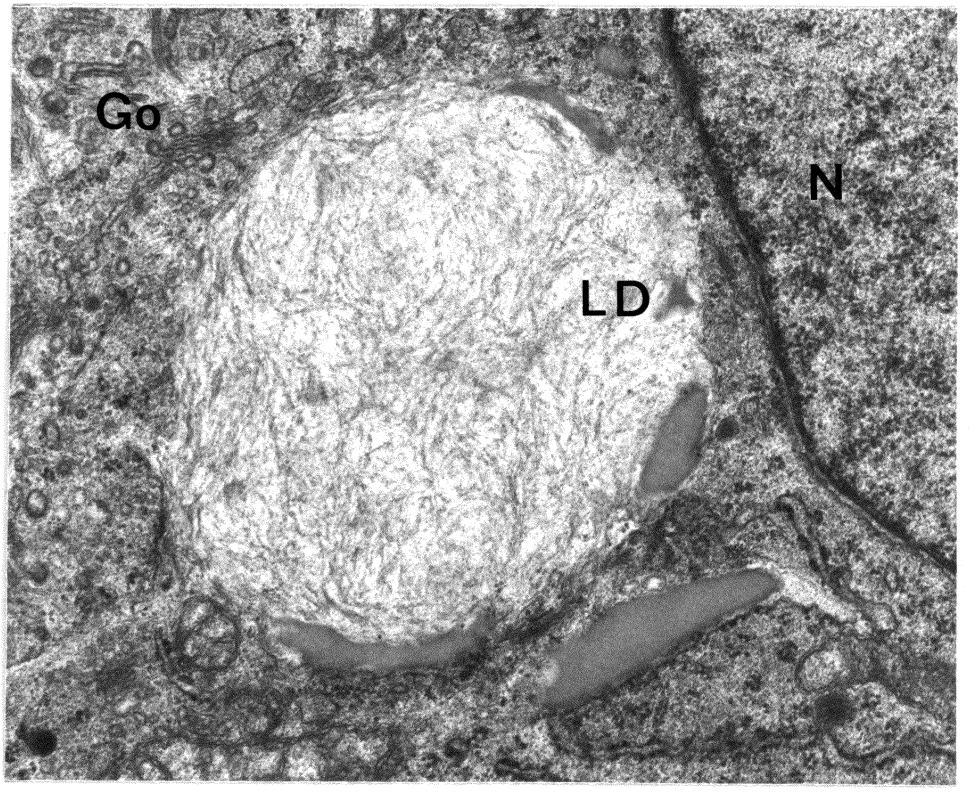

Mic
RB

Plate 17
UV Sperm Gland - Fertile Hen

Fig. 1. A lipid droplet (LD) located adjacent to the nucleus (N). Golgi apparati (Go) are located near the periphery of the lipid droplet. (16,000 X)

Fig. 2. A lipid droplet (LD) adjacent to a nucleus (N). A golgi apparatus (Go) is located on the periphery of the lipid droplet. Note the lipid droplet depresses the nuclear membrane. (6,600 X)


Go
N
LD

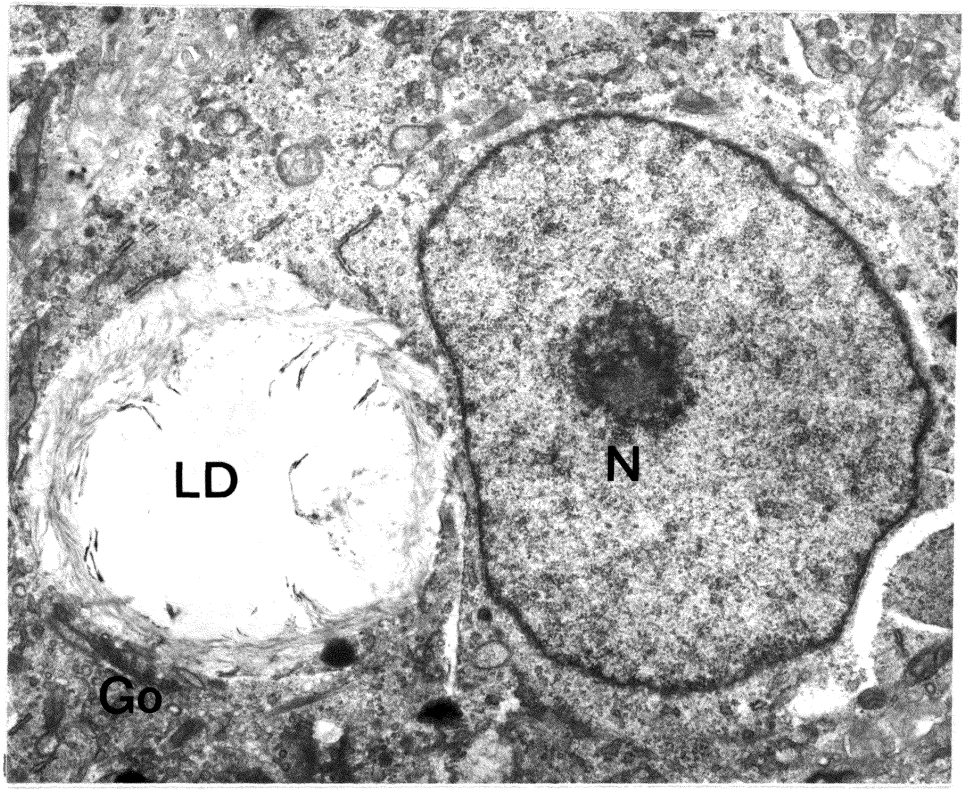

LD
N
Go

Plate 18
UV Sperm Gland - Fertile Hen

A cross section of a UV gland with small lipid droplets (LD) dispersed apically. Note that the nuclei (N) are juxatoposed to the basal lamina (BL). (4,000 X)

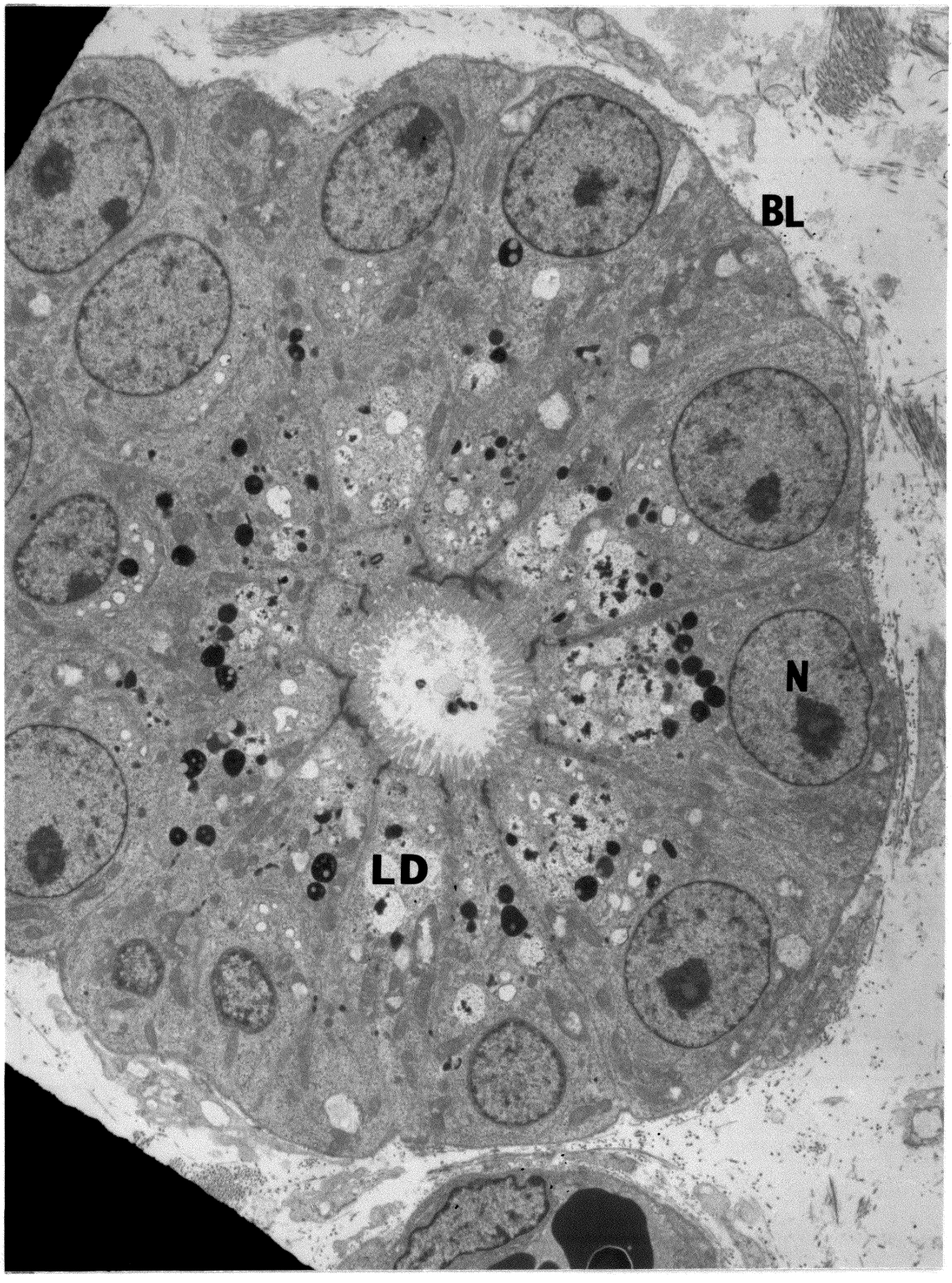
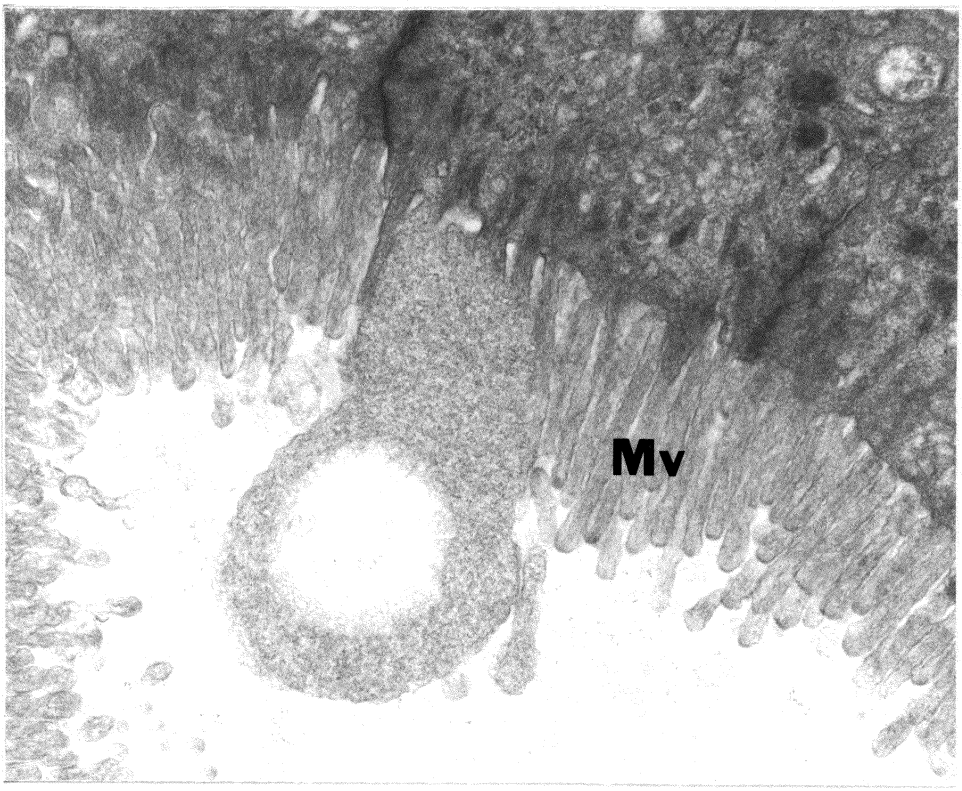

BL
N
LD

Plate 19
UV Sperm Gland - Fertile Hen

Fig. 1. Microvilli engaged in what appears to be large scale apocrinesecretion into the lumen. (13,000 X)

Fig. 2. Microvilli engaged in what appears to be apocrine blebing (arrows) into the lumen. The lumen contains spermatozoa(Sp) and secretory debris (Sd). (8,300 X)


Mv

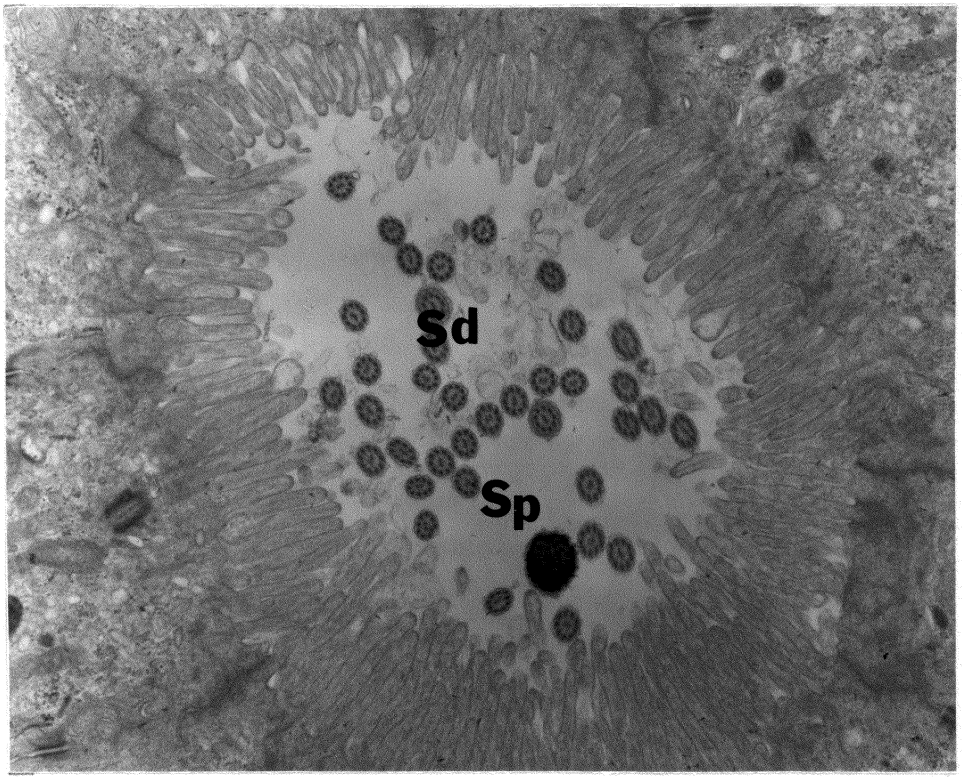
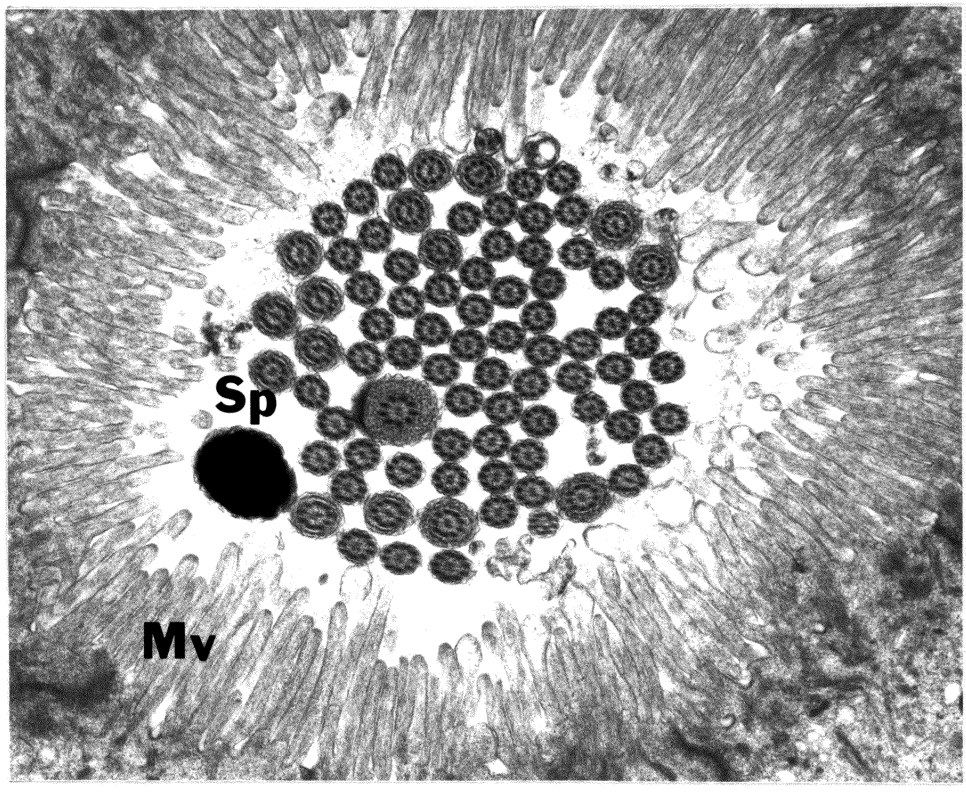

Sd
Sp

Plate 20
UV Sperm Gland - Fertile Hen

Fig. 1. Microvilli (Mv) projecting into a UV gland lumen which contains spermatozoa (Sp) cut in cross section. (10,000 X)

Fig. 2. Spermatozoa (Sp) contained within a lumen of a UV sperm gland. (5,000 X)

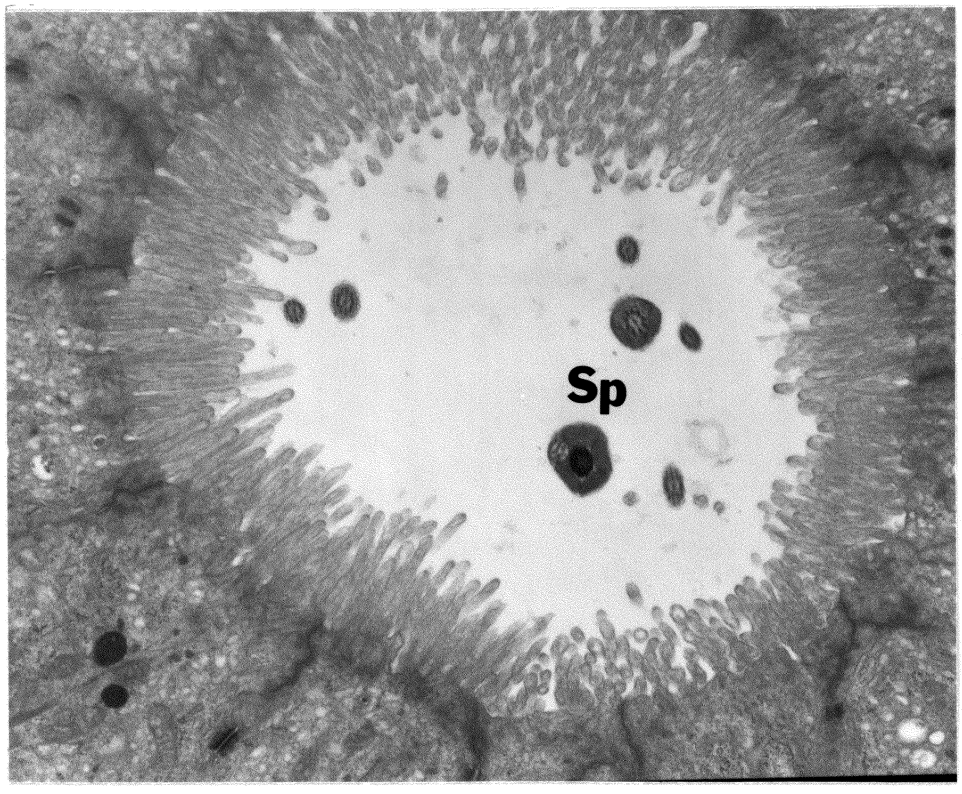

Sp
Mv


Sp

Plate 21
UV Sperm Gland - Fertile Hen

Fig. 1. A sparse accumulation of widely spaced spermatozoa (Sp) within a UV gland lumen. (6,600 X)

Fig. 2. A dense accumulation of spermatozoa (Sp) within the lumen of a UV gland. Sperm cell contact is suggestive of agglutination.


Sp

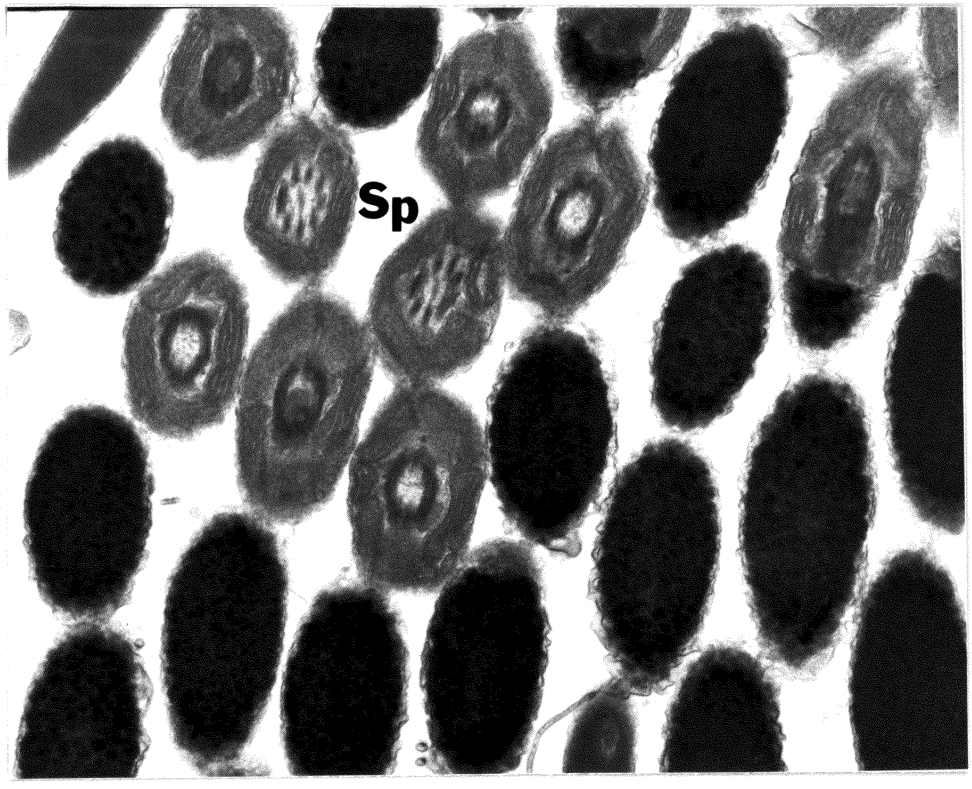
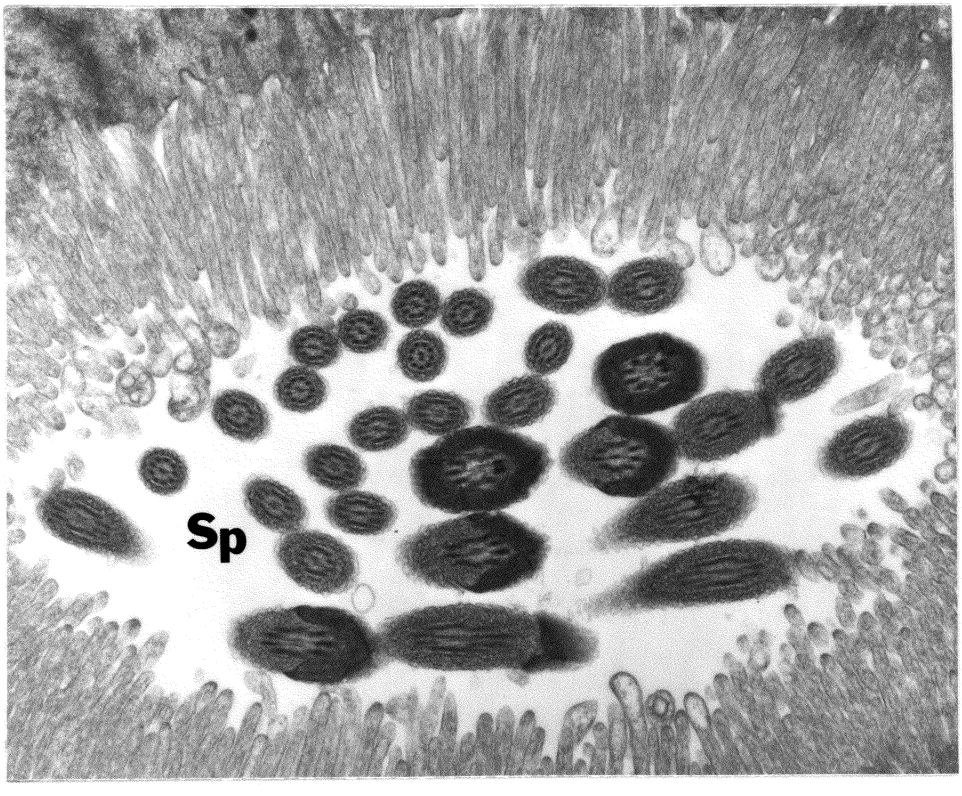

Sp

Plate 22
UV Gland - Fertile Hen

Fig. 1. Spermatozoa (Sp) present in the lumen of a UV gland. Note the sporatic intimate contact between spermatozoa. (10,000 X)

Fig. 2. Spermatozoa (Sp) present in the lumen of a UV gland. Note the relatively abbreviated microvilli which is characteristic of villi nearer a gland orifice. (10,000 X)


Sp

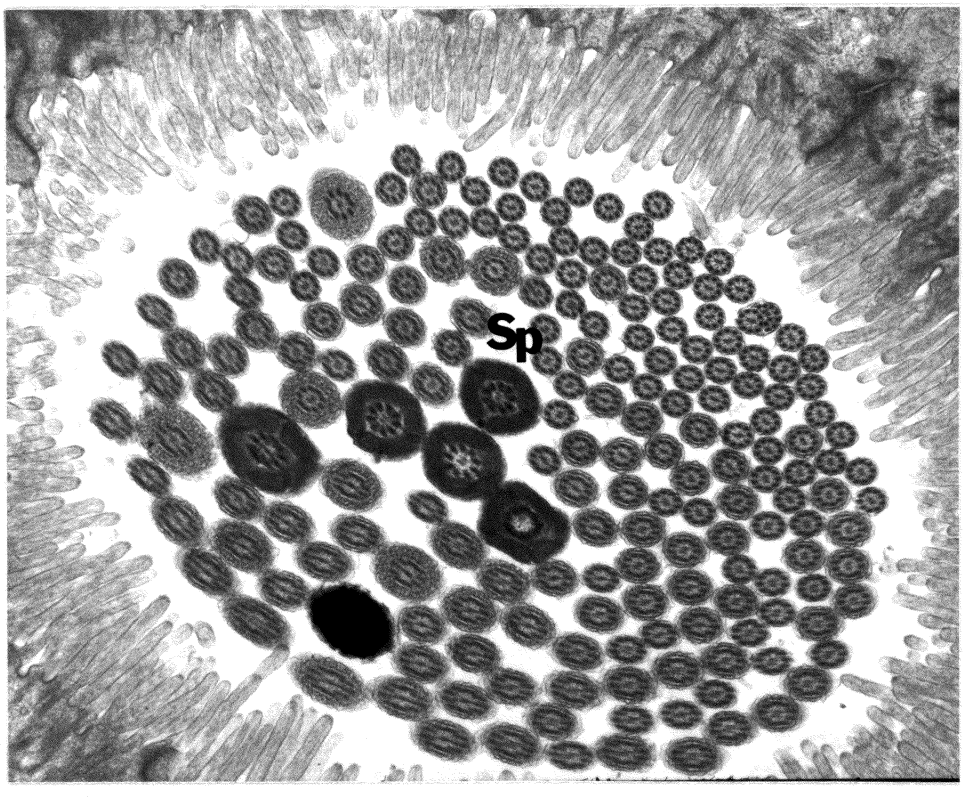
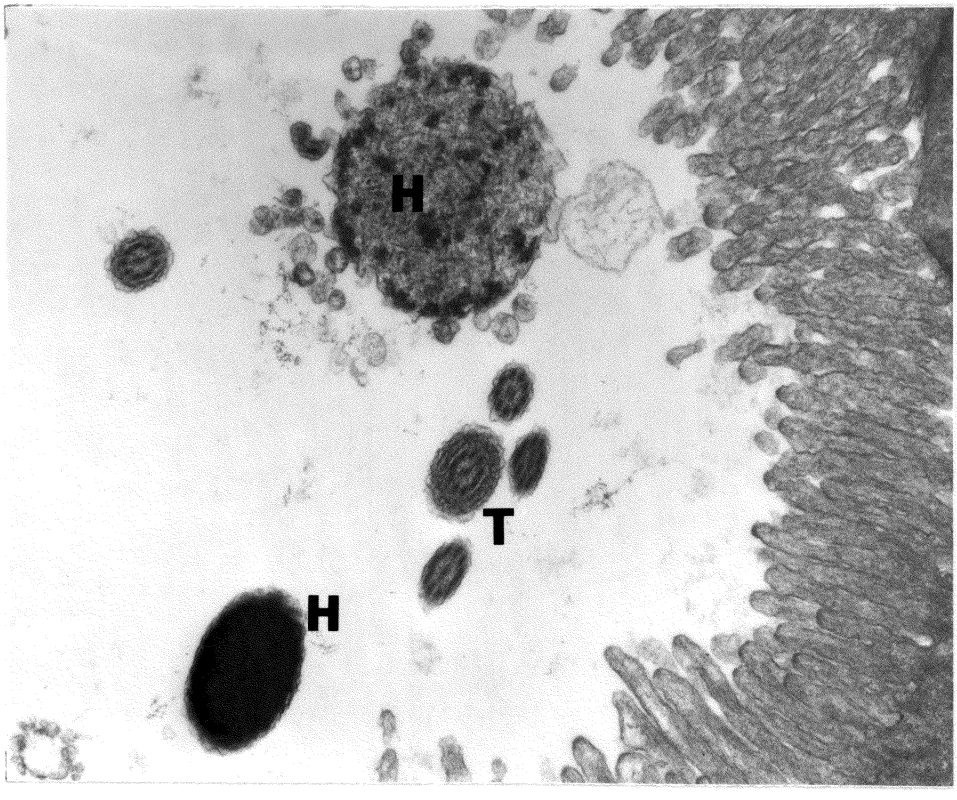

Sp

Plate 23
UV Sperm Gland - Fertile Hen

Fig. 1. Spermatozoa (Sp) present in the lumen of a UV gland. Two sperm heads (H) are seen, one is normal in appearance while the other (arrow) is undergoing degeneration. Remaining spermatozoa present in the lumen are sectioned through various levels of the tail (T). (13,000 X)

Fig. 2. Spermatozoa at varying stages of degeneration within the lumen of a UV gland. Free filaments (F) are also present. (10,000 X)


H
T
H


F

Plate 24
UV Sperm Gland - Fertile Hen

Lymphocyte (Lym) present in the connective tissue (CT) surrounding a UV gland. Present within the lymphocyte are a distinctive nucleus (N) and mitochondria (Mi). (16,600 X)

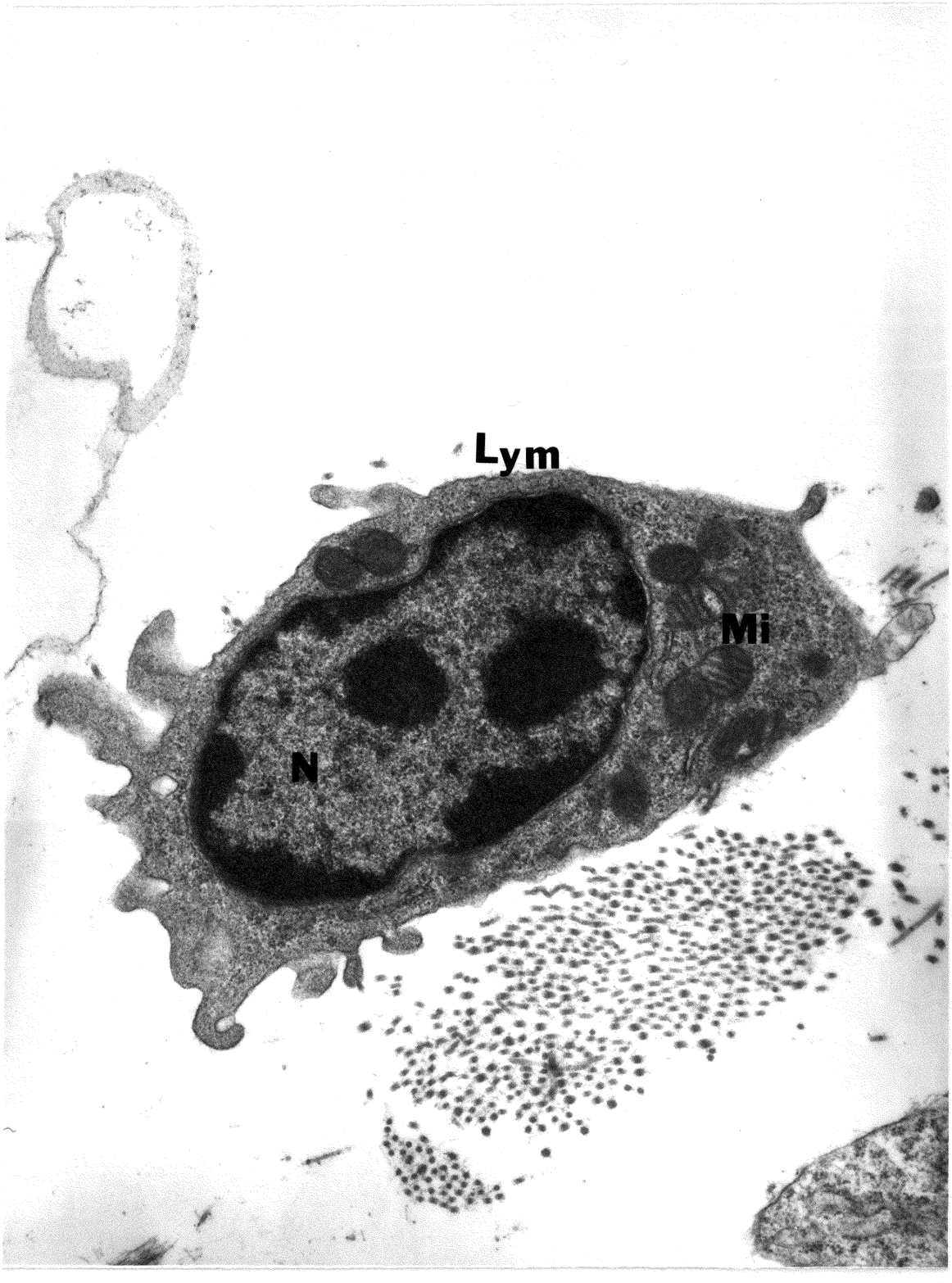

Lym
Mi
N

Plate 25
UV Sperm Gland - Fertile Hen

Lymphocyte (lym) infiltrating between lateral walls (LW) of adjacent glandular cells. Also seen are the nucleus (N) and basal mitochondria (Mi) of a UV gland cell. (32,000 X)

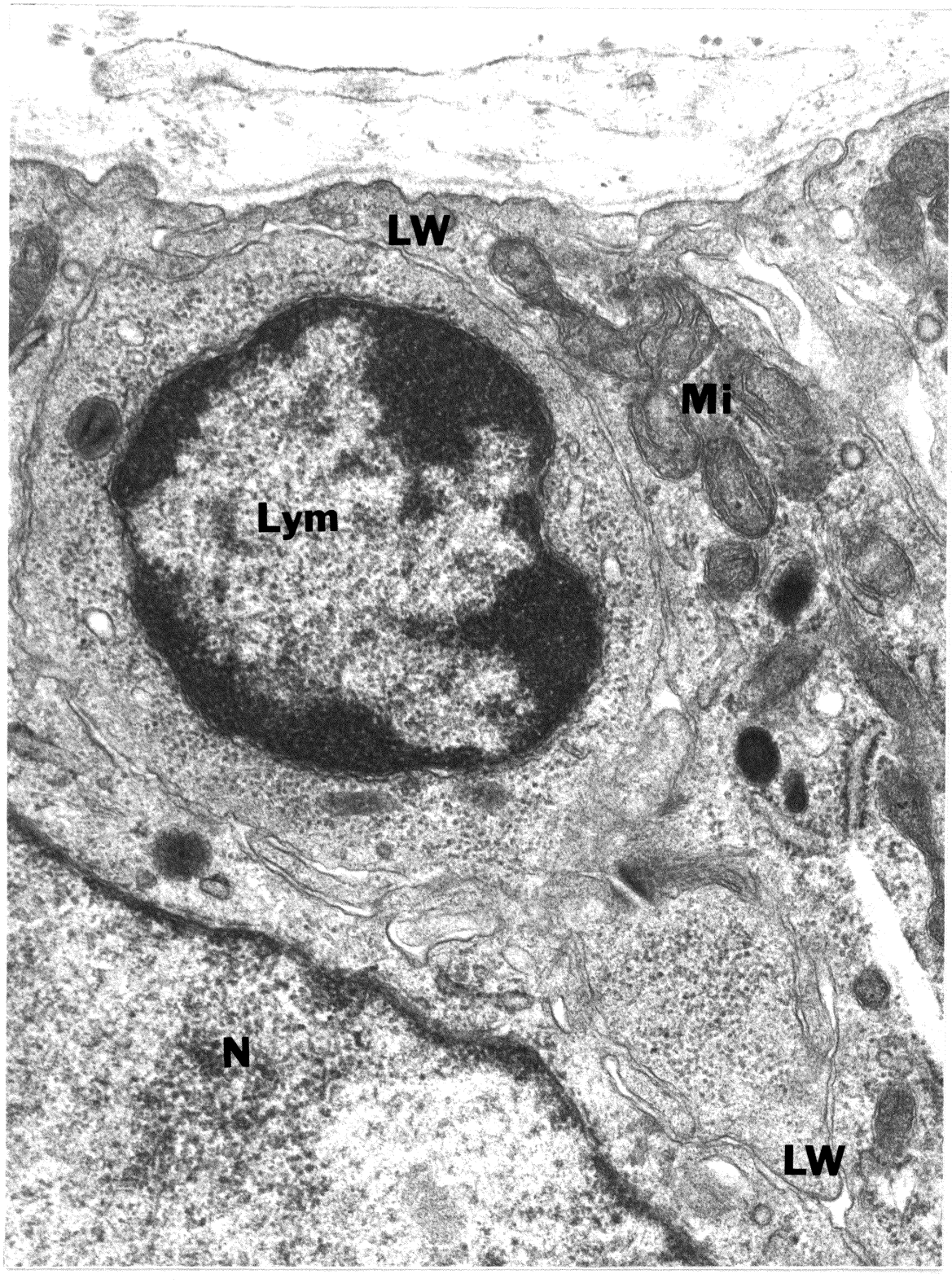

LW
Mi
Lym
N
LW

Plate 26
UV Sperm Gland - Fertile Hen

Plasma cell (PC) present in the connective tissue (CT) surrounding a UV gland. Located within the plasma cell are the nucleus (N), extensive rough endoplasmic reticulum (RER) and mitochondria (Mi). (13,200 X)

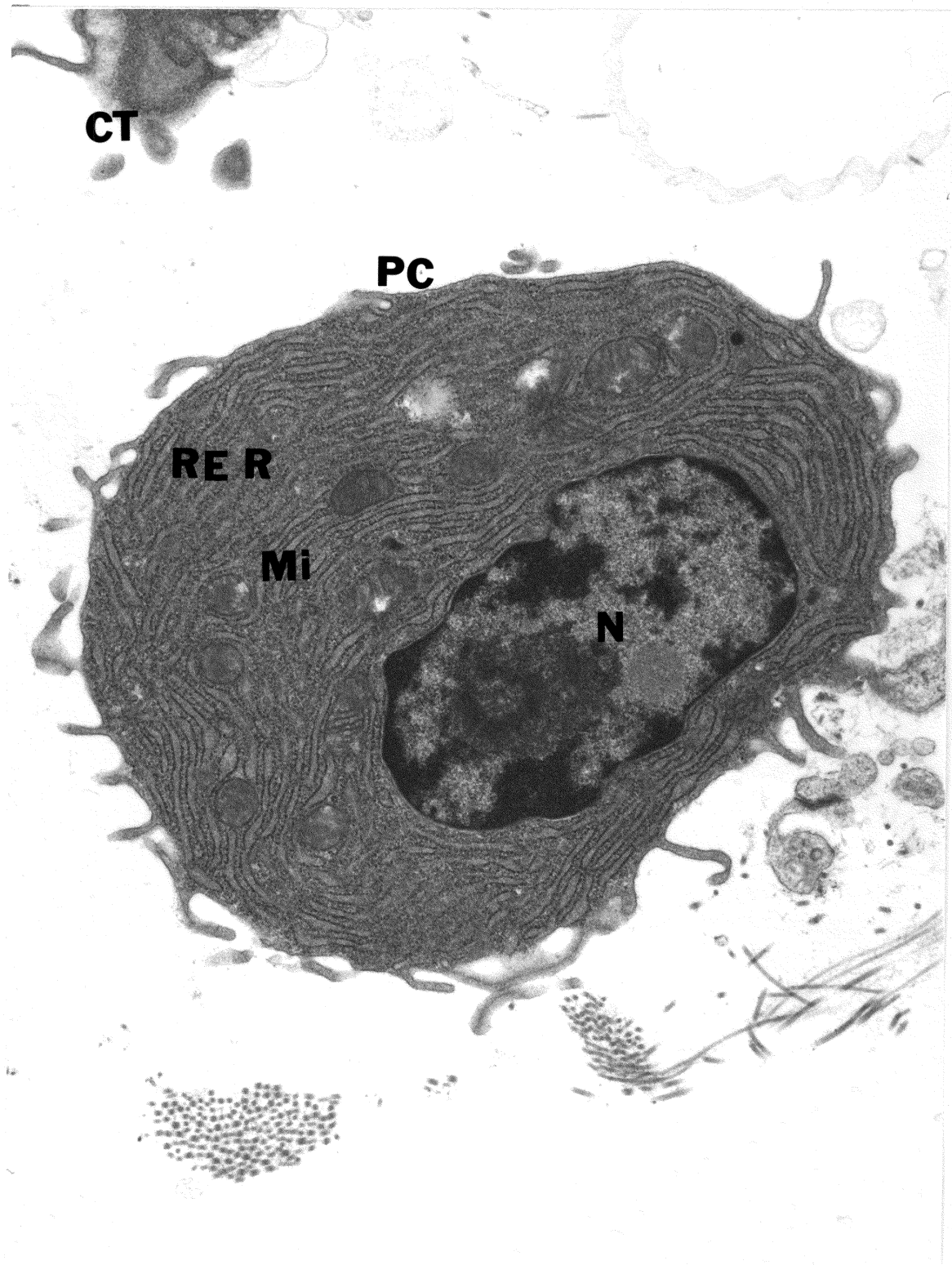
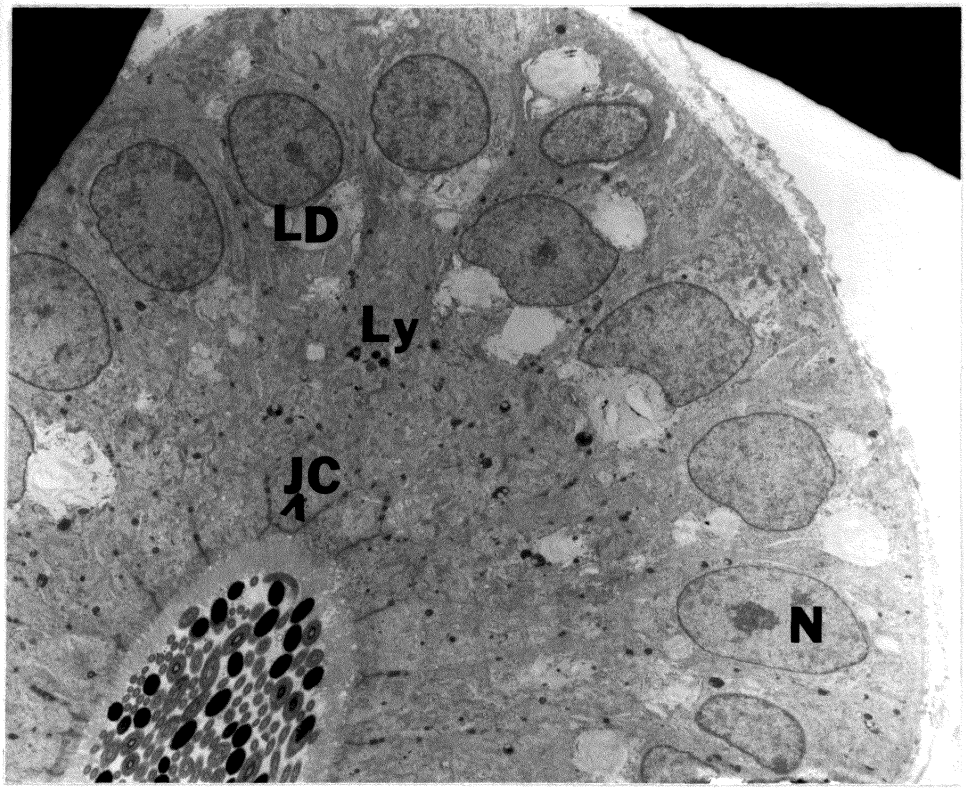

CT
PC
RE R
Mi
N

Plate 27
UV Sperm Gland - Prelay

Fig. 1. Cross section of a UV gland collected immediately prior to the initiation of egg production. Nuclei (N) are located basally. Large lipid droplets (LD) are seen perinuclearly. An abundance of spermatozoa are present in the glandular lumen. Note the limited lysosomes (Ly) and clearly demarcated junctional complexes (JC). (2,000 X)

Fig. 2. Cross section of cells comprising a UV gland. A basally located lymphocyte (Lym) is seen among UV gland cells even at this early stage of the reproductive cycle. Large lipid droplets (LD) are found perinuclearly. Cells appear to be engaged in secretion (arrows) into the lumen which contains spermatozoa (Sp). (3,300 X)


LD
Ly
JC
N

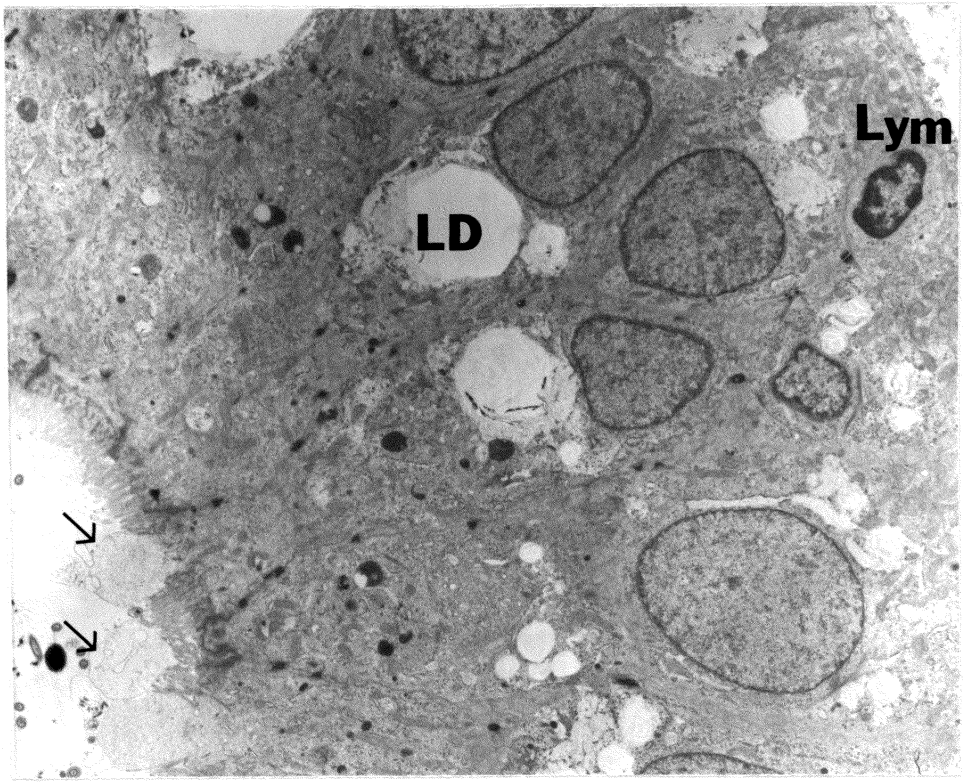

Lym
LD

Plate 28
UV Sperm Gland - Prelay

Cells of a UV gland collected from a hen immediately prior to the initiation of egg production. Two basally located lymphocytes (Lym) are observed within the gland proper. Large characteristic lipid droplets (LD) are located supranuclearly. Microvilli (Mv) project into an empty lumen (Lu). (6,600 X)

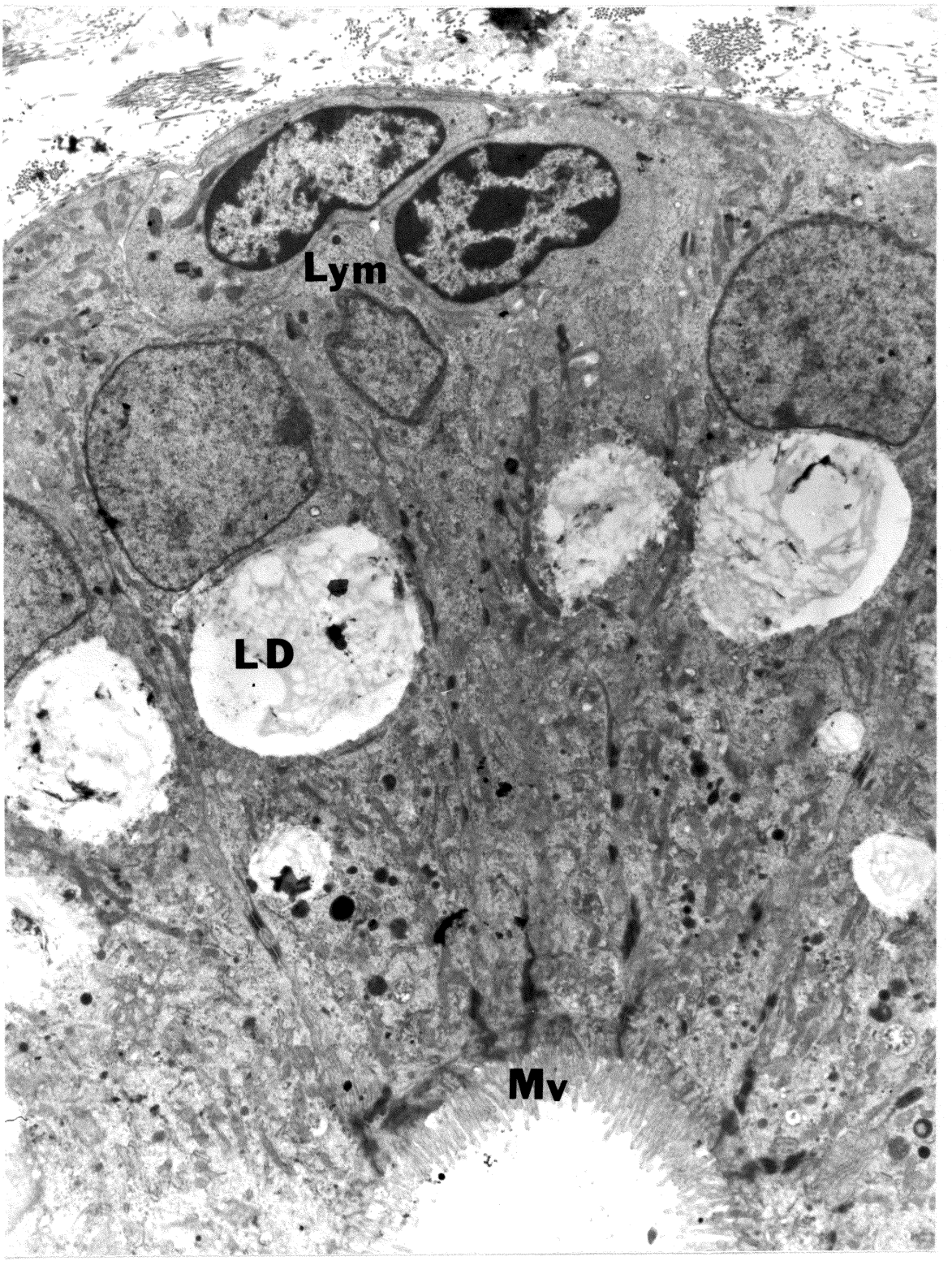
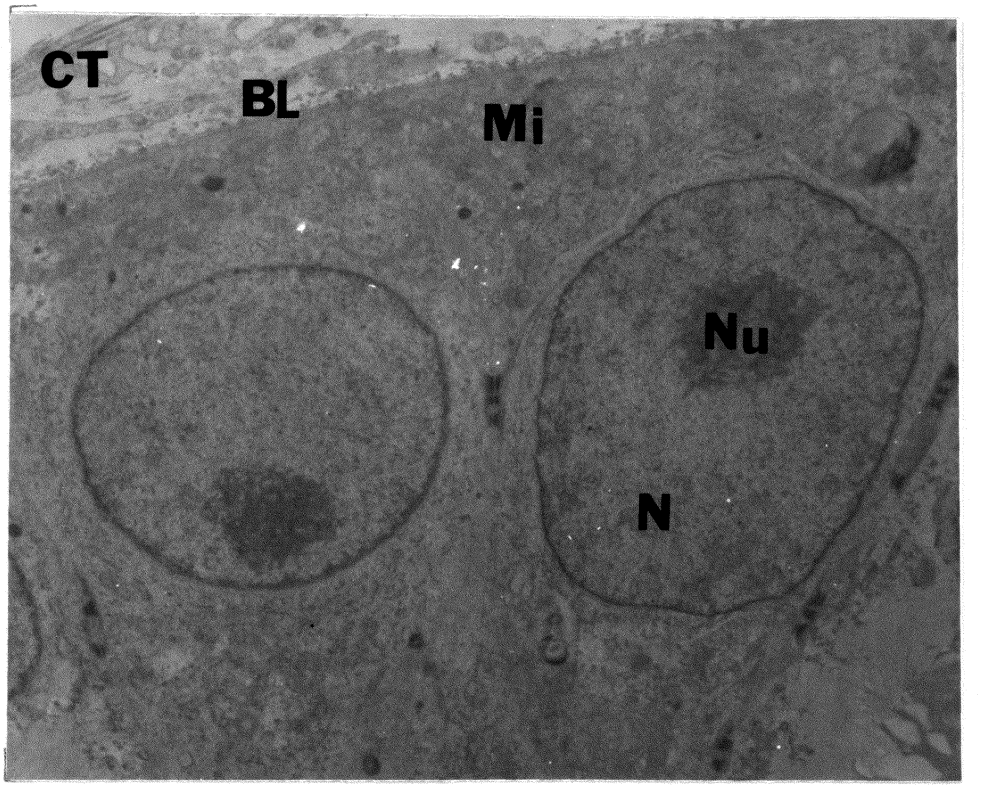

Lym
LD
Mv

Plate 29
UV Sperm Gland - Prelay

Fig. 1. Basal aspects of UV gland cells are shown. The basal lamina (BL) separates UV gland cells from the connective tissue (CT). Aggregates of mitochondria (Mi) are also seen. Nuclei (N) are principally composed of euchromatin (Eu). Nucleoli (Nu) areapparent within a nucleus. (6,600 X)

Fig. 2. Apical cellular aspects of a UV gland are shown. Microvilli (MV) project into the glandular lumen. Lysosomes (Ly), scattered rough endoplasmic reticulum and mitochondria are also present. (6,600 X)


CT
BL
Mi
Nu
N

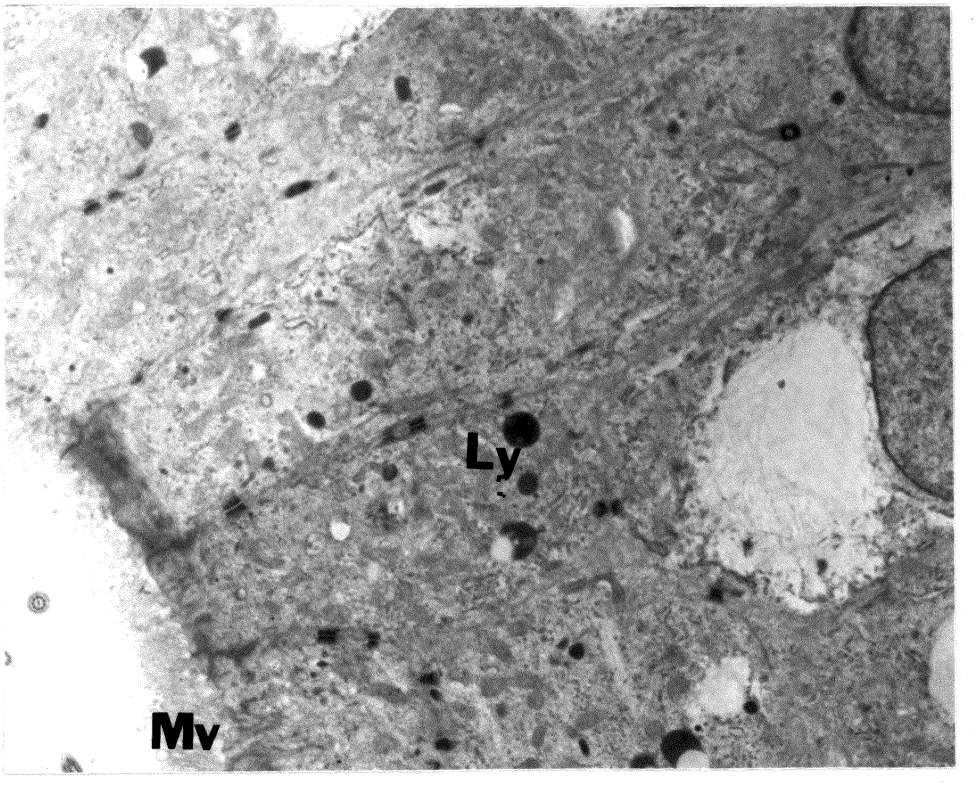
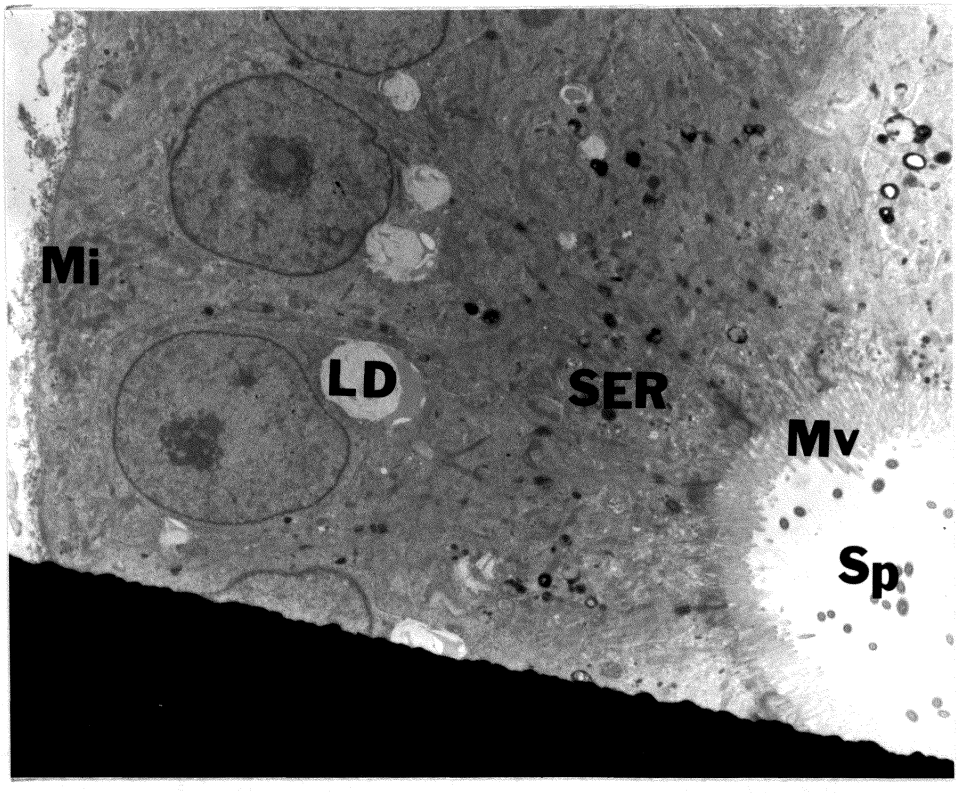

Ly
Mv

Plate 30
UV Sperm Gland - Fertile Hen

Fig. 1. Tissue collected from a fertile hen at the end of the laying season. Aggregates of mitochondria (Mi) occur basally. Lipid droplets (LD) are found supranuclearly. Within the apical cytoplasm lysosomes (Ly) and smooth endoplasmic reticulum (SER) are observed. Microvilli (Mv) project into a lumen which contains spermatozoa (Sp). (3,300 X)

Fig. 2. UV gland cells from a fertile hen at the end of the laying season. Aggregates of mitochondria (Mi) are seen in the basal cytoplasm. Lysosomes (Ly) lie apically. (3,300 X).


Mi
LD
SER
Mv
Sp

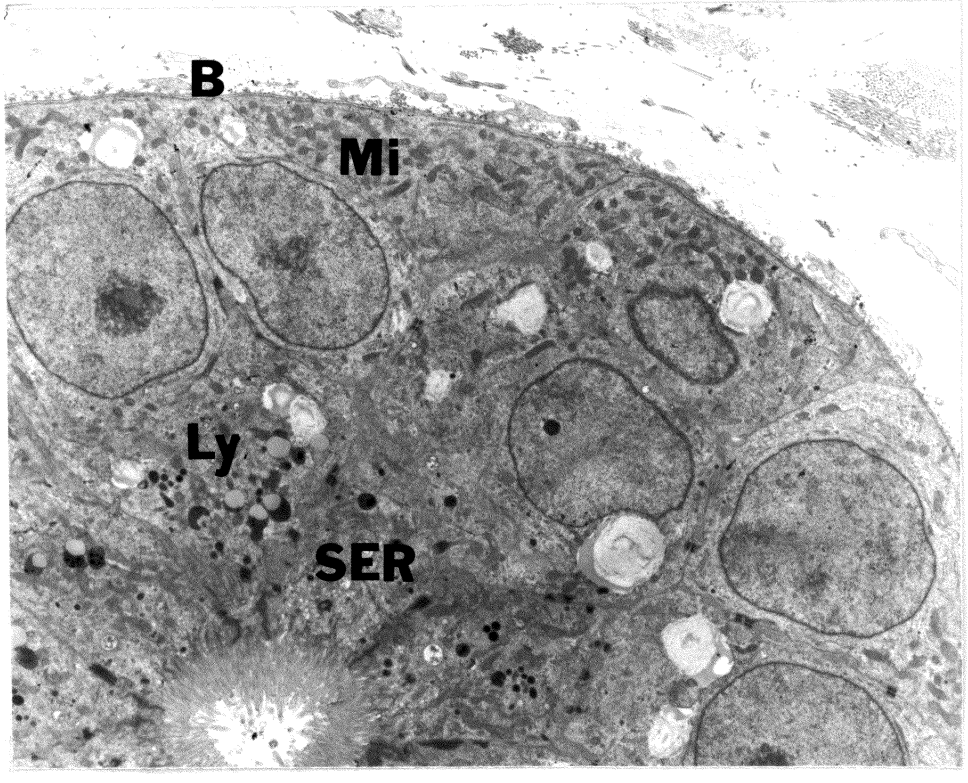

B
Mi
Ly
SER

Plate 31
UV Sperm Gland - Fertile Hen

Section through cells from a sperm gland collected at the end of the laying season. Mitochondria (Mi) are located apically and basally. The apical cytoplasm contains golgi apparati (Go), scattered rough endoplasmic reticulum (RER), smooth endoplasmic reticulum (SER) and lysosomes (Ly). (10,000 X)

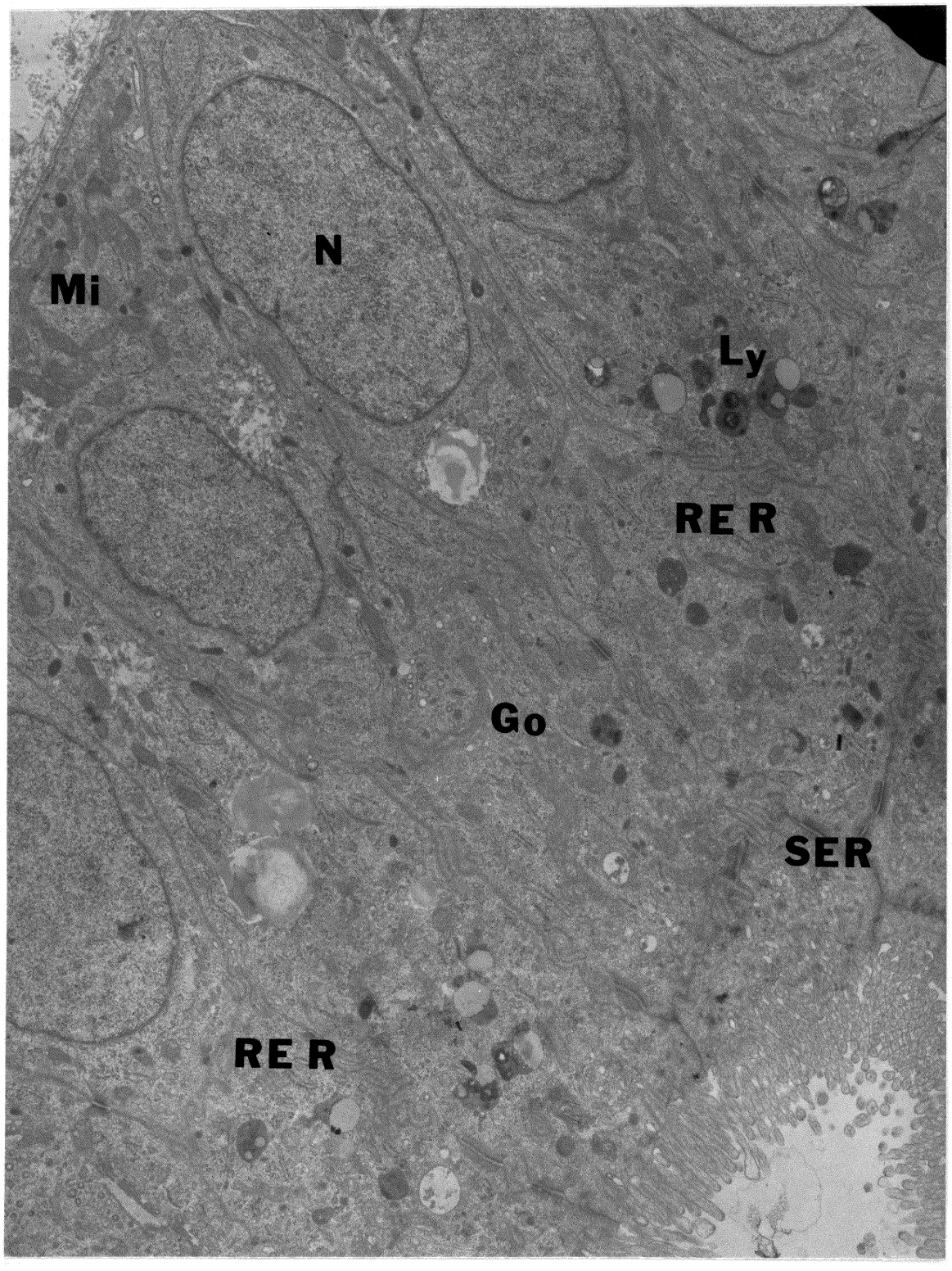
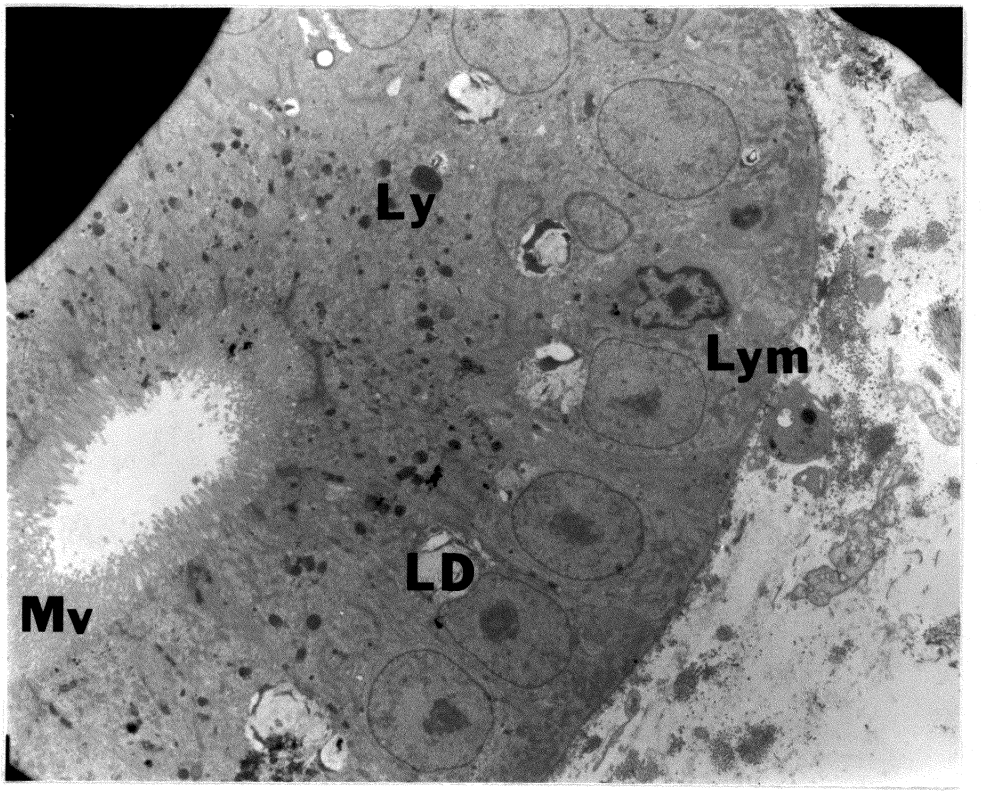

Mi
N
Ly
RE R
Go
SER
RE R

Plate 32
UV Sperm Gland - Infertile Hen

Fig. 1. Cross section of a UV gland from an infertile hen. Two lymphocytes (Lym) are visible, one in the surrounding connective tissue (CT), and the other within the gland proper. Large lipid droplets (LD) lie supranuclearly as in fertile hens. The apical cytoplasm contains an abundance of lysosomes (Ly) while aggregates of mitochondria (Mi) are localized basally. Microvilli (Mv) project into an empty lumen (Lu). (2,000 X)

Fig. 2. Accumulations of mitochondria (Mi) lie basally. The apical cytoplasm contains lysosomes (Ly). Microvilli (Mv) project into a lumen which contains limited spermatozoa (Sp). (3,300 X)


Ly
Lym
LD
Mv

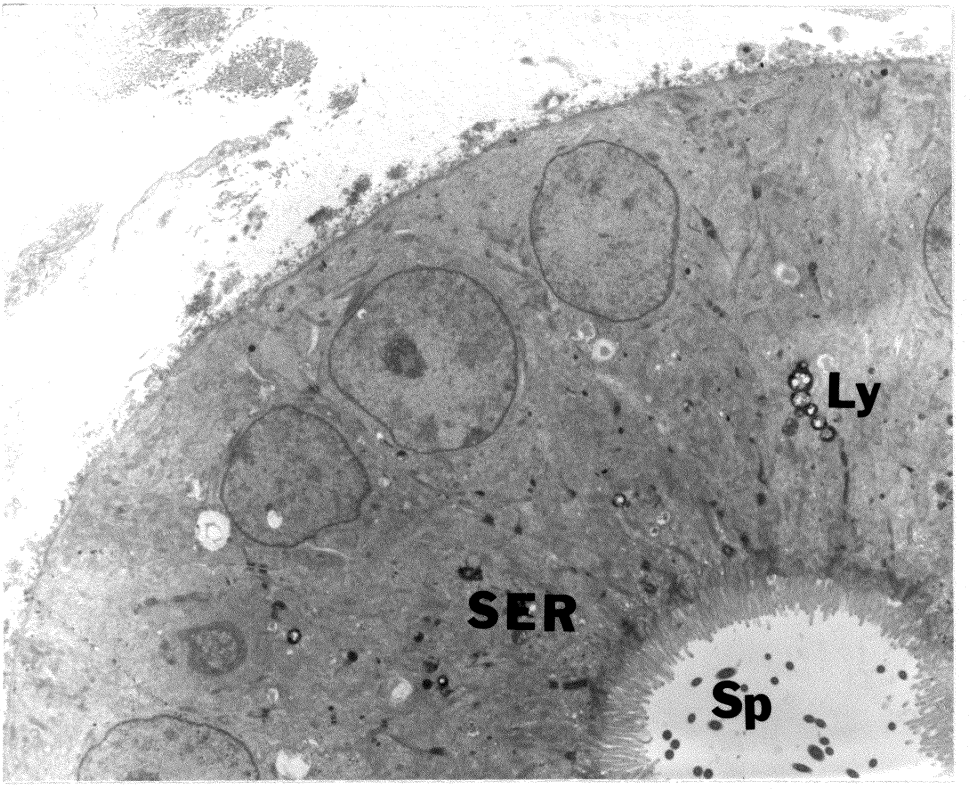
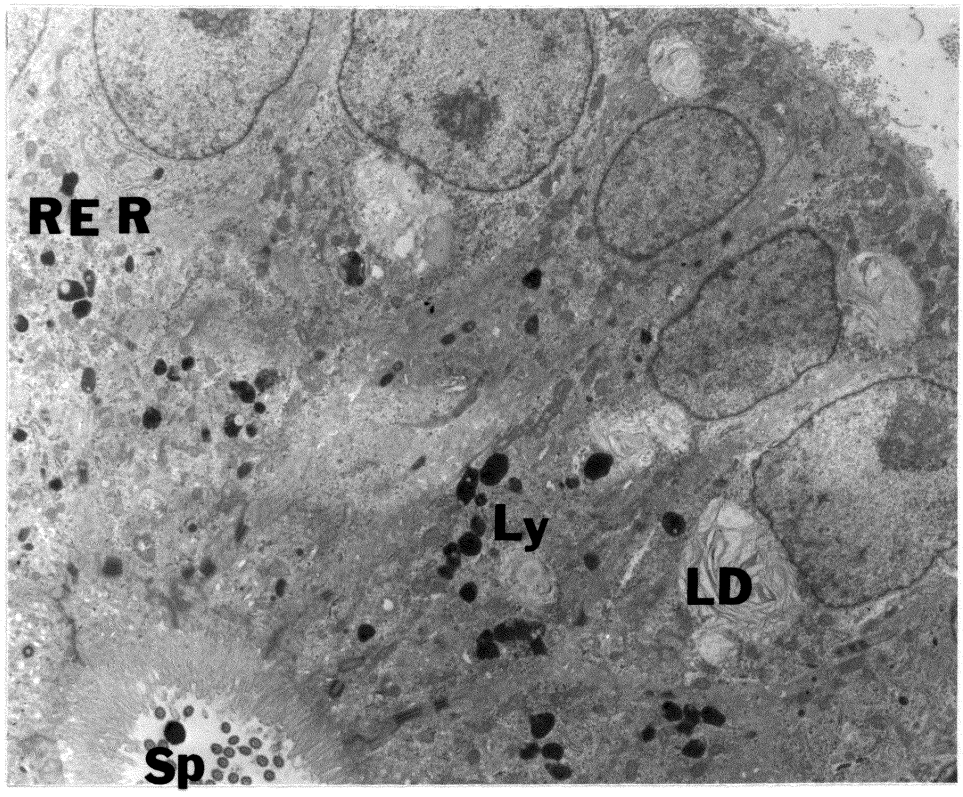

Ly
SER
Sp

Plate 33
UV Sperm Gland - Infertile Hen

Fig. 1. UV gland from a poorly fertile hen. Short mitochondria (Mi) are observed basally while elongate mitochondria (Mi) are seen apically. The apical cytoplasm contains lipid droplets (LD), lysosomes (Ly), golgi apparati (Go) and scattered rough endoplasmic reticulum (RER). Limited spermatozoa (Sp) are present in the glandular lumen. (5,000 X)

Fig. 2. Cells comprising a UV gland from an infertile hen. Aggregates of mitochondria (Mi) are seen basally and an ample quantity of lysosomes (Ly) are distributed apically. While the lumen is devoid of spermatozoa, secretory debris (arrows) is present. (2,600 X)


RE R
Ly
LD
Sp

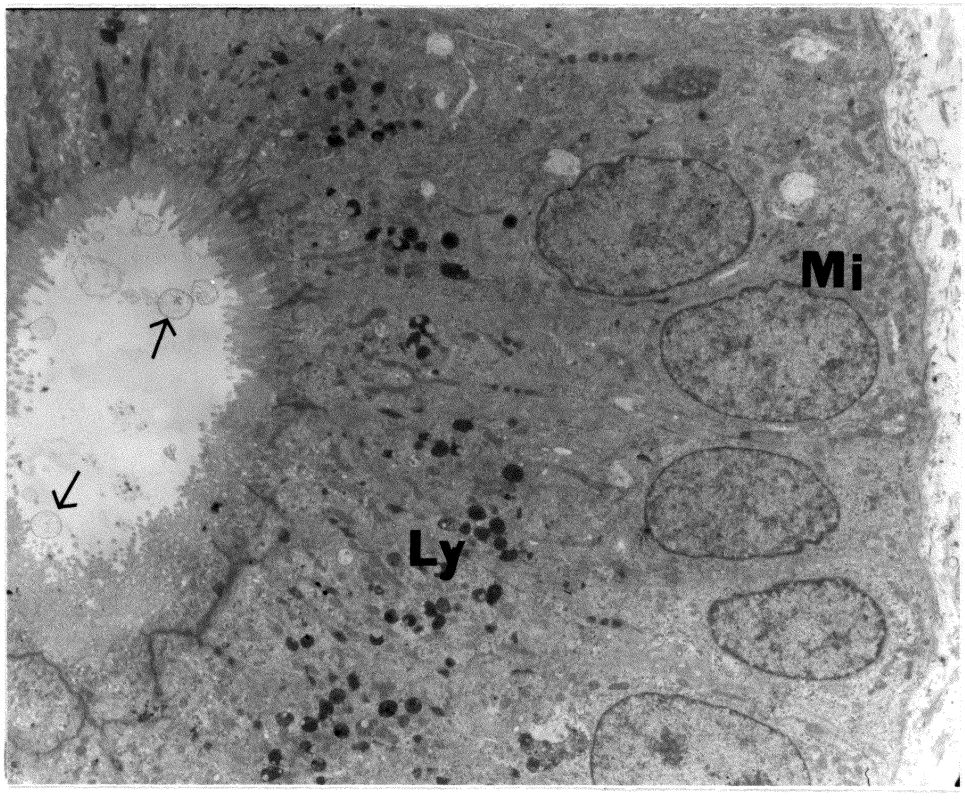
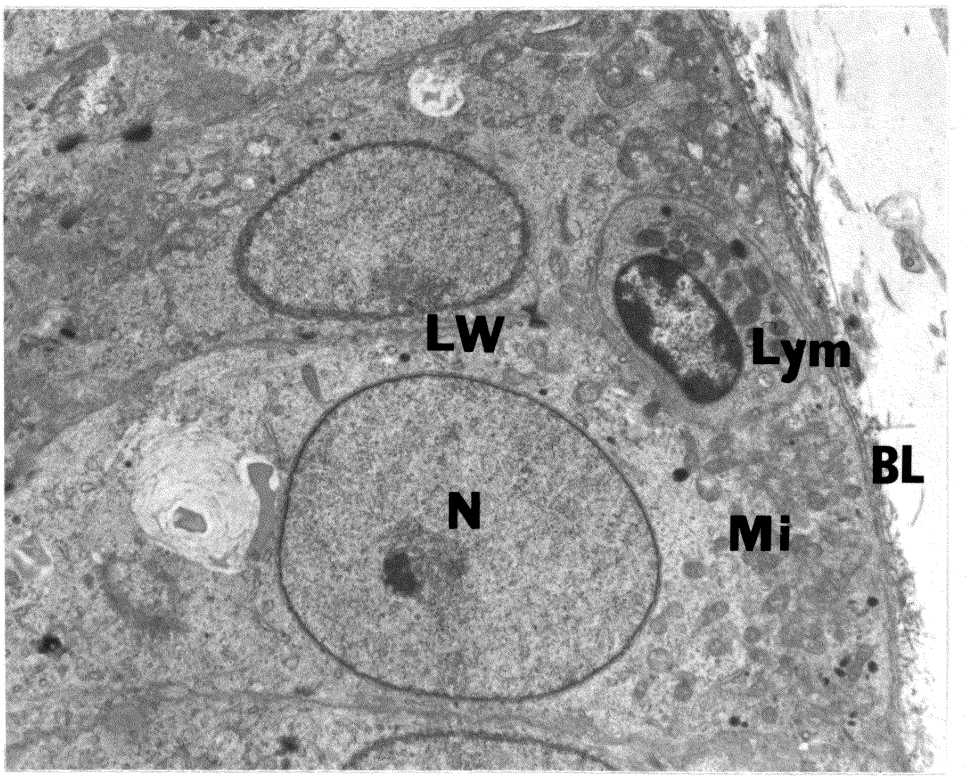
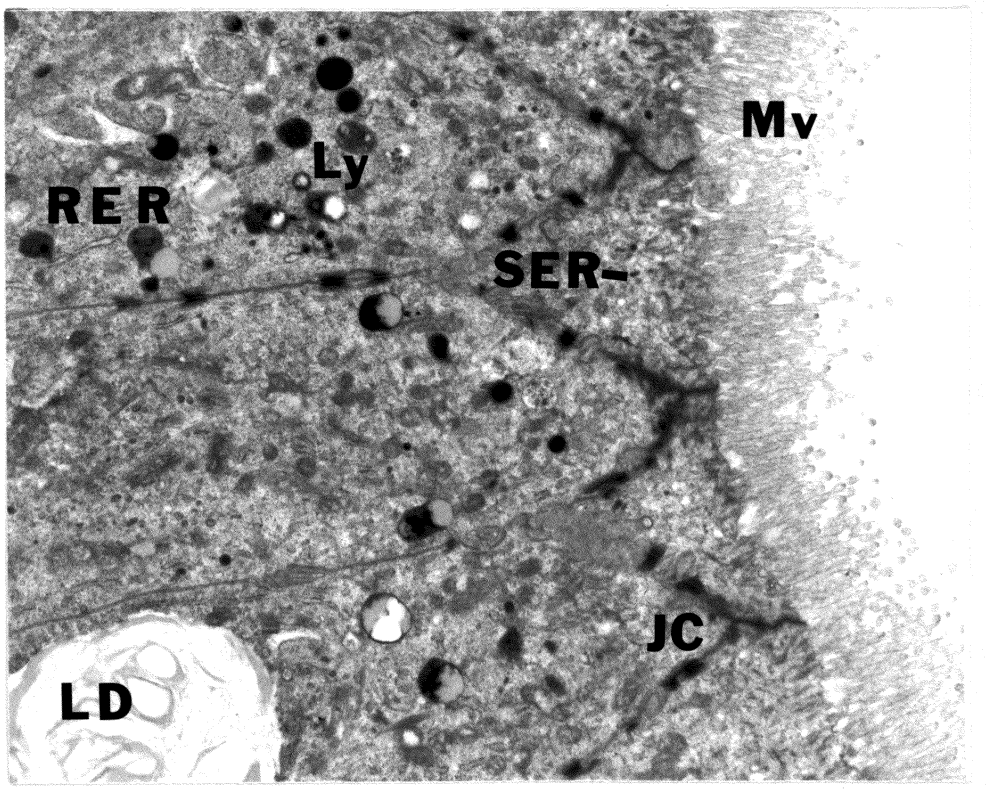

Mi
Ly

Plate 34
UV Glands - Infertile Hens

Fig. 1. Basal aspects of UV gland cells from an infertile hen. Short round mitochondria (Mi) are aggregated between the nuclei (N) and basal lamina (BL). A lipid droplet (LD) is observed supranuclearly. A lymphocyte (Lym) can be seen between the lateral walls (LW) of adjacent cells. (7,800 X)

Fig. 2. Apical aspects of UV gland cells from an infertile hen. Golgi apparati (Go) lysosomes (Ly), sparse rough endoplasmic reticulum (RER) and smooth endoplasmic reticulum (SER) are seen in the cytoplasm. Microvilli (Mv) project into the lumen. Junctional complexes (JC) are readily evident. (10,000 X)


LW
Lym
BL
N
Mi


Mv
Ly
RER
SER
JC
LD

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ULTRASTRUCTURAL ANALYSES OF UTEROVAGINAL SPERM STORAGE GLANDS OF TURKEY BREEDER HENS

by

George T. Schuppin

(ABSTRACT)

The ultrastructure of the uterovaginal sperm storage glands of turkey hens was characterized. The ultrastructural morphology of these cells was monitored over an entire egg laying season. In addition, comparisons were made between glands from fertile and infertile hens.

Based on ultrastructural observations, it was determined that cells of the uterovaginal sperm storage glands exhibit only a moderate level of metabolic activity. The limited presence of intracellular secretory products, along with an infrequently observed cellular release of secretory material, suggested that the effect of glandular secretion on spermatozoal survival was minimal. The density and degree of development of the apical microvilli, however, suggested that a primary function of glandular cells may be absorption of lumenal contents.

The length of time that a hen has been producing eggs did not influence the ultrastructure of storage gland cells. The ultrastructural morphology of glands from hens collected immediately prior to commencement of egg production was similar to that of glands taken from hens that had completed

an entire season of egg production. Thus, the uterovaginal sperm glands were fully developed when hens started to lay eggs and remained fully developed for as long as the hens continued to lay.

Aside from the observation that very few spermatozoa could be found in the uterovaginal sperm glands of infertile hens, no detectable ultrastructural differences were observed between glands from fertile and infertile hens. The uterovaginal sperm glands from infertile hens were observed to maintain the same degree of structural integrity and level of functionality as glands from fertile hens. Furthermore, there was no evidence of glandular microbial infections, nor was there evidence of an adverse immunological response at the level of the sperm glands.