CHAPTER III

RESULTS

1. Male reproductive organs of *Chrysomya megacephala*

The internal reproductive organs in male *C. megacephala* comprised pairs of testes, vas deferens and accessory glands, one ejaculatory duct and one sperm pump (Figures 10A, 10B). The testes were oval-shaped, orange-brown in color, with the terminal end connecting to the vas deferens. The vas deferens was a thin, transparent and long simple tube that opened into the anterior end of the thin medial ejaculatory duct, which was relatively long in length (Figures 10A, 10B). The terminal end of the ejaculatory duct was inserted into the sperm pump, connecting to the external genitalia (Figure 10B). The anterior end of the ejaculatory duct was slightly enlarged and globular (Figures 10A, 10B); where the terminal ends of the paired vas deferens and paired accessory glands opened into opposite sites (Figures 10A, 10B).

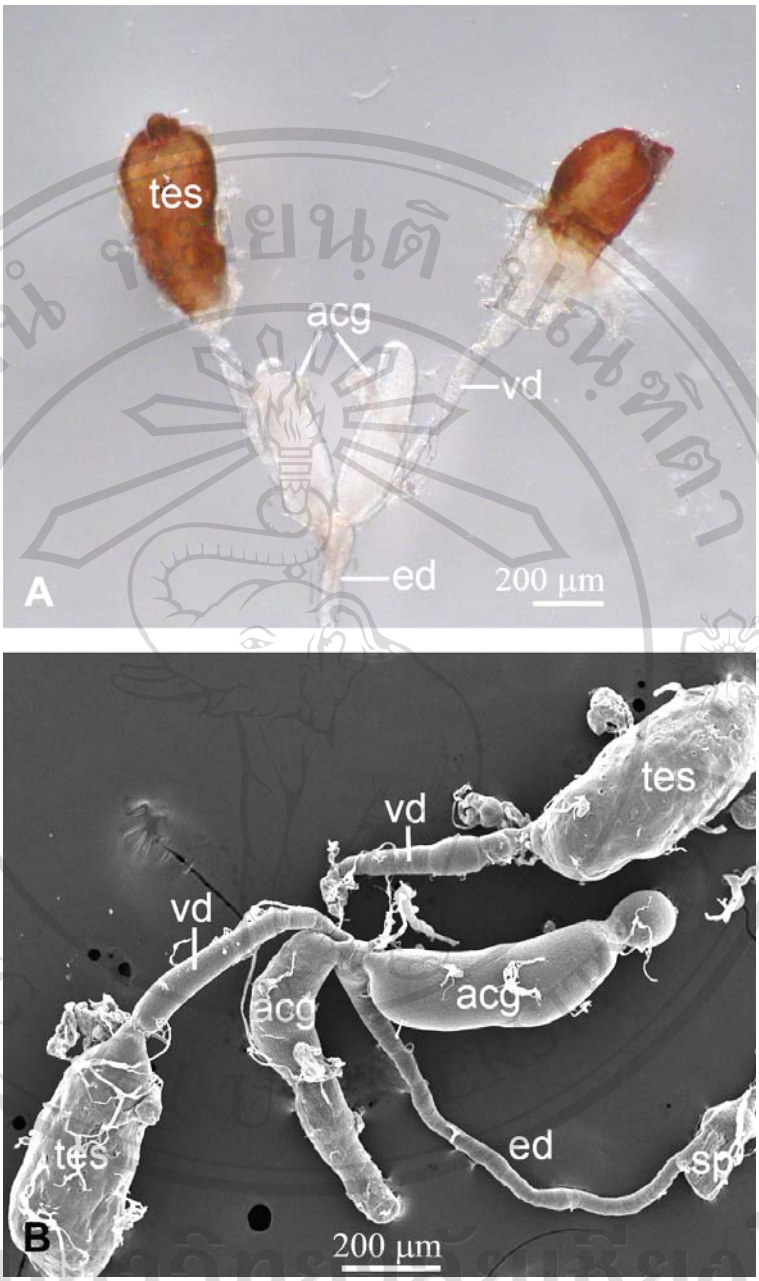

Figure 10 (A) Whole internal reproductive tract in male *C. megacephala* showing paired testes (tes), paired vas deferens (vd), paired accessory gland (acg), medial ejaculatory duct (ed) and sperm pump (sp). (B) SEM image of internal reproductive tract showing testes (tes), vas deferens (vd), accessory gland (acg), ejaculatory duct (ed) and sperm pump (sp).

1.1 Testis

Light microscope: Dissection revealed that the testes of *C. megacephala* are located in the posterior region of the abdomen and have an overall oval shape. They maintain their ellipsoidal form for a day or more after emergence, and then a characteristic constriction occurs about a third of the length from the base of the testes (Figure 11). Moreover, this constriction extends apically changes the form of the testes remarkably.

A comparison of the measurements of left and right testis revealed that there was no significant difference in either width or length as $P = 0.315$ and $P = 0.684$, respectively. Since there was no significant difference between the testes the medianof the two sides is illustrated in Figures 12A and 12B. There is an increase in testis length from day three until day five when the testis length declines until day seven. This pattern of increasing and decreasing length was repeated for the duration of the study (Figure 12A). Mediantestis width fluctuated in a less predictable manner than medianlength but the widest testis width was observed on day seven (Figure 12B).

The color of the testes of flies just after emergence is a pale orange. The color changes to become a reddish orange in 1 old-day flies. The color of the testes changes continuously from reddish orange to brown or to fuscous (Figure 13).

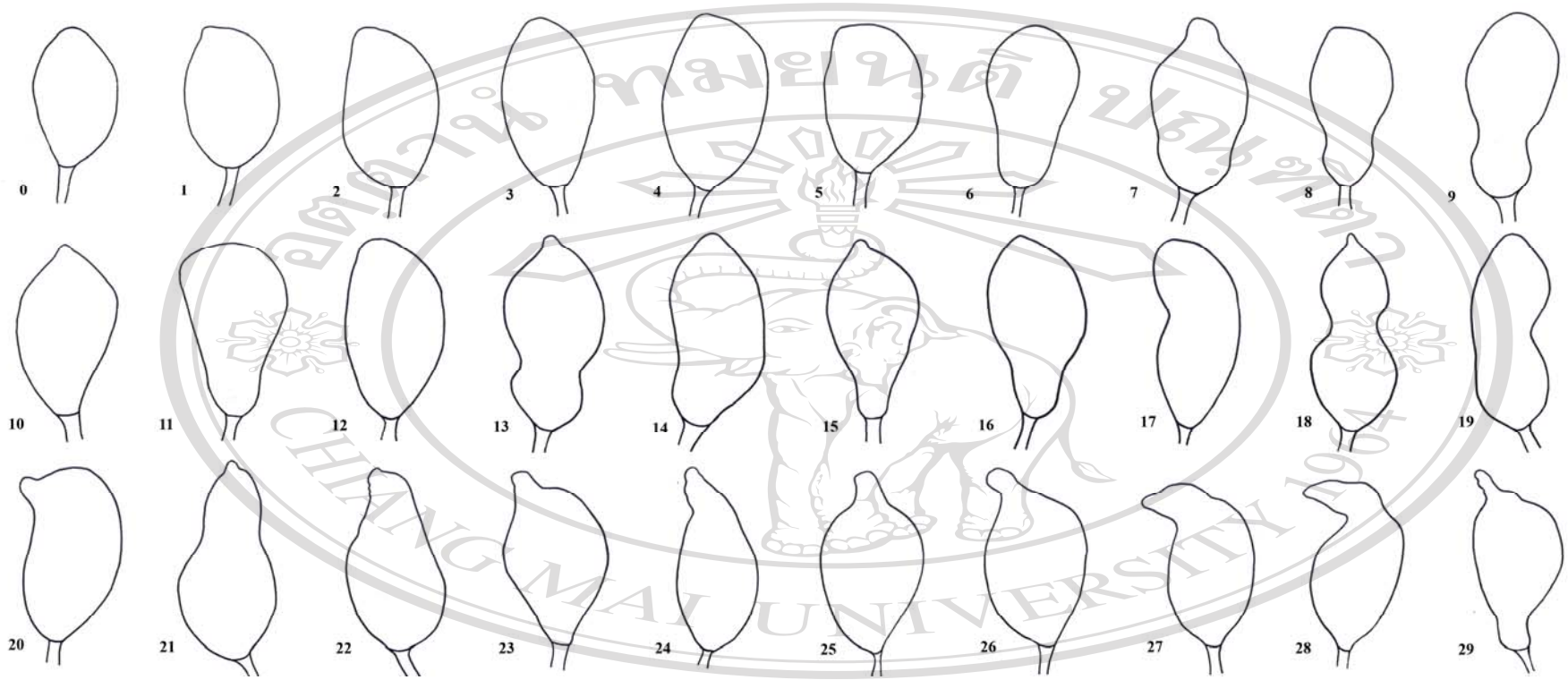

Figure 11 The illustrated of change the shape of the testes of *C. megacephala* from just after emergence (day 0) until 29 days old. The first row show illustrates day 0 to 9 day-old flies (from left to right), the second row displays 10 day-old to 19 day-old flies and the third row shows 20 day-old to 29 day-old flies. Of interest is the increase in length from day 0 to day 3 and the development of a constriction on day 7. (n = 150; selected images are used here that best illustrate maturation and development of the testes).

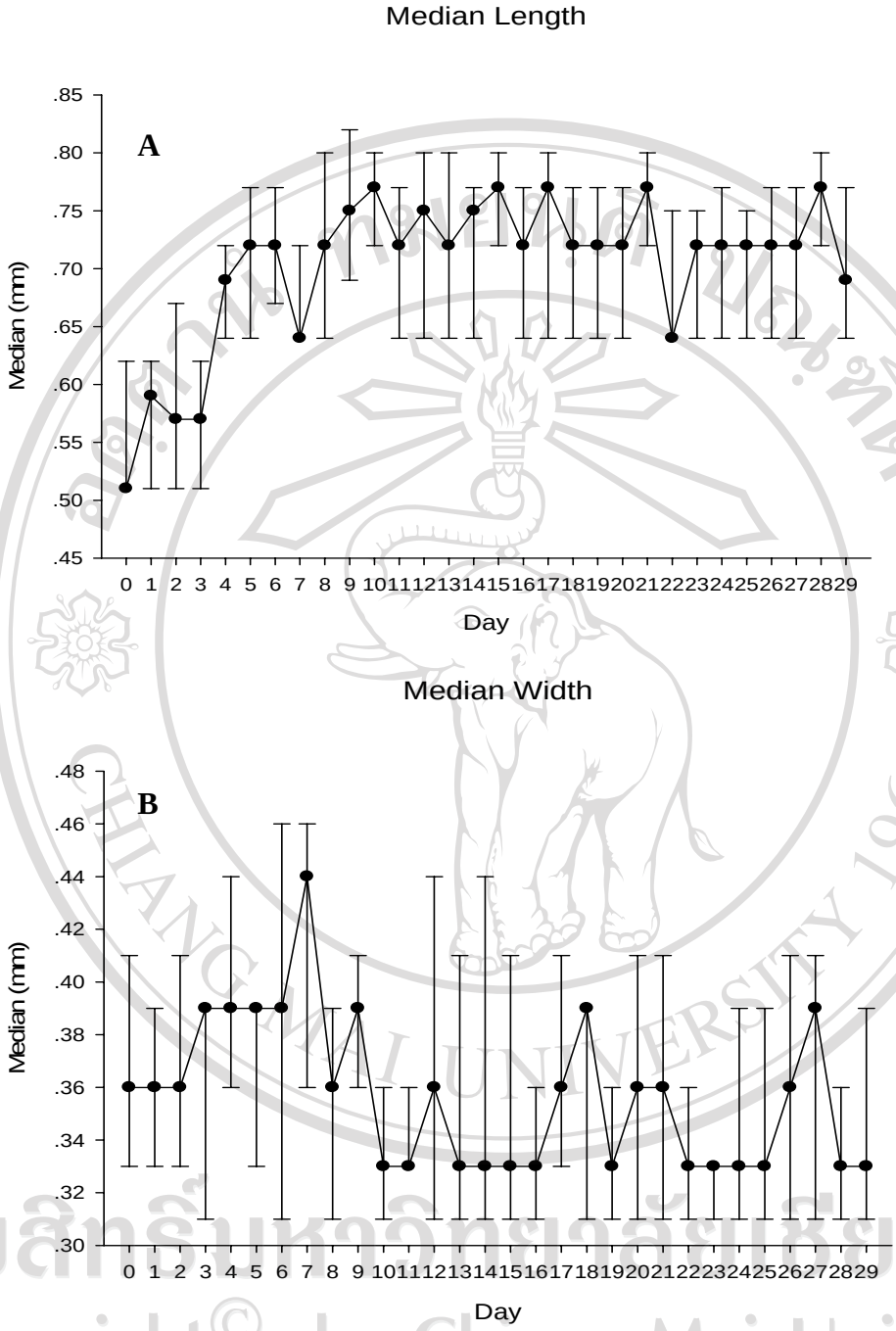

Figure 12 Median length (A) and width (B) of the testes of *C. megacephala* from just after emergence (day 0) until 29 days old illustrating the change in both length and width of the testes throughout the male fly lifetime.

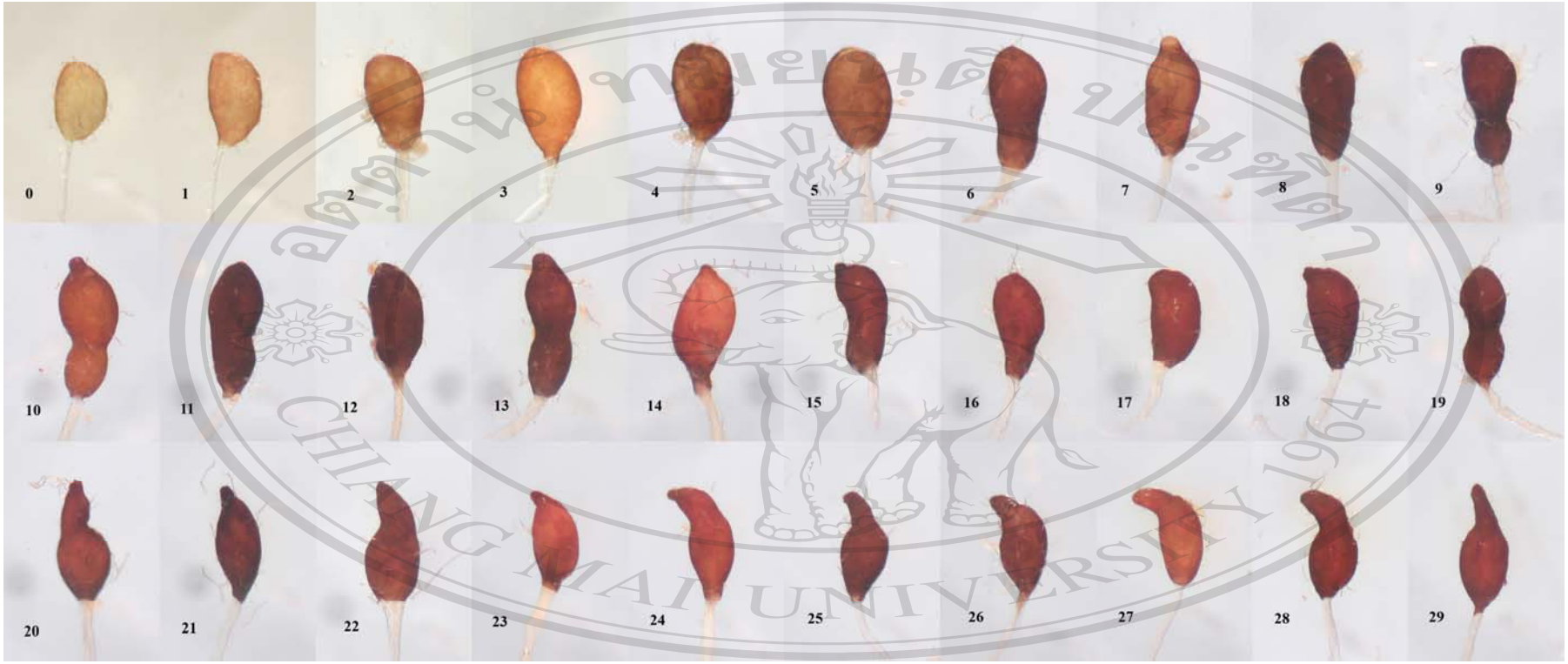

Figure 13 Light microscopy shows the progressive color change of the testes of *C. megacephala* just after emergence (day 0) until flies were 29 days old. The first row shows the color of the testes on emergence day (day 0) to 9 days old (from left to right), the second row shows 10 days old to 19 days old and the third row shows 20 days old to 29 days old. (*n* = 150; selected images are used here that best image maturation and development of the testes).

Scanning electron microscope: The scanning electron micrograph of testes shows a smooth surface and is occasionally penetrated by tracheoles (Figure 14A). Spermatozoa are released in bundles, which are twisted helically and show an unordered morphological arrangement (Figure 14B). The spermatozoa can be divided into two regions: the head characterized by an elliptical shape and the tail which appears wavy along its entire length (Figure 14C).

Transmission electron microscope: TEM micrographs showed the wall of the testes of 3 day-old *C. megacephala* is actually formed by an external layer, a peritoneal sheath, a basement membrane, and a follicular epithelium (Figures 15A, 15B). Rounded grains containing pigment, with varying density levels are spread in the cytoplasm (Figure 15B) of this layer.

The testes of 3 day-old adult males are characterized by three stages of developing spermatozoa. The first stage is the beginning of the first division of maturation, the primary spermatocyte. They are pear-shaped with large nuclei (Figure 16A). The ratio of nucleus and cytoplasm is 1:1, with chromatin that is dispersed inside the nucleus (Figure 16B). An alternate appearance of chromatin in the spermatocyte nucleus was also present (Figure 16A). In the second division of maturation, the secondary spermatocyte is characterized by the presence of elongated clusters of chromatin in the globular shaped cells (Figure 16D). The primary and secondary spermatocytes can be differentiated in the transmission electron micrograph (Figure 16C), where the third division of maturation is recognized by the appearance of bundled nuclei with a visible nuclear membrane and cytoplasm loose (Figures 16E, 16 F).

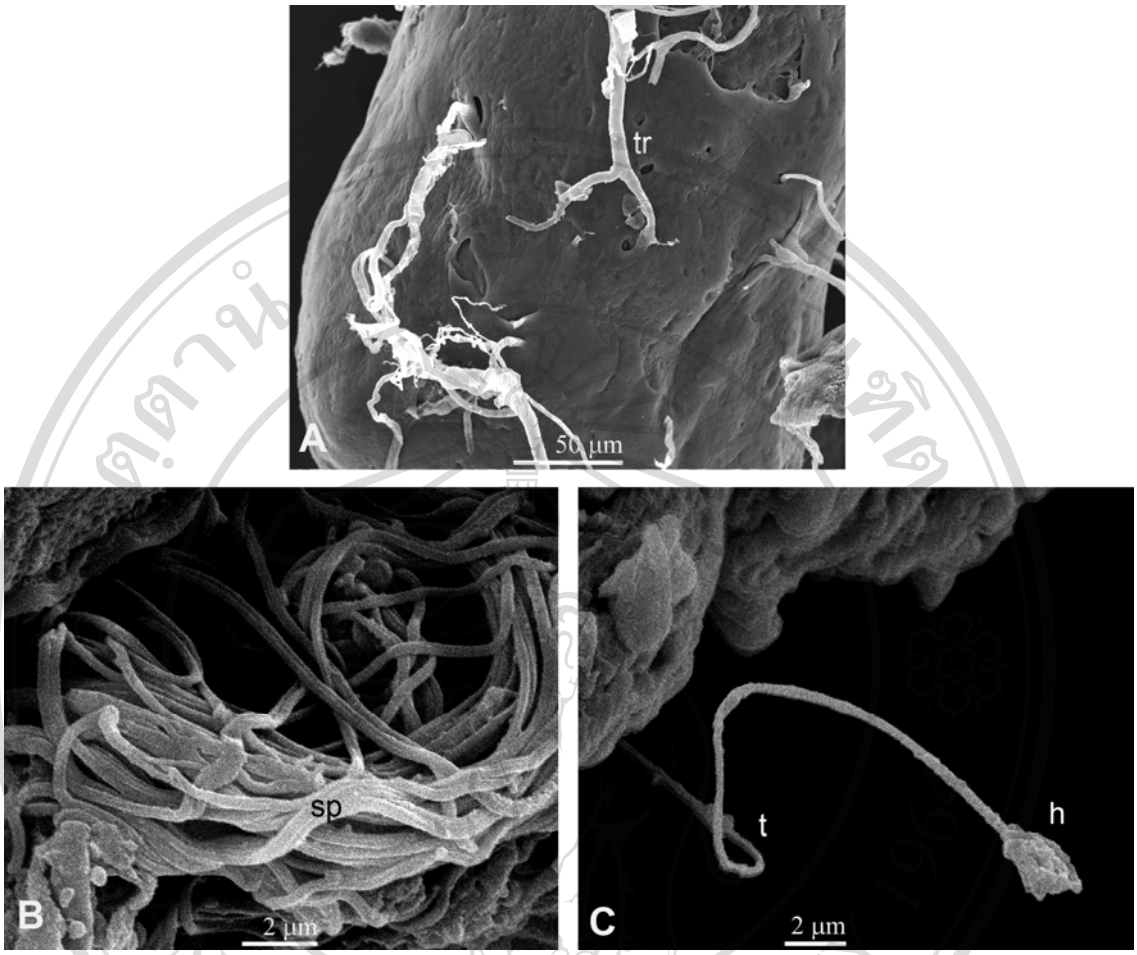

Figure 14 SEM micrographs of internal male reproductive system *C. megacephala* (A) Testis showing the smooth surface and is occasionally penetrated by tracheoles (tr). (B) Spermatozoa (sp) are released in bundles, which are helically twisted and showed a very unordered morphological arrangement. (C) The spermatozoa, with elliptical shape head (h), and wavy long tail (t).

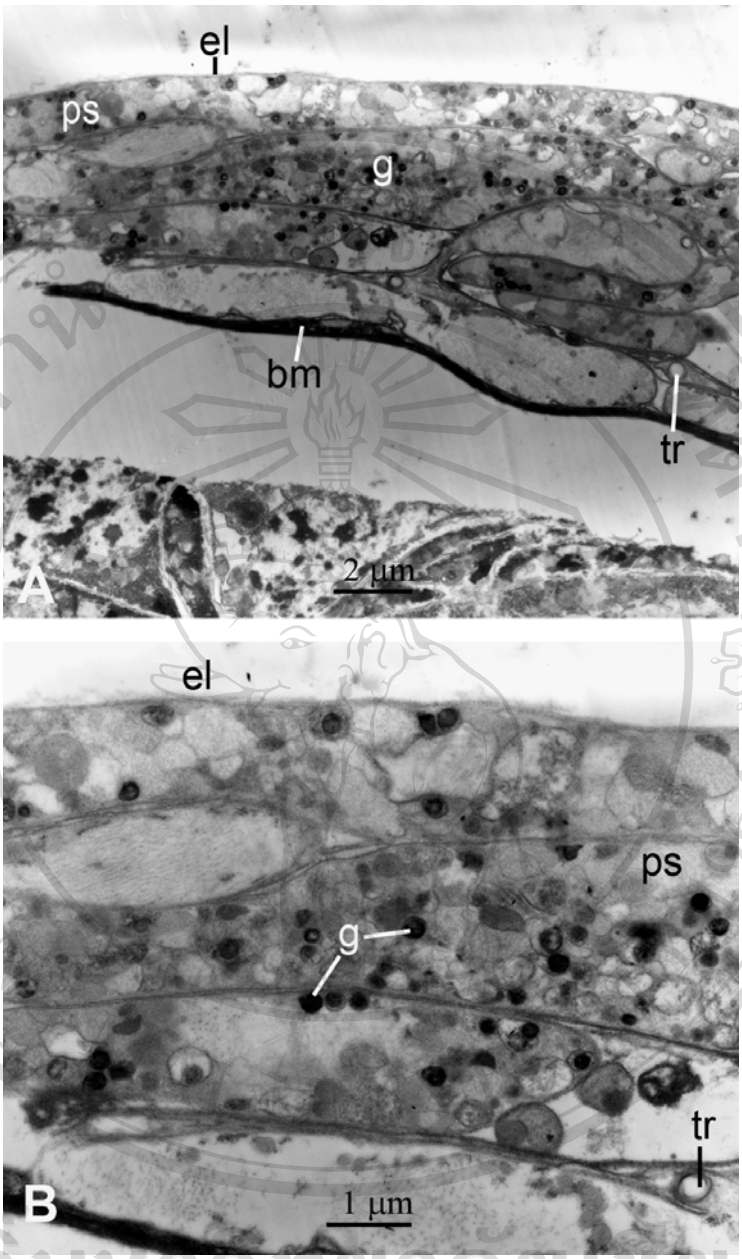

Figure 15 TEM micrographs of the testis of a 3 day-old of *C. megacephala*. (A) Transverse section showing the strata forming the testis wall. The follicular epithelium (el) and basement membrane (bm). (B) Higher magnification of the testes wall showing the different densities of the grains (g) in the peritoneal sheath (ps). Tracheoles (tr) inserting under the epithelial cells.

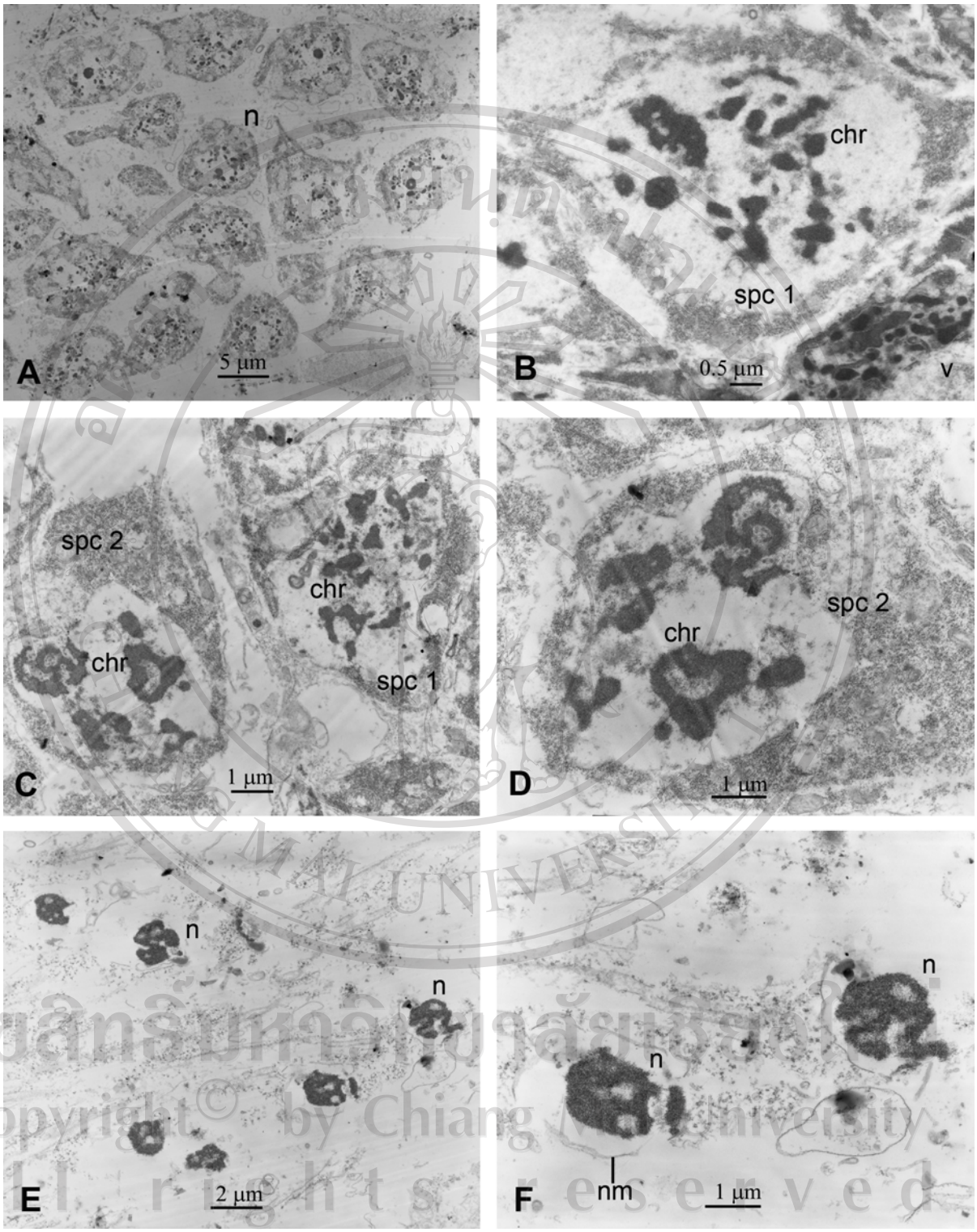

Figure 16 TEM micrographs of the testes of 3 old-day male adult *C. megacephala*. (A-B) Cross-section through the testes showing the first division of maturation, and the spermatocyte has a pear-shape with a large nucleus (n). (B) Primary spermatocyte (spc1) characterized by group chromatin material (chr) inside the nucleus. V = vacuole. (C) Primary (spc1) and secondary (spc2) spermatocyte. (D) Cross-section through the testes showing the second division of maturation. Secondary spermatocyte (spc2) characterized by four clusters of chromatin within nucleus. (E) Cross-section through the testes showing the third division of maturation, characterized by individual spermatozoa, each having nucleus (n) and elongated flagellum. (F) Higher magnification of spermatozoa showing nucleus (n) encircling with nuclear membrane (nm) and elongated flagella.

1.2 Male accessory glands

Light microscopy: The accessory glands, which were separated from the vas deferens, appeared as a thick, white paired structure and the terminal end opened into the anterior end of the ejaculatory duct (Figure 10A).

The morphometric results of five specimens using LM yield evidenced no significant difference in the median of either length (Figure 17A) or width (Figure 17B) of the left and right glands. The glands were uniform in width and diameter (Figure 17B). The age of the males significantly affected the size of the accessory glands, which increased in length and width in the 3-day-old flies (Figures 17A, 17B). The median length of the gland was highest in 5-day-old males (Figure 17A); whereas of the median width was highest in 7-day-old ones (Figure 17B).

Scanning electron microscopy: The accessory gland was a simple type, characterized by a slender tubule, elongated and sac-like, with an apical rounded end (Figure 18A). Some specimens, however, were seen as slightly constricted at the sub-apical part of the gland, and revealed better by SEM images (Figure 18B, arrows). Tracheoles inserted into the gland were frequently observed (Figures 18A, 18B).

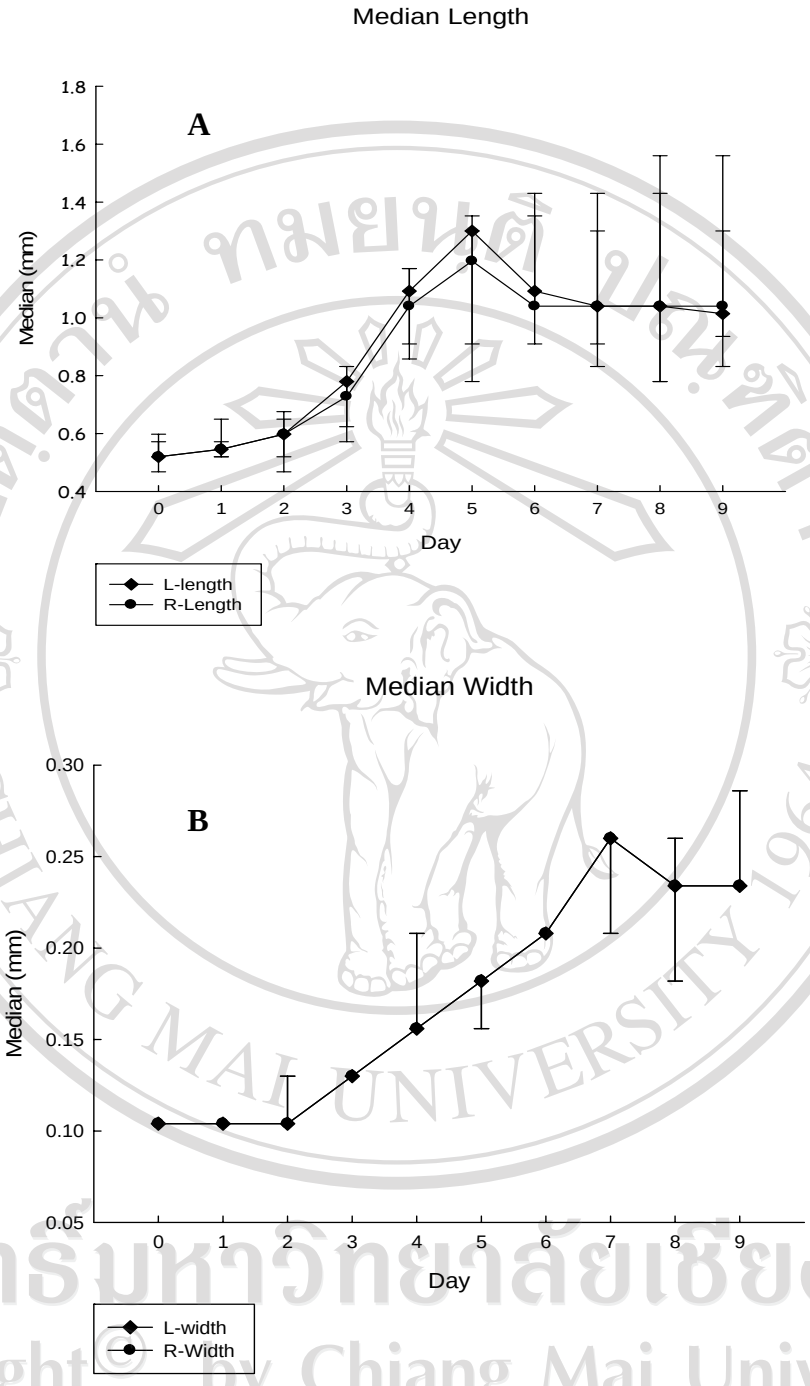

Figure 17 Median length (A) and width (B) of the male accessory glands of *C. megacephala* from just after emergence (day 0) until 9 days old illustrating the change in both length and width of the glands.

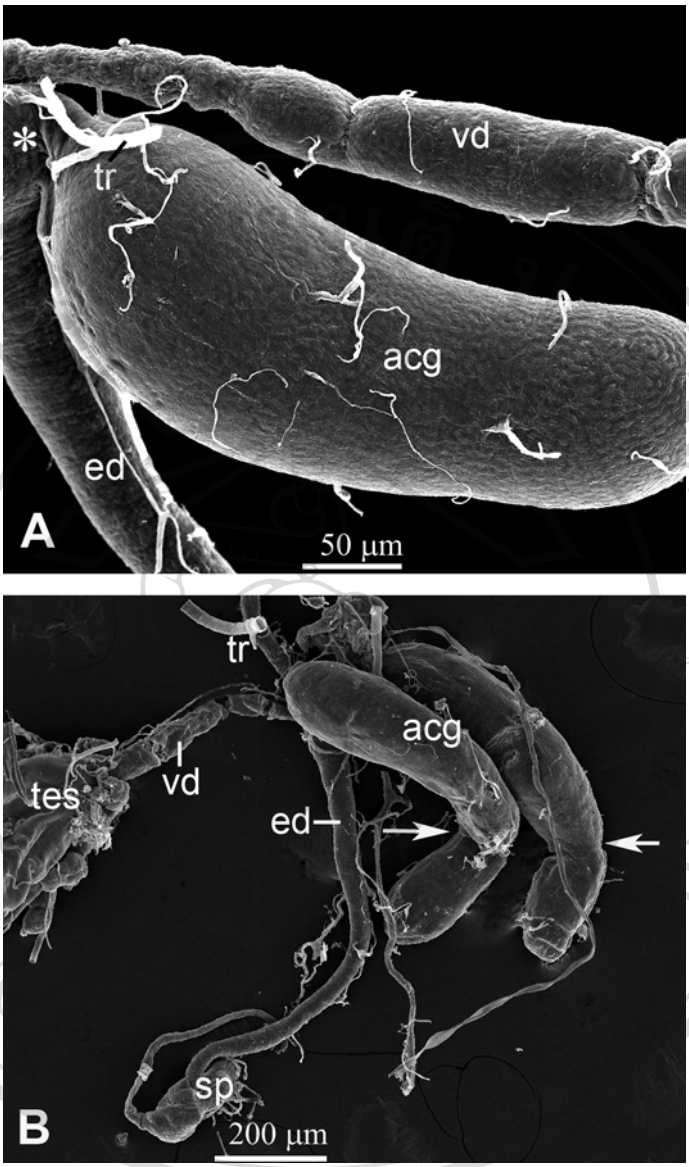

Figure 18 SEM micrographs of the accessory glands of male *C. megacephala*. (A) The accessory gland (acg) characterized by a slender tubule, elongated sac-like with apical rounded end. Tracheole (tr) inserted into the gland. Asterisk indicates the anterior end of the ejaculatory duct (ed), which has slightly globular enlargement where the terminal ends of vas deferens (vd) and accessory glands (acg) open separately into the opposite site. (B) Tubular accessory gland (acg) with sub-apical constriction (arrows) in some specimens. tes, testis; ed, ejaculatory duct; vd, vas deferens: tr, tracheole; sp, sperm pump.

Transmission electron microscopy: TEM analyses of the 3-days-old gland showed that the basal region was vacuolated; recognized by variable sizes of subcuticular cavities, whereas, the apical region was connected to the gland lumen (Figures 19A, 19B). When focused on the glandular cells, they appeared columnar bearing a large, oval-shaped nucleus with a prominent eccentric nucleous (Figure 19B). The cytoplasm of the glandular cells comprised several organelles, including numerous rough endoplasmic reticulum (RER), mitochondria, variable sizes of secretory vesicles, free polyribosomes and large numbers of vacuoles. A large nucleus contained one pack of chromatin, with a visible double-membrane nuclear envelope, which was occasionally porous (Figure 19B, arrows). Mitochondria, mostly oval and spherical in shape, were numerous but variable in size, bearing apparent cristae and membrane (Figures 19B, 19D). The RER looked active in the functional phase, appearing in two forms; mainly in the short swollen cisterns and less often in parallel stacks (Figure 19C). A group of electron-translucent content, probably the primary secretory granules, was frequently observed adjacent to the stacks or between the short swollen RER cisterns (Figure 19C). RER, which comprised the secondary secretory granules, characterized by varying electron-condensed aggregation from light to medium density within the membrane-bound inclusions, were occasionally found (Figure 19C). In some sections, large areas filled with, most probably, tertiary secretory granules were discernable in a commonly tight pack within or among glandular cells, but more evidently near the lumen (Figure 20A). When focused on the lumen, TEM sections of the 3-day-old glands revealed variable morphology of the tertiary secretory granules, giving rise to a progressive development. First, the lumen was mostly filled with moderated electron-dense materials with variable holes, and its

rim internally lined by small, heavy electron-dense materials (Figure 20B). Second, the moderately compact electron-dense materials disintegrated into numerous scattered small chains (Figure 20C). Some sections exhibited fusion of these secretory materials to form polymorphic electron-dense materials within a delicate sac (Figure 20D).

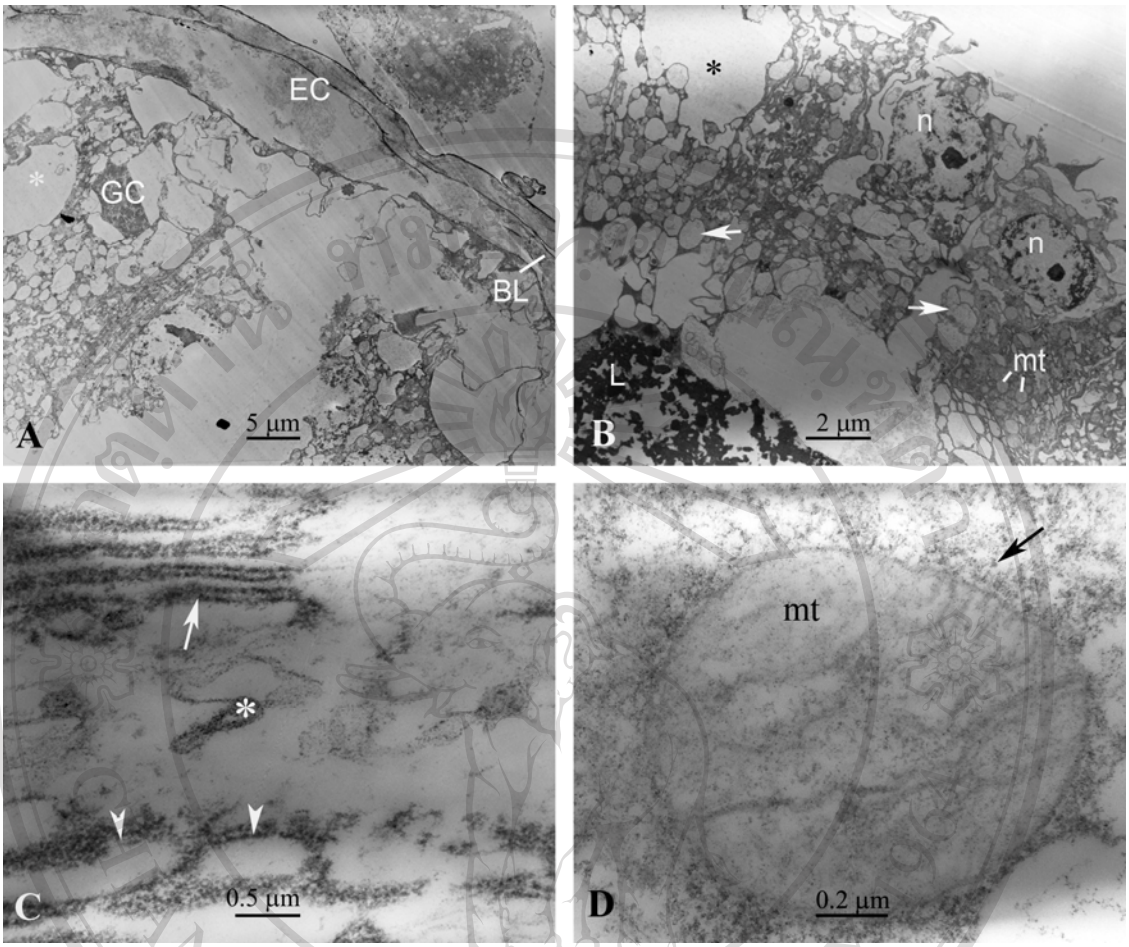

Figure 19 TEM micrographs of accessory gland composition dissected from 3-day-old male *C. megacephala*. (A) Cross-section through the middle of the gland showing epithelial cells (EC), basal lamina (BL) and glandular cells (GC). (B) Columnar glandular cell displaying a large, oval-shaped nucleus (n) and cytoplasm with numerous electron-lucent secretory vesicles (arrows). Numerous mitochondria (m) are visible. Asterisk indicates subcuticular cavity. (C) Rough endoplasmic reticulum observed in two forms; mainly in the short swollen cisterns (arrowheads) and less often in parallel stacks (arrow). Asterisk indicates electron-translucent content adjacent to rough endoplasmic reticulum. (D) Mitochondria (mt) and free polyribosome (arrow).

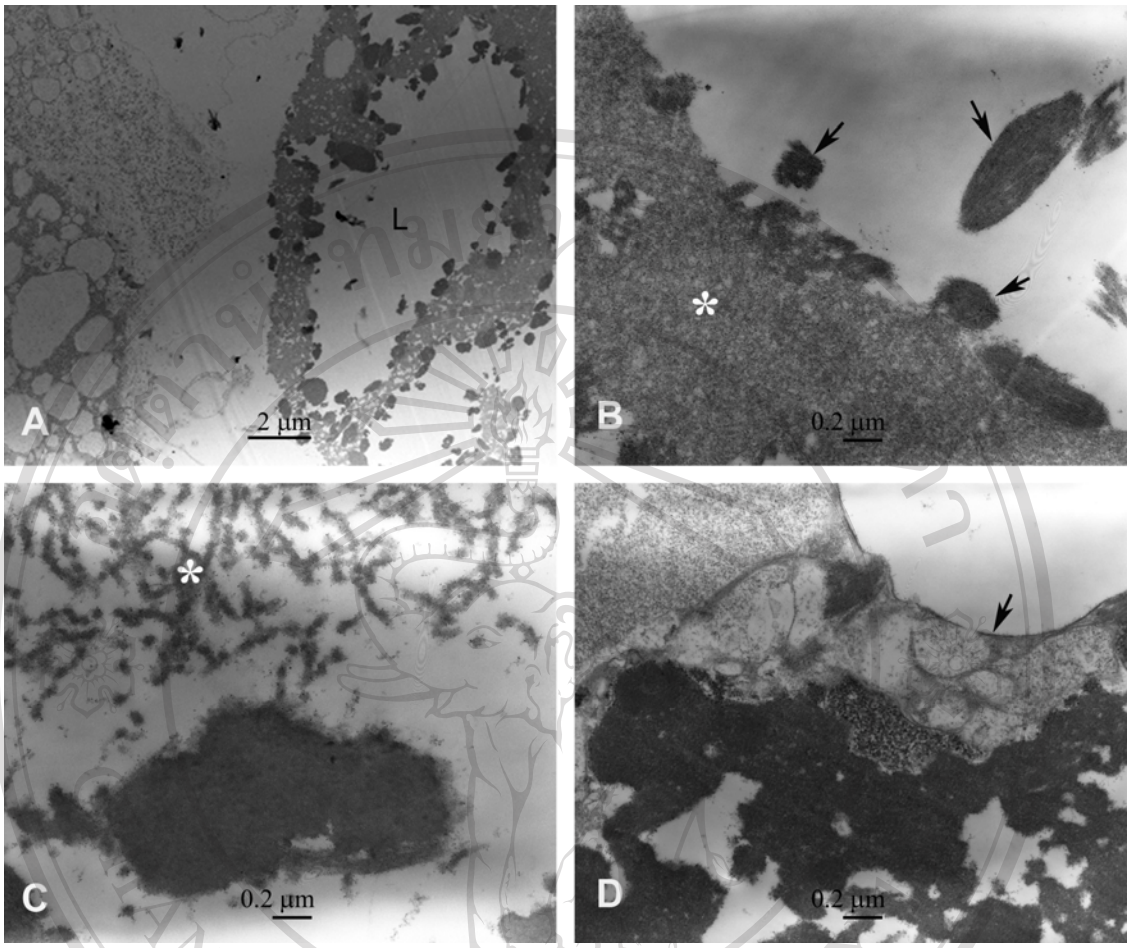

Figure 20 TEM micrographs of accessory glands dissected from 3-day-old male *C. megacephala*. (A) Cross-section displaying the gland lumen (L) with secretory materials. (B) Higher magnification of secretory materials (in Figure A) displaying densely packed secretory materials characterized by compaction of the moderated (asterisk) and heavy electron-dense materials (arrows) attached to the compacted ones. (C) Some sections revealing disintegration of the compact moderated electron-dense materials into numerous scattered small chains (asterisks). (D) The progression of some sections demonstrating fusion of the secretory materials within a delicate sac (arrow).

1.3 Vas deferent and ejaculatory duct

Light microscopy: The vas deferens of *C. megacephala* are a simple paired set of ducts that connect the testes with the ejaculatory duct. The terminal portion of the ejaculatory duct terminates to the exterior via the external organ, the aedeagus.

A comparison of the measurements of left and right vas deferens revealed that there was a significant difference in length as $P < 0.05$ for the overall median (paired *t*-test). The left vas deferens (median = 0.91 mm) was longer than the right (median = 0.86 mm) but the original anatomical position with respect to left and right of the vas deferens was not preserved during dissection so it is not possible to be certain that this corresponds to the internal orientation within the fly. The median length of the left and right vas deferens are presented in Figure 21A which shows that despite an overall difference in medianlength development was similar and there is only a single date where there was a significant difference between left and right on day 3 (non-overlapping variation). However, since the positions of right and left were not maintained and could have shifted from day to day this finding may be uninformative. The width of the vas deferens remained constant, measuring 0.06 mm for the entire observation period (Figure 21B).

Figures 22A and 22B illustrate the median length and width of the ejaculatory duct. The median ejaculatory duct length fluctuated but slowly increased similarly to the vas deferens with a peak in length on about day 5 (Figure 22A). Median duct width did not fluctuate and remained 0.078 mm for the duration of the study (Figure 22B).

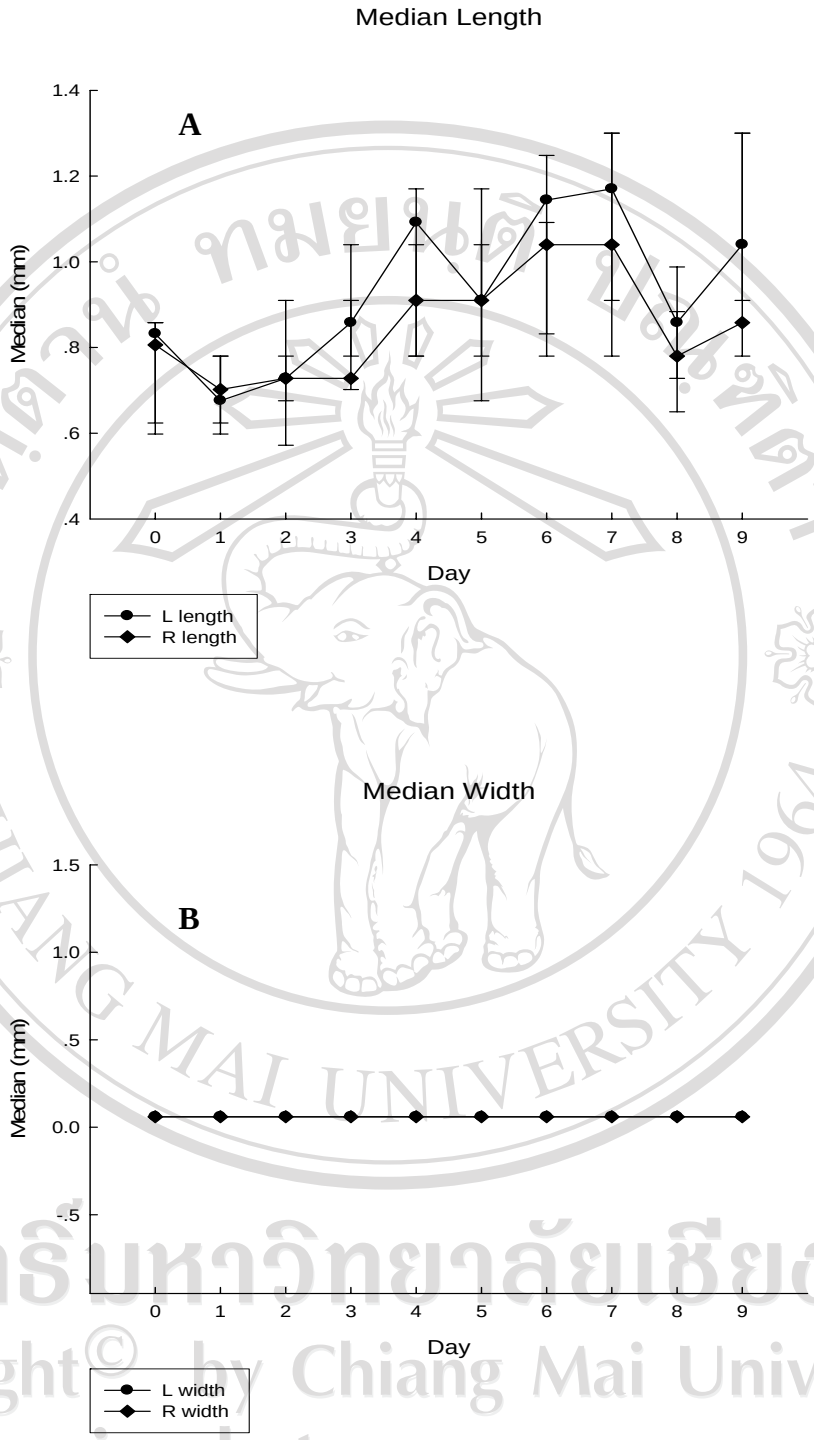

Figure 21 Median length (A) and width (B) of the vas deferens of *C. megacephala* from just after emergence (day 0) until 9 days old illustrating the change in both length and width of the ducts.

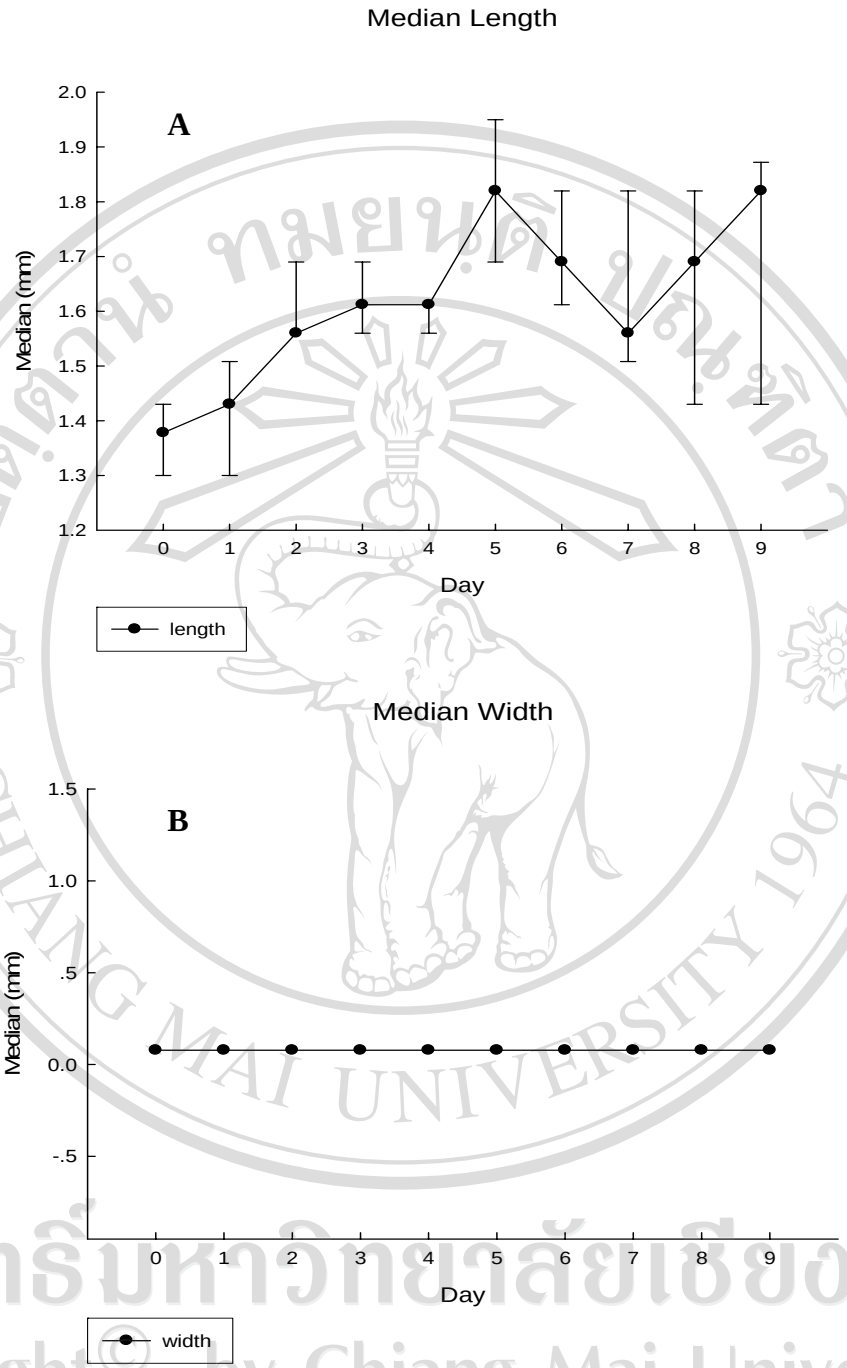

Figure 22 Median length (A) and width (B) of the ejaculatory duct of *C. megacephala* from just after emergence (day 0) until 9 days old illustrating the change in both length and width of the ducts.

Scanning electron microscopy: SEM images of male *C. megacephala* confirmed the vas deferens connection of the testes to the ejaculatory duct (Figures 10A, 10B, 18A and 18B) The images showed that the vas deferens and the ejaculatory duct are also occasionally penetrated by tracheoles (Figures 18A, 18B and 25A). SEM magnification also showed a distinct pattern consistent with longitudinal muscles visible on the surface of the ejaculatory duct (Figure 25A).

Transmission electron microscopy:

The vas deferens: The vas deferens of *C. megacephala* consist of three layers including an epithelial cell layer which is the most external layer, a connective tissue layer and a plasma membrane layer adjacent to the duct lumen (Figures 23A, 23B). The epithelium is composed of cylindrical cells which are separated from the connective tissue layer by a thick basement membrane (Figures 23B and 23C). The muscular layer and tracheoles are visible among the epithelial cells (Figures 23B and 23C). The connective tissue layer was largely composed off electron-transparent content (Figures 23A and 23B).

The plasma membrane layer located adjacent to the duct lumen (Figure 23B) is rich in rough endoplasmic reticulum (Figures 23A, 23B and 24A). The plasma membrane image showed a cell with a visible nucleus, numerous rough endoplasmic reticulum, Golgi complexes, mitochondria and secretions (Figure 24A). The nucleus of the cell imaged in the plasma membrane revealed irregularly shaped, scattered chromatins in the cytoplasm (Figures 23A, 23D). The nuclear envelope is visible as a double-membrane surrounded by mitochondria with visible cristae and free polyribosomes (Figures 24C, 24D). The Golgi complexes are composed of saccules

and have more or less electron-dense vesicles of visible size (Figure 24A). The plasma membrane layer of the vas deferens also contained myelin figures and rough endoplasmic reticulum (Figure 24B). The secretions were loosely packed fibrous materials (Figure 24A) which were visible in the lumen as well (Figure 23A).

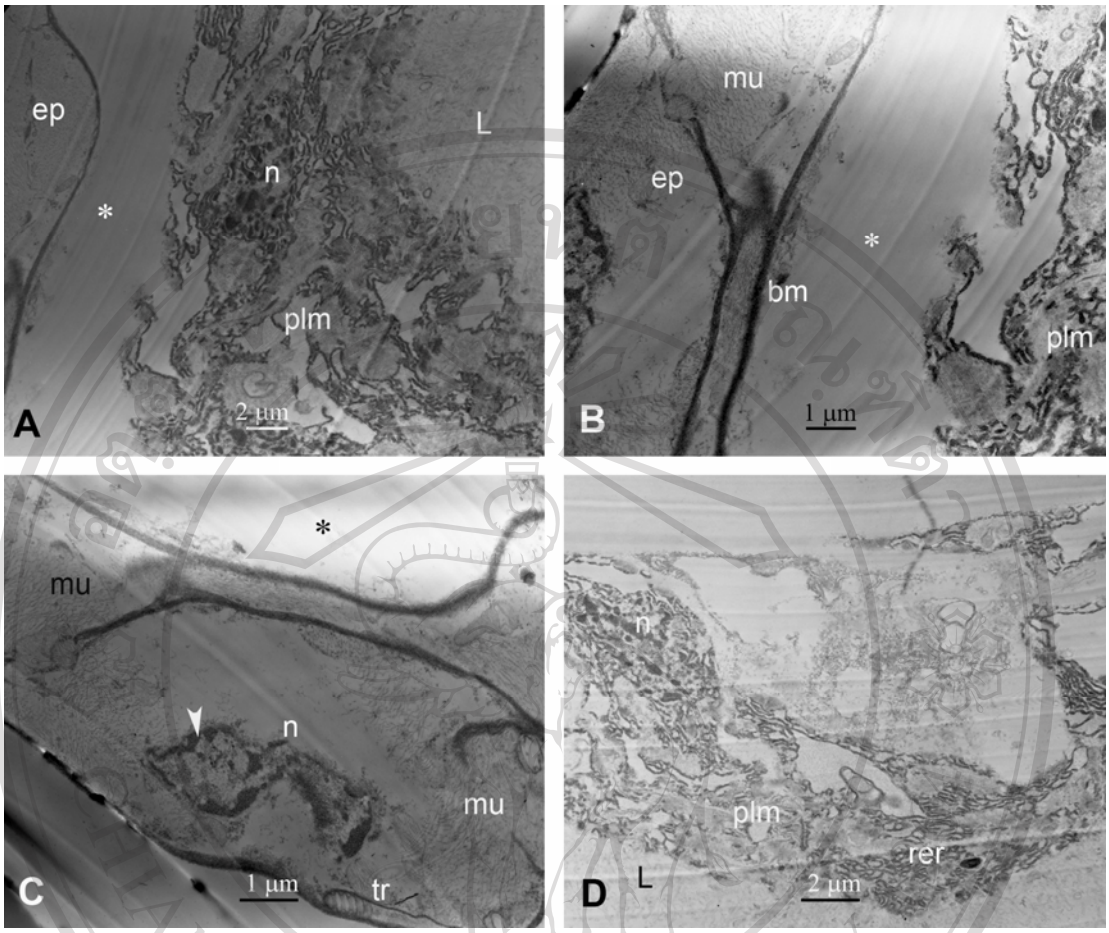

Figure 23 TEM micrographs of vas deferens of *C. megacephala*. (A-B) The vas deferens consists of three layers including the epithelial cell layer (ep) which is the most external layer, a connective tissue layer (asterisk) and a plasma membrane layer (plm) adjacent to the duct lumen (L). Muscular layer (mu) is adjacent to epithelial cell layer (ep). (C) The epithelial cell with irregular nucleus (n) having electron-dense material in the nuclear envelope (arrowhead). Muscular layer (mu) and tracheoles (tr) are visible. (D) The plasma membrane layer (plm) with nucleus (n) adjacent to the duct lumen. Rough endoplasmic reticulum (rer) also present in the cytoplasm.

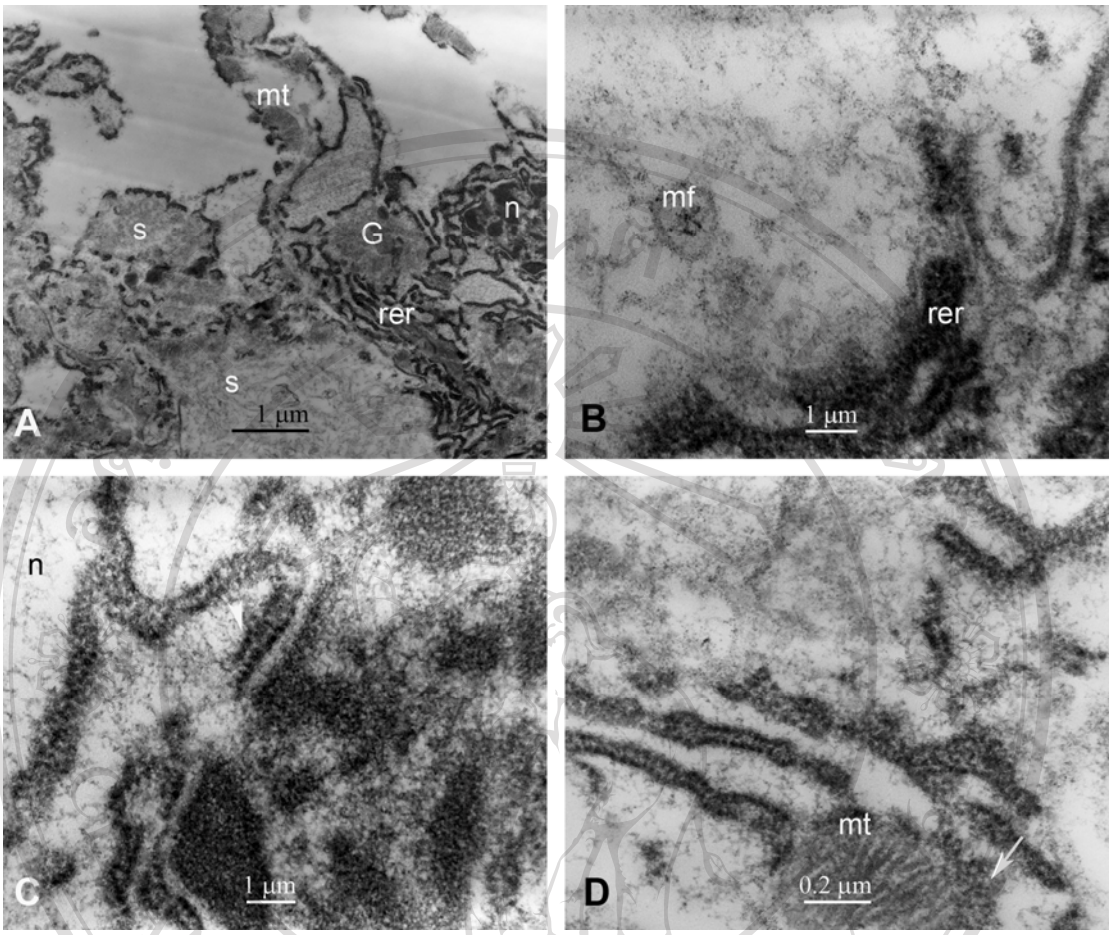

Figure 24 TEM micrographs of vas deferens of *C. megacephala*. (A) The plasma membrane layer showing the nucleus (n), rough endoplasmic reticulum (rer), Golgi complexes (G), mitochondria (mt) and secretion (s). (B) The plasma layer contains myelin figures (mf) and rough endoplasmic reticulum (rer). (C) A nucleus (n) with nuclear envelope presenting folds (arrowhead). (D) Mitochondria (mt) and free polyribosome (arrow).

The ejaculatory duct: The overall view transverse sections of the ejaculatory duct of *C. megacephala* show it is composed of epithelial and luminal cuticle (Figure 25B). The epithelial cells are flat with round nuclei and the outer epithelial layer forms the concave of microvilli (Figures 25B, 25C). Beneath the epithelial cells is the luminal cuticle (Figure 25B). An image of the region between the epithelial cells showed that it contained a microfilament and secretions with visible fibrous materials (Figure 25D). The double-membrane of the nuclei is visible in Figure 25E. The images revealed numerous mitochondria surrounding the rounded nuclei (Figure 25F). However, despite evidence of a muscular layer in the SEM images we found no evidence of this layer in the ejaculatory duct of *C. megacephala* using TEM imaging. Besides nuclei, mitochondria, microvilli and microfilament between the epithelial cells, the ejaculatory duct of this blowfly did not have any special features. Interestingly, the spermatozoa were not present in either the vas deferens or the ejaculatory duct of *C. megacephala*.

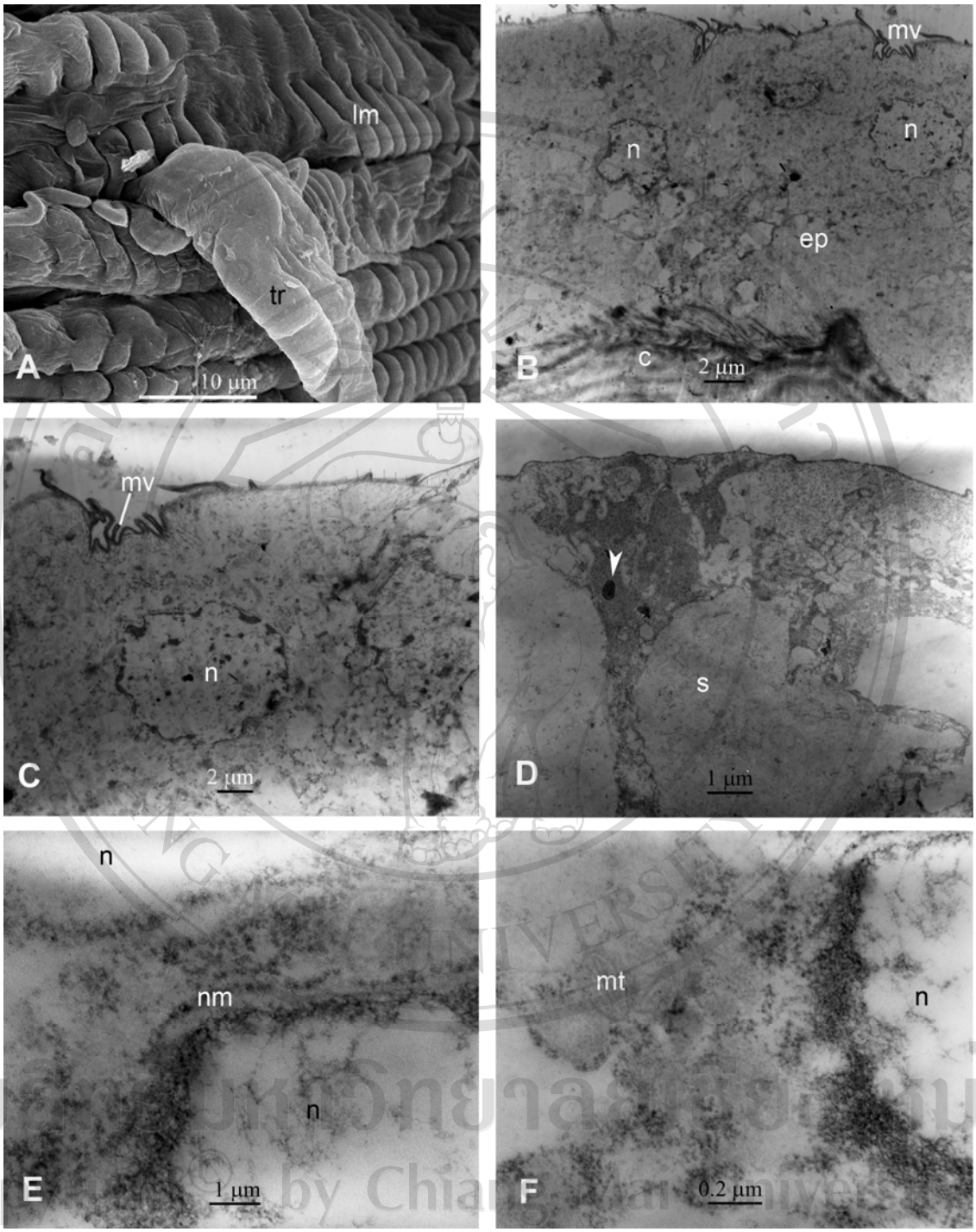

Figure 25 Electron micrographs of ejaculatory duct of male *C. megacephala*. (A) SEM micrograph on surface of the ejaculatory duct showing longitudinal muscle (lm), penetrated with tracheoles (tr). (B-F) TEM micrographs. (B) Cross section of ejaculatory duct showing the epithelial (ep) and luminal cuticle (c). (C) Apical zone of an epithelial cell showing a large nucleus (n) and microvilli (mv). (D) The region between two epithelial cells revealing a microfilament (arrowhead) and secretion (s). (E) The nuclear-membrane (nm) of a nucleus (n) in an epithelial cell. (F) Distinct mitochondria (mt) nearby nucleus (n).

2. Female reproductive organs of *Chrysomya megacephala*

The female internal reproductive organs of *C. megacephala* consist of 2 ovaries, 2 lateral oviducts, a common oviduct, 3 spermathecae and 2 accessory glands (Figures 26A, 26B). The round ovaries are placed dorsalateral to the alimentary canal, enclosed in a peritoneal sheath. The surface of the ovary was mesh surface, with usually penetrated by tubular tracheoles (Figures 26A, 26B). The short lateral oviducts, which highly convoluted, were fused to form a common oviduct leading to vagina. There were 3 rounded spermathecae of which 2 were loosely bound together. The spermathecae with tubercular surface consisted of pyriform shape with ducts entering the vagina dorsally (Figures 26A, 26B). A paired of long tubular accessory glands, opened into the dilated anterior vagina (Figures 26A, 26B).

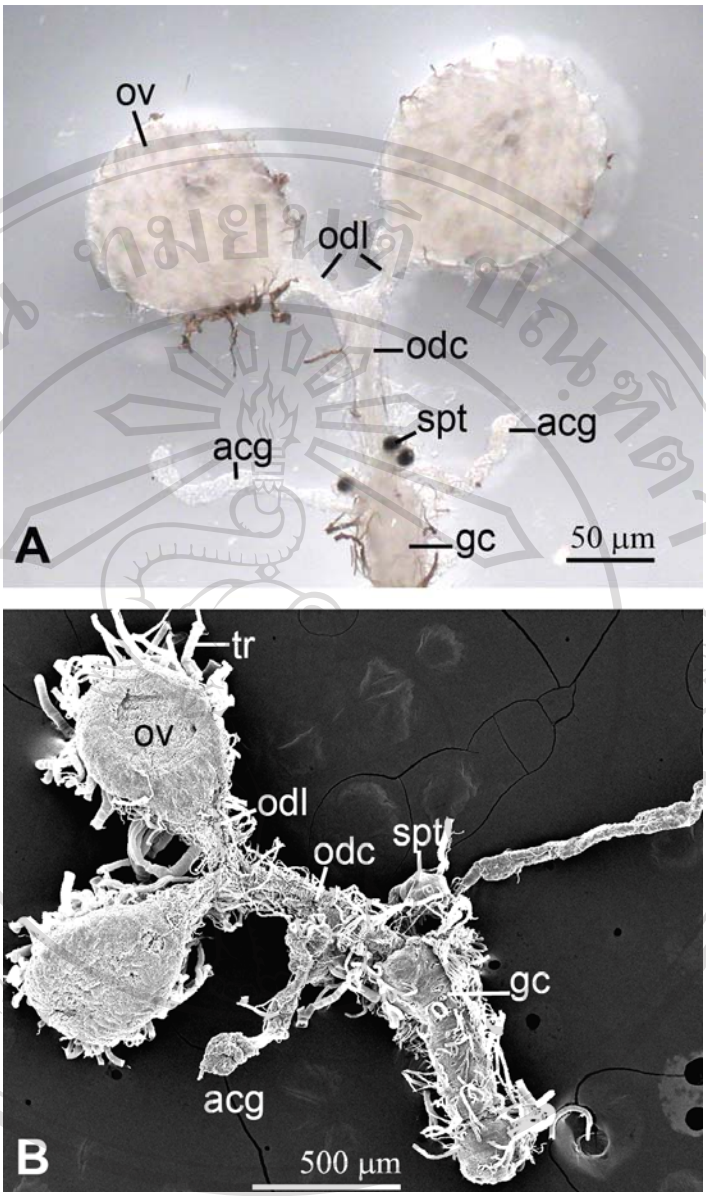

Figure 26 Micrographs of internal reproductive organs of female *C. megacephala*. (A) Light micrograph displaying paired ovaries (ov), lateral oviduct (odl), single common oviduct (odc), 3 spermathecae (spt), paired accessory glands (acg) and genital chamber (gc) or vagina. (B) SEM micrograph showing 2 ovaries (ov), 2 lateral oviducts (odl), a common oviduct (odc), 3 spermathecae (spt), 2 accessory glands (acg) and genital chamber (gc).

2.1 Ovary and ovariole

2.1.1 Ovarian morphology: Comparisons of the morphometric changes in the ovaries of 3-day-old and 9-day-old *C. megacephala* were made under light microscopy and scanning electron microscopy as shown in Figures 27A and 27B, respectively.

The two ovaries of 3-day-old *C. megacephala* are small round bodies that are white in color (Figure 27A) and the ovarioles can be observed within each ovary during egg development (Figure 29A). Each ovary in the 3-day-old fly is sheltered by an ovarian envelope, which consists of a tough outer epithelial sheath surrounding the entire ovary (Figures 29A, 29B). The ovaries of 3-day-old blow flies are also tightly enveloped by a large and well developed tracheal system, which becomes looser as the eggs mature (Figures 27A, 29A).

The ovaries of 9 day-old females appear larger and more developed than those of 3 day-old females. They reached a maximum observed size of nearly 4 mm in diameter by 9 days of age and the eggs now fully filled the ovary (Figure 27B). The ovarioles remain sheltered by the ovarian envelope, however the whole structure appears thin with many holes (Figure 29B). The tracheal system also appears to be greatly reduced in 9-day-old blow flies (Figure 27B).

The median ovary length and width of the left and right sides was approximately equal, which was confirmed by the fact that there was no significant difference between sides in length or width as $P = 0.260$ and $P = 0.159$ (paired *t*-test), respectively. The relationship between the morphometric characters of the ovaries and female age are presented in Figures 28A, 28B. There was a slow increase in the length

and width of ovaries at day 0 (just after emergence) until day five then there was a rapid increase in size of the ovary from day five to day nine (Figures 28A, 28B). These features are correlated with changes in ovariole size during egg development, which is described in the following section.

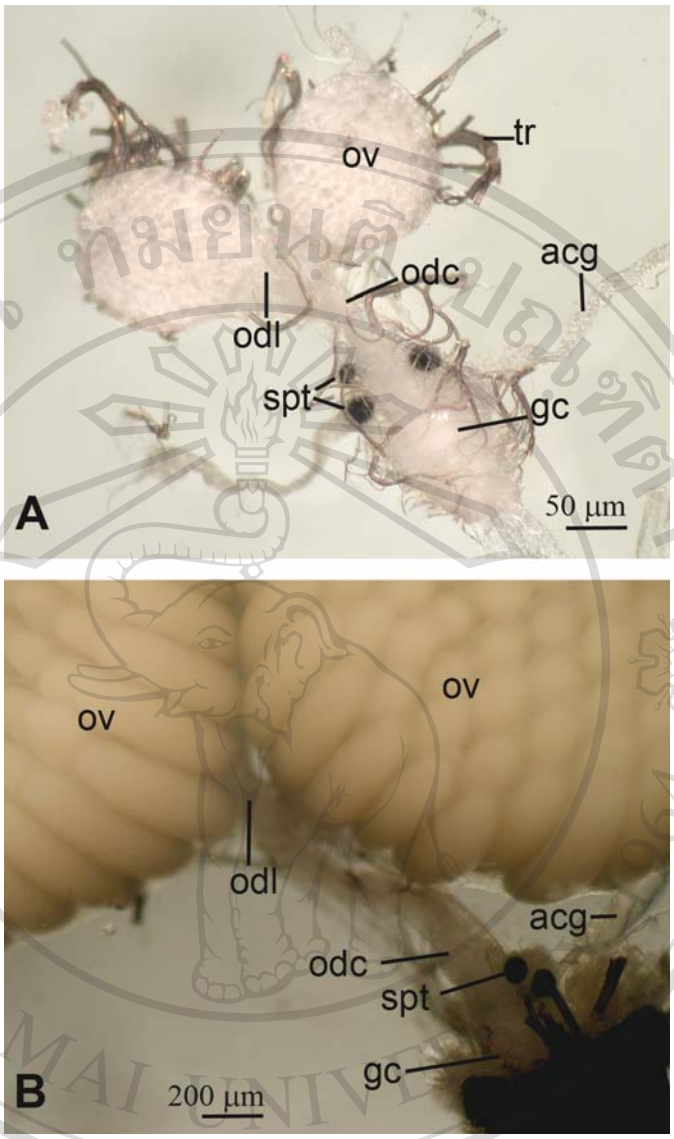

Figure 27 Light micrograph of internal reproductive organs of female *C. megacephala*. (A) Reproductive system of a 3-day-old flies showing a pair of ovaries (ov), a pair of lateral oviducts (odl), a common oviduct (odc), a pair of accessory glands (acg), 3 spermathecae (spt) and the genital chamber (gc). Tracheoles (tr) inserted into the ovary and other organs. (B) Reproductive system of a 9-day-old flies, each ovary (ov) contains mature eggs. Other organs included lateral oviducts (odl), common oviduct (odc), accessory glands (acg), spermathecae (spt), genital chamber (gc).

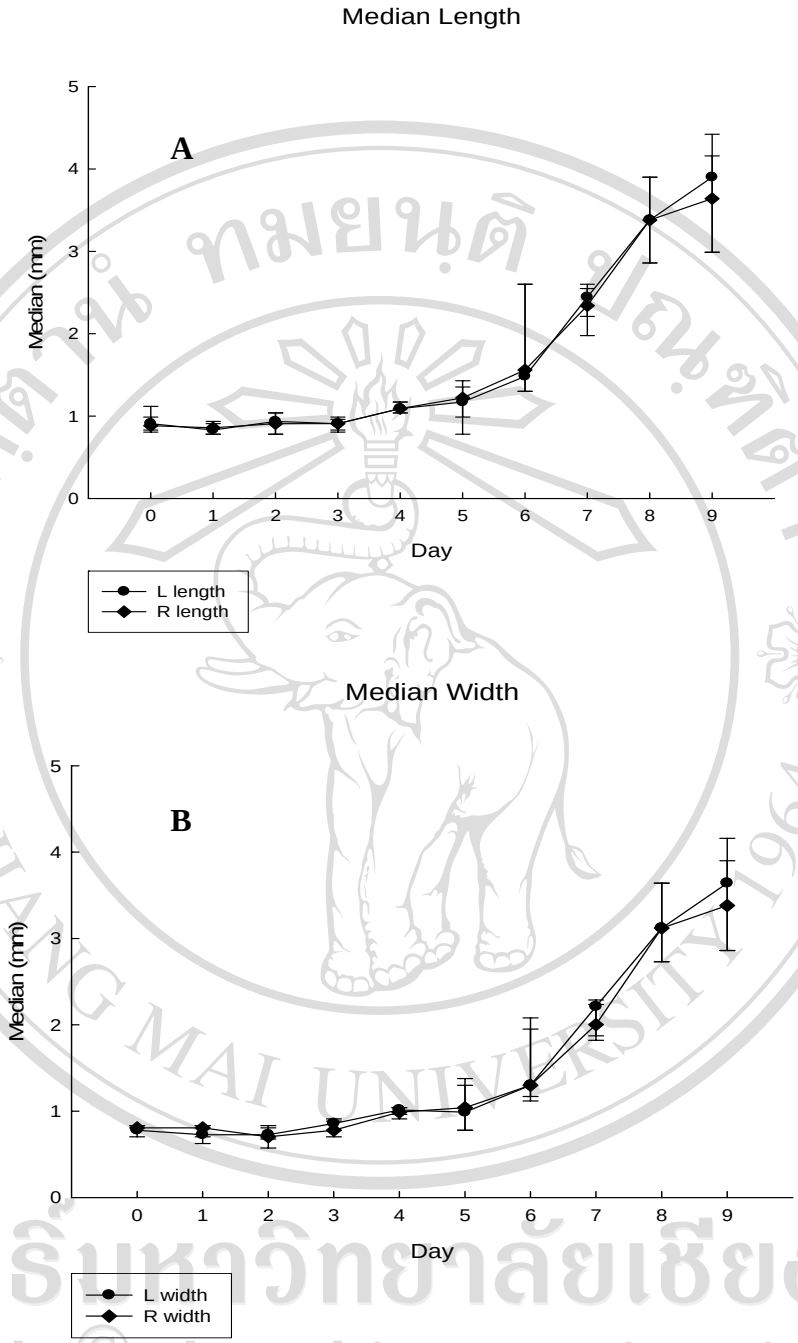

Figure 28 Median (+/- range) length (A) and width (B) of the ovaries of *C. megacephala* from just after emergence (day 0) until 9 days old illustrating the change in both length and width of the ovaries

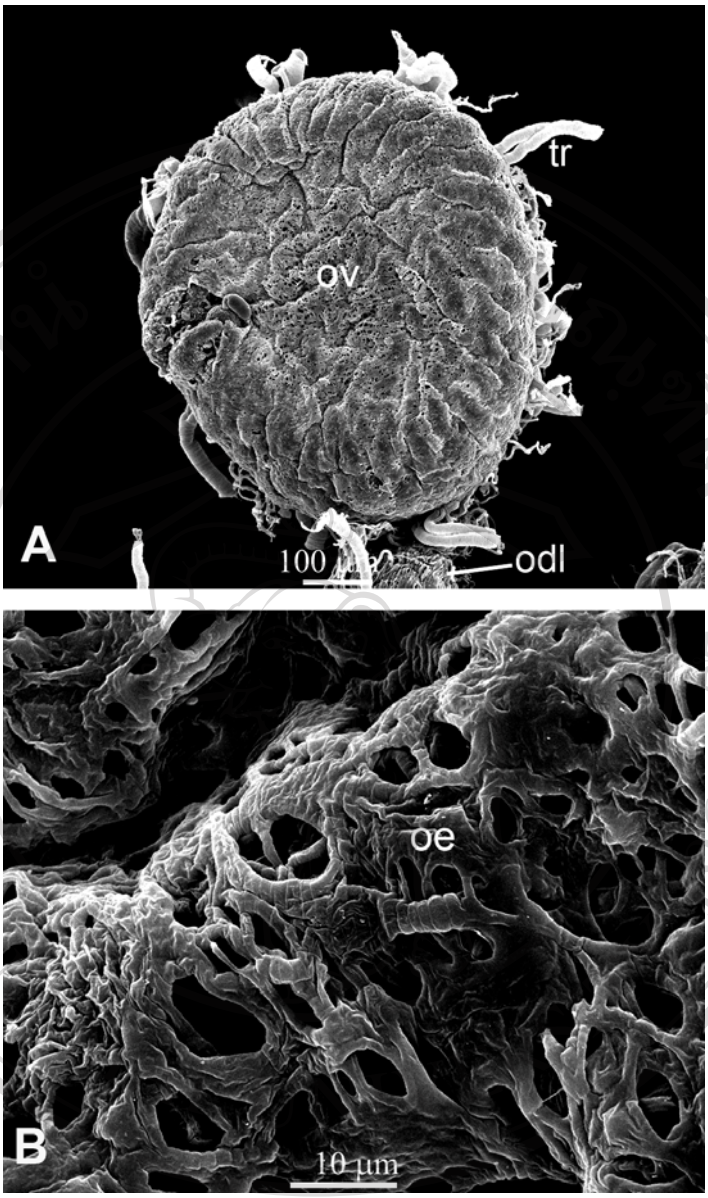

Figure 29 SEM micrographs of the ovaries of 3-day-old *C. megacephala*. (A) Overview of one ovary (ov) connecting with lateral oviduct (odl) and trachleoles (tr). (B) Ovarioles are sheltered by an ovarian envelope (oe), which is a tough outer epithelial sheath surrounding the whole ovary.

2.1.2 Changes in the ovariole during egg development:

Changes in the ovariole: The stages described here for *C. megacephala* from the basis of an aging technique that could be used to differentiate the ages of field collected female blow flies. In this experiment, the ovarioles of *C. megacephala* were observed for changes from day 0 (just after emergence) until 9-days-old under ambient temperature conditions of 18-27°C during the study period. The observations of the ovarian stages were conducted using fresh preparations in which the ovarioles were placed on a slide and covered with a cover slip. They were then examined and measured under an ordinary light microscope. The ovarioles of *C. megacephala* are of the polytrophic ovariole type (Bansal and Murad, 1987; Buning, 1998) and the characterization of each stage of ovariole maturation was conducted in a similar manner to the way in which these stages have been outlined in other blow fly species; including *Lucilia cuprina* (Diptera: Calliphoridae) (Vogt et al., 1974; Beattie and Cheney, 1979), *Chrysomya bezziana* (Diptera: Calliphoridae) (Spradbery and Sands, 1976), *Cochliomyia hominivorax* (Diptera: Calliphoridae) (Adams and Reinecke, 1979), *Chrysomya putoria* (Diptera: Calliphoridae) (Avancini and Prado, 1986) and *Chrysomya albiceps* (Diptera: Calliphoridae) (Mustafa, 1990). The correspondence between the classifications used to describe the ovarian stages of other blowflies with that of *C. megacephala* is provided in the following section.

Stage I (Day 0-2: Figure 30): Flies that have just emerged until three days old display the same ovarioles stage, in which each ovariole has a piriform germarium with a developing cyst. It is assumed that the oogonium divides into a cystoblast that

will continue this division process. The follicles are not yet well differentiated from the germarium.

Stage II (Day 3: Figure 30): The follicle is nearly spherical and distinct from the germarium. The cystocytes are clearly visible and situated in the basal region of the follicle which is surrounded by the follicular cells.

Stage III (Day 4: Figure 30): The follicle is now completely separated from the germarium with its only connection being by an interfollicular stalk and it is now nearly spherical in shape. The cystocytes are completely surrounded by the follicular epithelial cells and a more distinct separation has appeared between the germarium and the follicle in the formation of the first egg chamber.

Stage IV (day 5-6: Figure 30): The follicle is spherical and the cystocytes have differentiated into nurse cells. The follicle has enlarged considerably and the nurse cells are dispersed in the follicle chamber. However, no yolk is visible in the follicle chamber.

Stage V (Day 7: Figure 30): By this stage the follicle has increased in size and is slightly ellipsoidal in shape. Yolk deposition has increased in the oocyte and is visible at the basal region of the follicle. The follicle epithelium is clearly visible and a second follicle has started to form in the germarium.

Stage VI (Day 8: Figure 30): The follicles are now oval in shape with yolk (oocyte) occupying up to one-third to one-half of the total follicle length. The epithelium around the oocyte has become columnar and the appearance of the follicular material around the nurse chamber remains similar to that of stage V.

Stage VII (Day 9: Figure 30): The follicles are elongate, and have greatly increased in length while only slightly increasing in width (Figure 32A and 32B). The

yolk (oocyte) now accounts for two-thirds of the total follicle length. The degenerating nurse cells occupy only the anterior cone of the follicle. The follicular cells are still present but of the squamous shape and are actively secreting the chorion.

Stage VIII (Day 10: Figures 31A, 31B): In this final stage the eggs are full size with the oocyte occupying the entire follicle and the nurse cells have disappeared. The eggs of *C. megacephala* are elongate and the egg size, as measured after staining with 1% potassium permanganate, was previously recorded at 1.40 mm in length and 0.40 mm in width (Sukontason et al., 2004). In the mature egg the micropyle with a narrow plastron is clearly visible as is a fine chorionic pattern (Figure 31B, fresh preparation).

Ovarioles measurements: Figures 32A and 32B shows the considerable increases in length and width of the follicle during egg development. There is a slight increase from day 0 (just after emergence) until 6-days-old. This is followed by a increase in both length and width from day 6 to day 9 (the last day that they were observed in this study; Figures 32A, 32B)

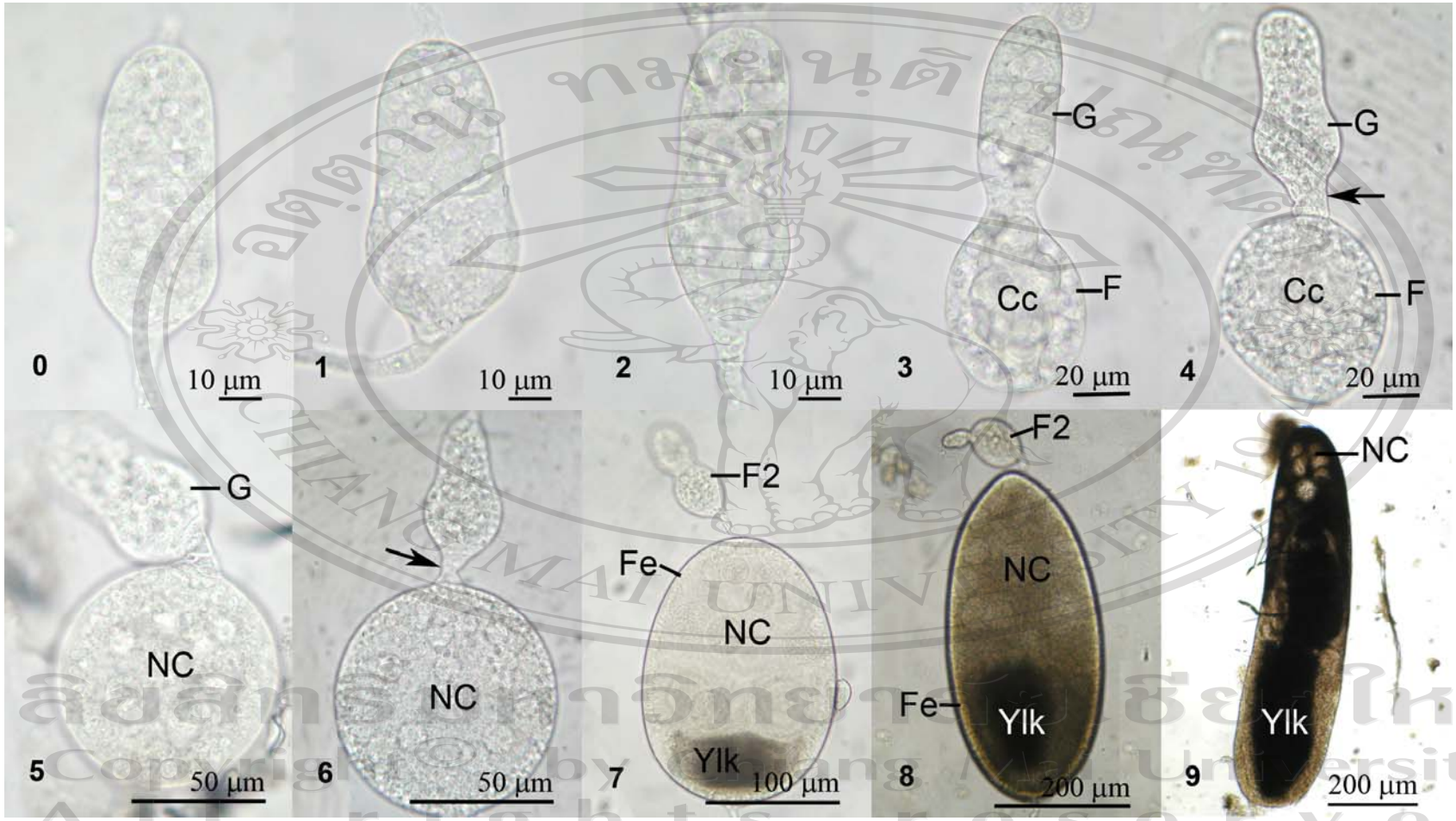

Figure 30 Light microscope images showing the development of the ovarioles of *C. megacephala* from just after emergence (day 0) until 9 days-old and illustrating the successive ovarian stages. The first row illustrates flies 0 to 4 days-old (from left to right), the second row displays flies 5 to 9 days-old. Stage I (day 0-2) showing the follicles not being well differentiated from the germarium. Stage II (day 3); follicle (F) showing germarium and the cystocytes (Cc) are clearly visible. Stage III (day 4); the first follicle is almost fully separated from the germarium (G) by an interfollicular stalk (arrow). Stage IV (day 5-6); follicle showing nurse cell (NC). Stage V (day 7); follicle elongates considerable the yolk (Ylk) at the basal region. The follicular epithelium (Fe) is clearly visible. Stage VI (day 8); One-third of the follicle is occupied by yolk (Ylk). Stage VII (day 9); Two-third of the follicle is occupied by yolk (Ylk). Note the secondary follicle (F2) at stage IV is visible in 7- and 8 day-old blow fly.

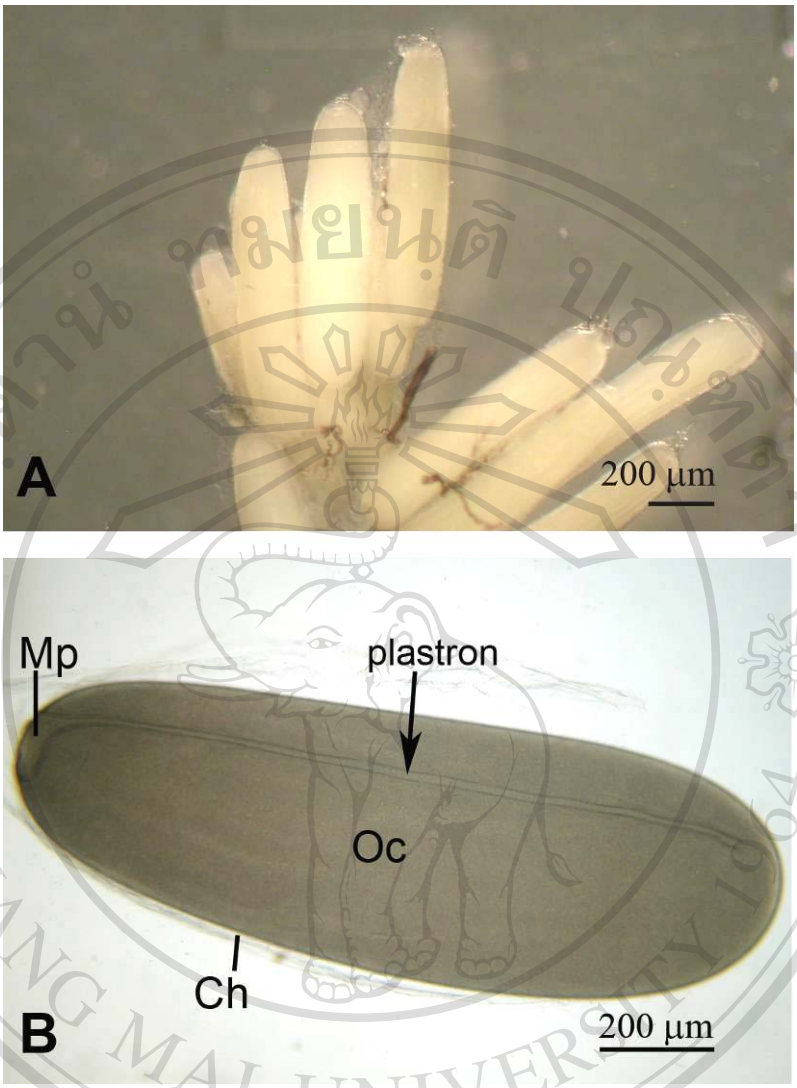

Figure 31 Light micrographs of a fully developed egg from a 10-day-old *C. megacephala*. (A) A group of eggs visualized under a dissecting light microscope illustrating the elongated overall shape of the eggs. (B) A whole egg showing the micropyle (Mp) with a narrow plastron, chorion (Ch) and oocyte (Oc)

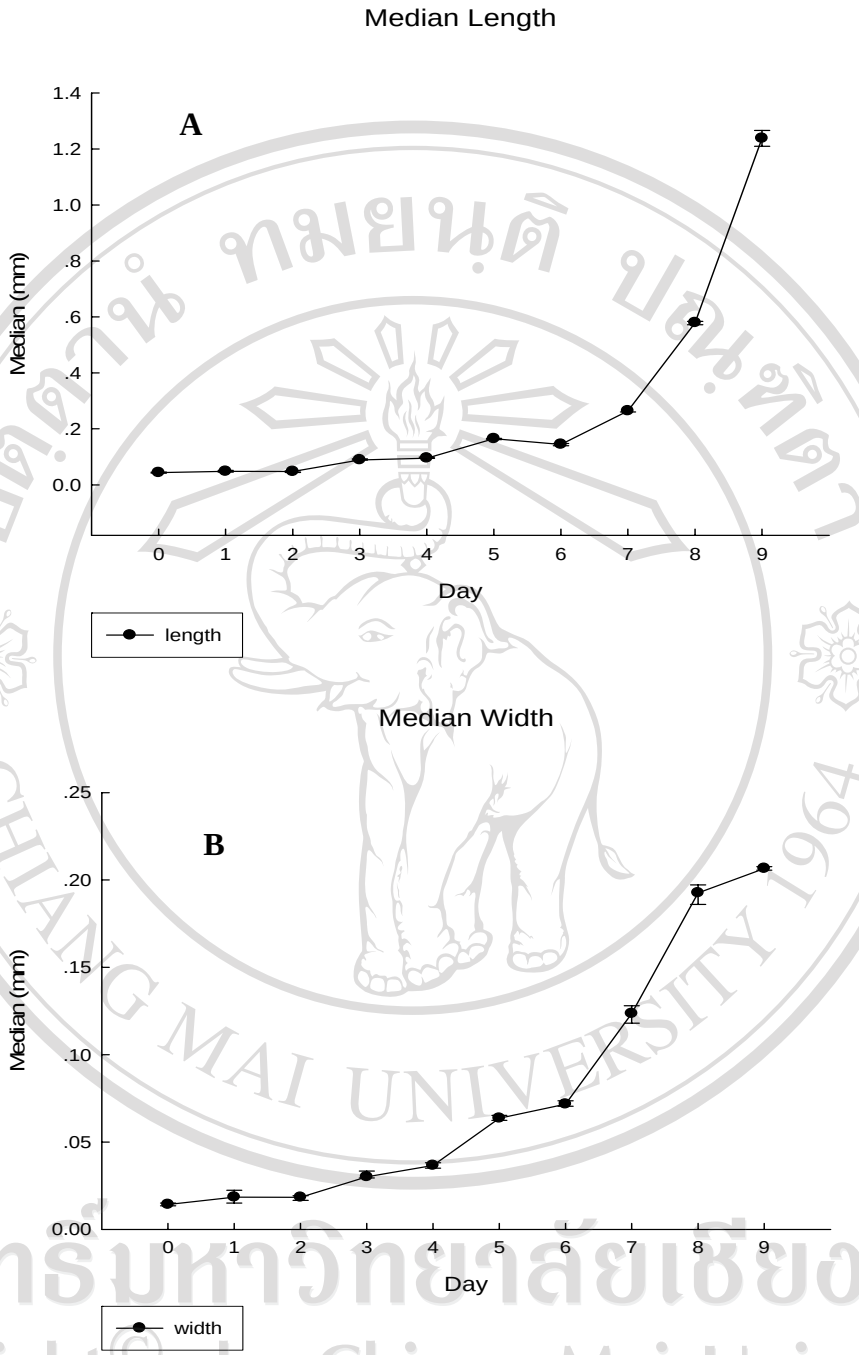

Figure 32 Median (+/- range) length (A) and width (B) of the ovarioles of *C. megacephala* from just after emergence (day 0) until 9 days-old illustrating the change in the ovarioles.

2.1.3 Development of the follicular epithelium in *C. megacephala*

This study presents the first transmission electron microscopic study of the development of the follicular epithelium in 3- and 7-day-old *C. megacephala*. The follicular epithelium surrounds the developing egg and is responsible for secreting the chorion. The cellular changes of the follicular epithelium are documented during egg development for 3-day-old and 7-day-old females in the following section.

Morphology of the follicular epithelium of 3-day-old flies: In three day old flies three layers of the developing chorion are discernable including the basal lamina, follicular cell layer and an transparent electron-dense space between this layer and the oocyte with nurse cells (Figures 33A, 33B). The follicular cells contain large nuclei are infiltrated with numerous tracheoles and a muscle layer is visible as well (Figure 33A). The nuclei are elongate along the cell axes and contain large nucleoli (Figure 33A). The cytoplasm of the oocyte contains visible mitochondria and rough endoplasmic reticulum (Figure 33B).

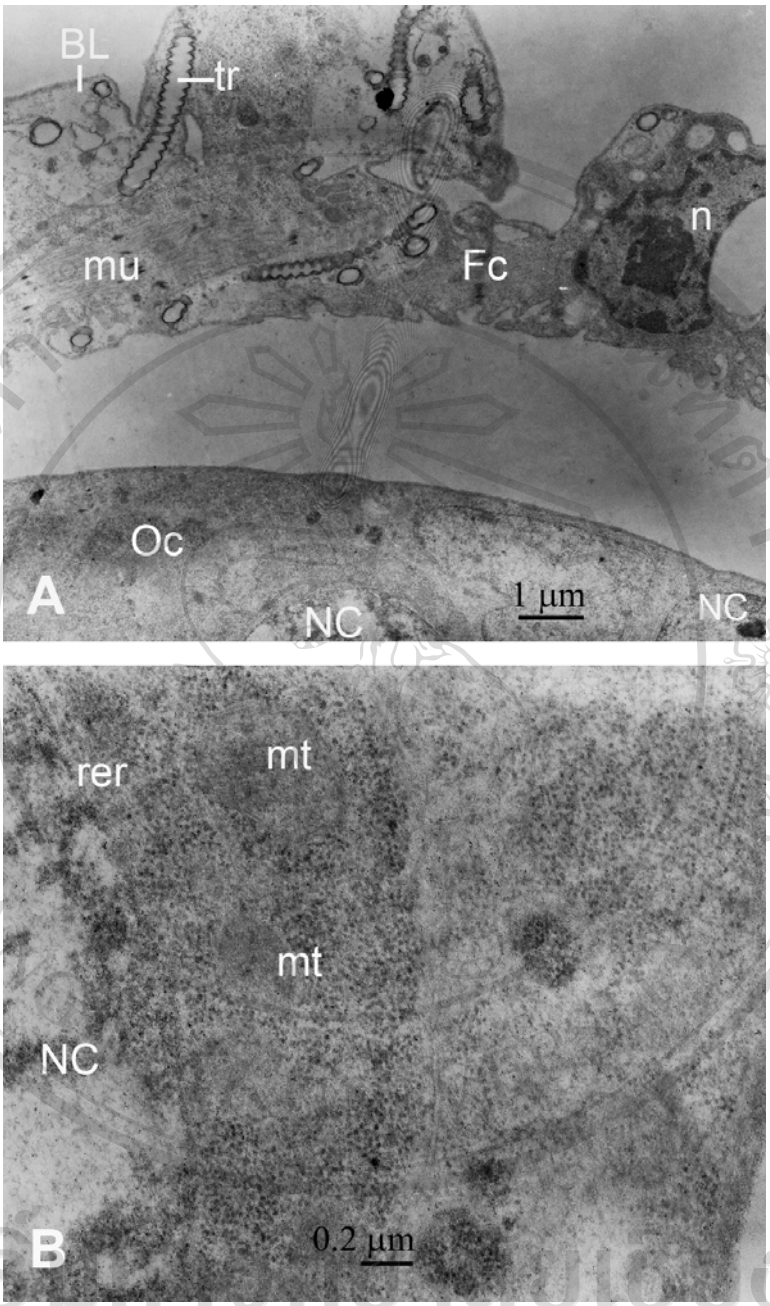

Figure 33 TEM micrographs of ovarioles of 3-day-old *C. megacephala*. (A) The chorion consists of the basal lamina (BL), follicular cells (Fc). Beneath the transparent electron-dense space is the oocyte (Oc) with nurse cells (NC). A follicular cell demonstrating the penetration of numerous tracheoles (tr), a muscle layer (mu) and elongated shaped of nucleus (n). (B) Oocyte showing mitochondria (mt) and rough endoplasmic reticulum (rer).

Morphology of the follicular epithelium of 7-day-old flies: In seven day old flies the formation of the chorion appears more complete as evidenced by the presence of the basal lamina, follicular cell layer, the outer layer of chorion and the inner layer of chorion (Figure 34A). The inner chorionic layer at the posterior pole of the egg has formed a shallow pit surrounded by a collar (Figure 34A). The bottom of this pit is the site of attachment for the egg (Figure 34A). The components of the oocyte are visible including the yolk body surrounded by the vitelline envelope (Figure 34B). The vitelline envelope is a fine granular layer quite close to the chorion (Figure 34B). Deep to the vitelline envelope the oocyte is filled with reserve materials such as yolk spheres and lipid droplets (Figure 34B).

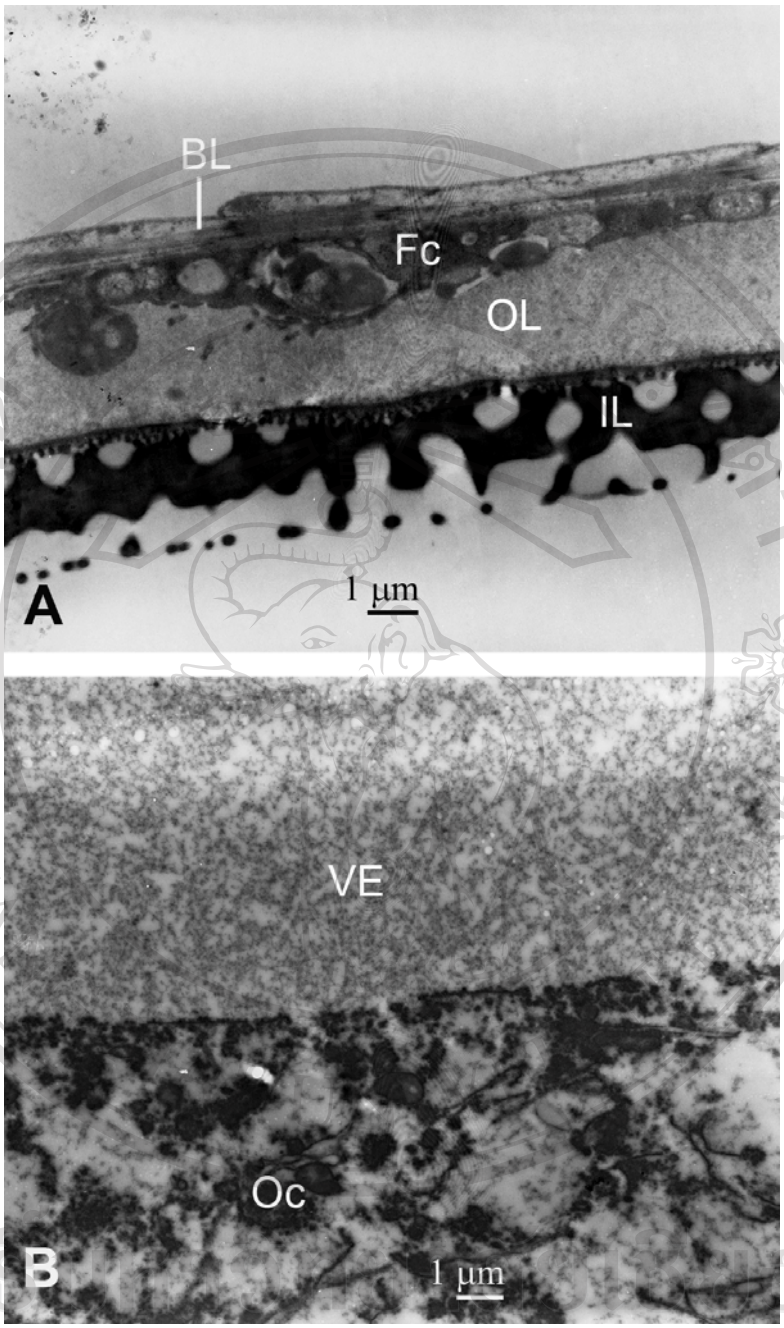

Figure 34 TEM micrographs of ovarioles of 7-day-old *C. megacephala*. (A) The developed chorion now consists of the basal lamina (BL), follicular cells (Fc), an outer layer of chorion (OL) and an inner layer of chorion (IL). (B) The oocyte (Oc), here illustrating the yolk body surrounded by the vitelline envelope (VE).

2.2 Female accessory glands

Light microscopy: The accessory glands of *C. megacephala* are long and tubular, opening into the dilated portion of the anterior genital chamber (Figures 26A, 26B). The development of the accessory glands was observed for the first 10 days of adult life, from just after emergence (day 0) until 9 days of age. Gland length was measured from the apex of the gland to the duct opening at the junction with the genital chamber. A comparison of the measurements of left and right glands revealed that there was a significant difference in length as $P < 0.05$ for the overall median (paired *t*-test). The right accessory gland (median = 2.22 mm) was longer than the left (median = 2.16 mm) but the original anatomical position with respect to left and right of the accessory gland was not preserved during dissection so it is not possible to be certain that this corresponds to the internal orientation within the fly. The median length of the left and right accessory glands are presented in Figure 35A. Gland width, measured at the widest of the apical bulb, revealed that there was no significant difference between the two sides as $P > 0.05$. Development of accessory gland width is illustrated in Figure 35B.

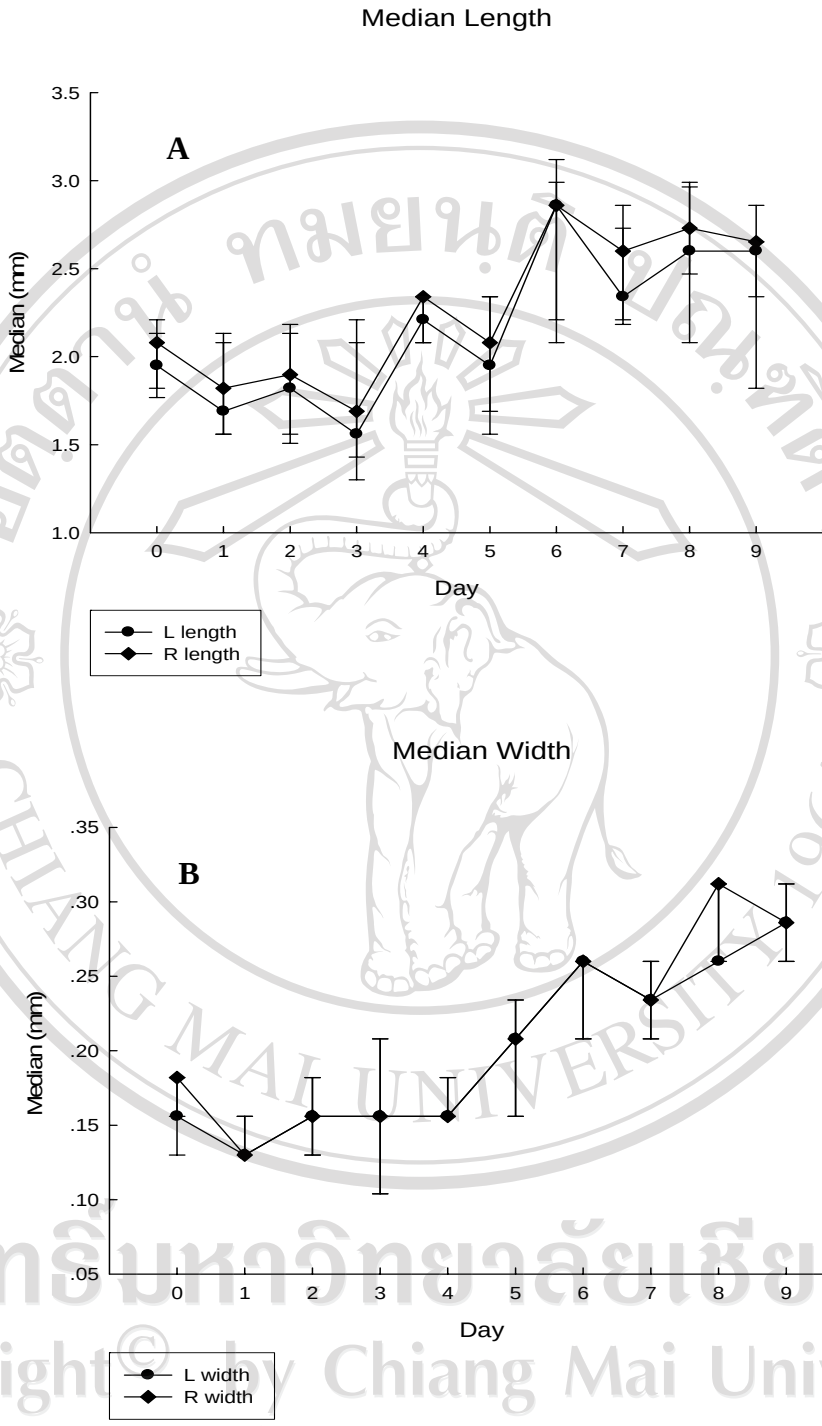

Figure 35 Median length (A) and width (B) of the female accessory glands of *C. megacephala* from just after emergence (day 0) until 9 days old illustrating the change in both length and width of the glands.

Scanning electron microscopy: SEM micrographs confirmed that the accessory glands are a paired structure opening to the proximal end of the genital chamber (Figure 26B). The level of detail revealed by the SEM images was much higher than that obtained with light microscopy. These images showed that each gland has two distinct portions including an apical bulb and a tubular duct (Figure 26B). The apical region is a dilated region forming an elliptically shaped apical bulb. This area is characterized by numerous papillae and is occasionally penetrated by tracheoles. The region of the accessory gland adjacent to the genital chamber is a long and tubular gland duct. The surface of the tubular duct is also covered in papillae with penetrating tracheoles (Figure 36). Neither of these features were observable under light microscopy.

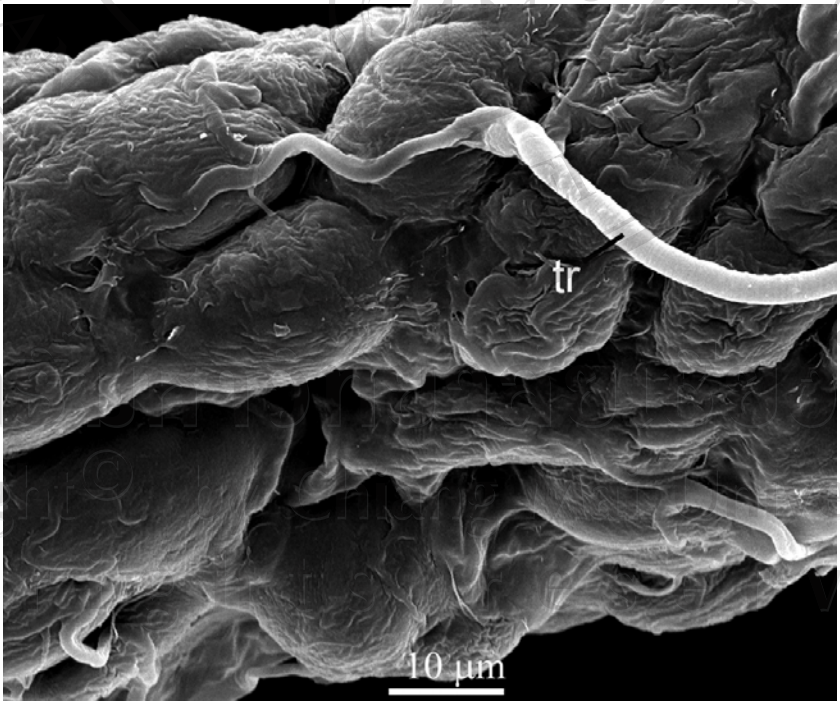

Figure 36 SEM micrograph of female accessory glands of *C. megacephala* showing basal region covered in numerous papillae and penetrated by tracheoles (tr).

Transmission electron microscopy of accessory glands of 3 day-old females: The epithelial cells of the apical bulb and tubular gland duct of 3 day-old flies are displayed in Figures 37A, 37B. The transverse section through the epithelial cells of the apical bulb of the gland at 3 days old illustrated in Figure 37A displays a single cell with a large central nucleus and a Golgi apparatus visible in the cytoplasm. Deep to the epithelial cell is the lining of the duct-forming cells and thin cuticle, which project toward the gland lumen (Figure 37A). The cistern cells, where large secretions are stored, are also visible adjacent to the epithelial cell (Figure 37A). The lumen of the cistern is apparently empty in this young female accessory gland and appears transparent electron dense (Figure 37A). Figure 37B displays a transverse section through the tubular portion of the gland, comprising the duct, of a 3day-old fly. This region contains secretory granules and vesicles of variable sizes that are present in the cytoplasm of the secretory cells in this region (Figure 37B). The cistern cells contain secretory materials to be delivered directly to the gland lumen (Figure 37B).

Replicated transverse sections along the long axis of the accessory gland of 3 day-old *C. megacephala* revealed cells in various stages of maturation which could be divided into three main stages. The first stage of maturation was characterized by the presence of numerous rough endoplasmic reticulum, secretory granules in the cytoplasm and cistern cells filled with secretory materials (Figures 38A, 38B). Each rough endoplasmic reticulum was characterized in this stage by having five distinct long channels (Figures 38A, 38B) The lumen of one of the imaged cistern cells contained fine fibrous material which is suggestive of a pore in the cuticle lining the lumen and thus a connection with the lumen of the gland (Figure 38C) (Dallai et al.,

2008). The second stage of maturation was characterized by the presence of a large cistern cell in the cytoplasm which was surrounded by mitochondria and secretory granules (Figure 38C). In the lumen of the cistern cell fibrous material was also observed as in the first stage (Figure 38C). The secretory granules seem to be connected with the lumen of the cistern cell suggesting that they release secretions into the lumen of cistern cell which are in turn released into the gland lumen through pores in the cuticle. In the third stage of maturation secretory granules, mitochondria, and rough endoplasmic reticulum were still observed in the cytoplasm but the rough endoplasmic reticulum of the third stage were more swollen in shape (Figure 39A). The cistern cells in this stage appeared to contain two types of secretions including one that was dark color and one that was pale color.

In the main accessory gland lumen homogenous dense secretions were present in low volume, with about 15% of the total area comprising secretions. There was also a transparent electron-dense space with between the epithelial cells and the gland lumen (Figure 39B).

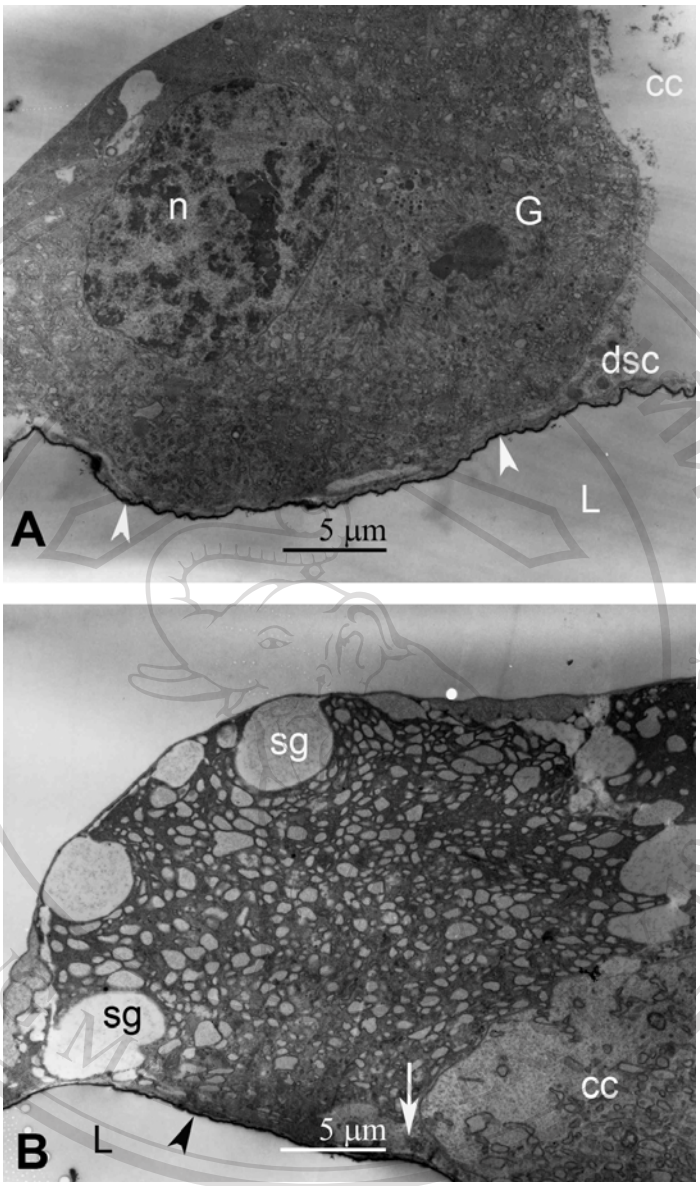

Figure 37 TEM micrographs of the epithelial cells of female accessory glands of 3 day-old *C. megacephala*. (A) Transverse section through epithelial cells of the apical bulb of gland showing the nucleus (n), Golgi apparatus (G), cistern cell (cc), duct-forming cells (dsc), cuticle (arrowheads) and gland lumen (L). (B) Transverse section through epithelial cells of the gland duct showing secretory granules (sg), a cistern cell (cc), duct-forming cells (arrow), and cuticle (arrowhead) adjacent to gland lumen (L).

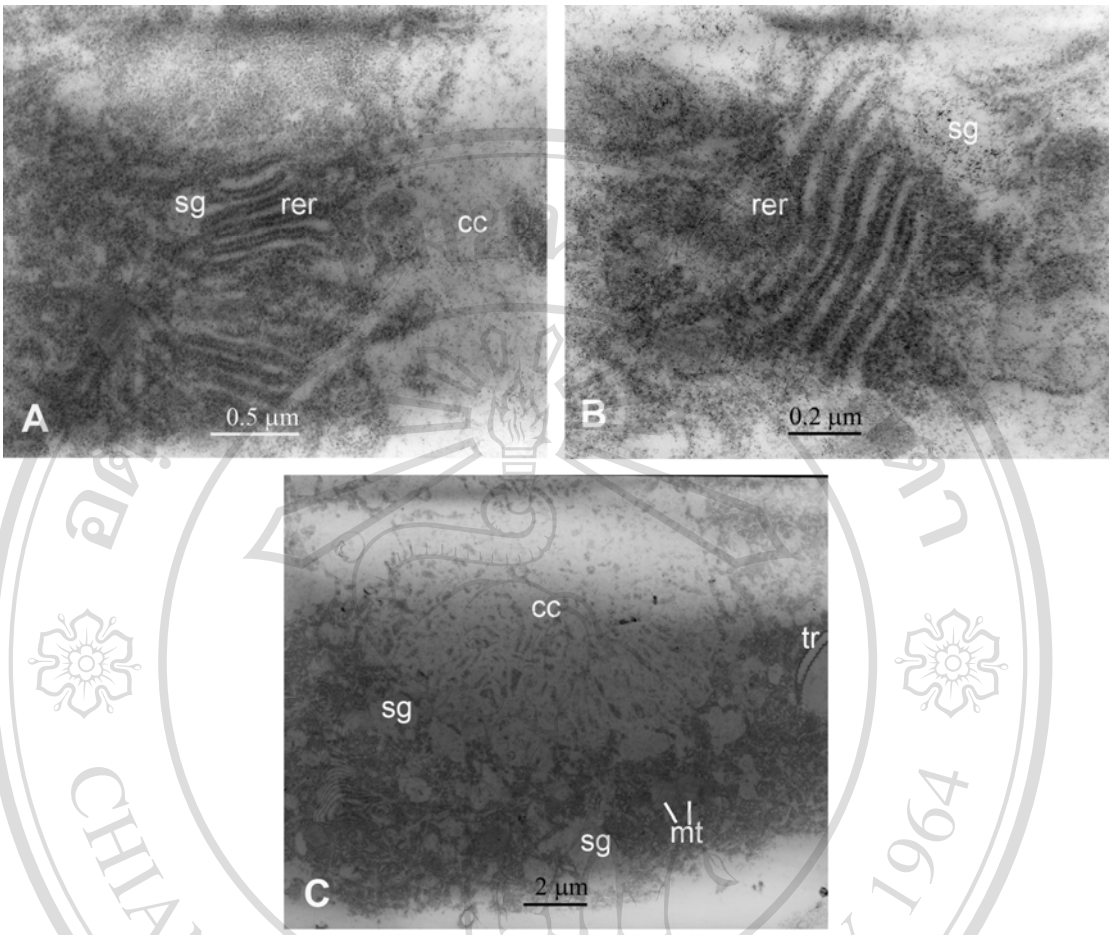

Figure 38 TEM micrographs of female accessory glands of 3 day-old *C. megacephala*. (A-B) The first stage of development of the accessory gland cells which show rough endoplasmic reticulum (rer), secretory granules (sg) and cistern cells (cc). (C) The second stage of development of the accessory gland cells showing mitochondria (mt), secretory glanules (sg), tracheole (tr) and the cistern cell (cc) with fibrous material inside.

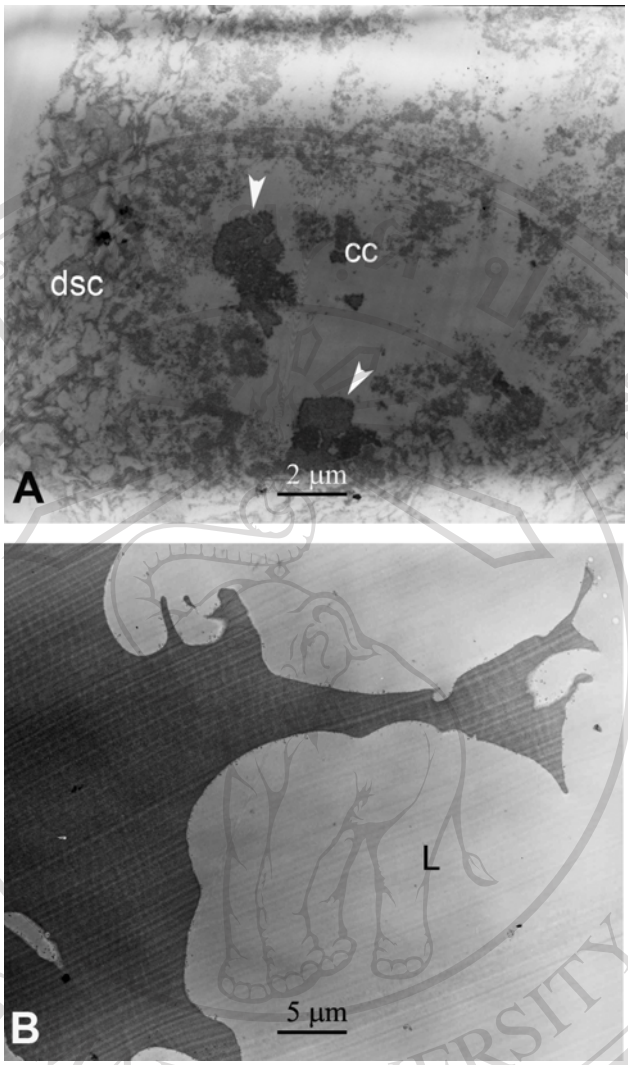

Figure 39 TEM micrographs of female accessory glands of 3 day-old *C. megacephala*. (A) The third stage of development of the accessory gland cells displaying duct-forming cells (dsc) and cistern cell (cc) with electron-dense secretions (arrowheads). (B) The accessory gland lumen (L) filled with dense secretions.

2.3 Spermathecae

Light microscopy: The spermathecae of *C. megacephala* are black and pyriform shaped with a duct entering the genital chamber dorsally (Figures 26A, 26B). The spermathecae of this blow fly are arranged in 1:2 or 2:1 arrangement bilaterally. On the side with two spermathecae, they are loosely bound together while the one on the other side is not attached (Figures 26A, 26B).

Scanning electron microscopy: SEM micrographs of the three spermathecae showed they have a tubercle covered surface, which is penetrated by tracheoles (Figure 40). The basal region of each spermatheca is connected via a spermathecal duct to the genital chamber. Longitudinal muscles are clearly visible on surface of the spermathecal duct (Figure 40).

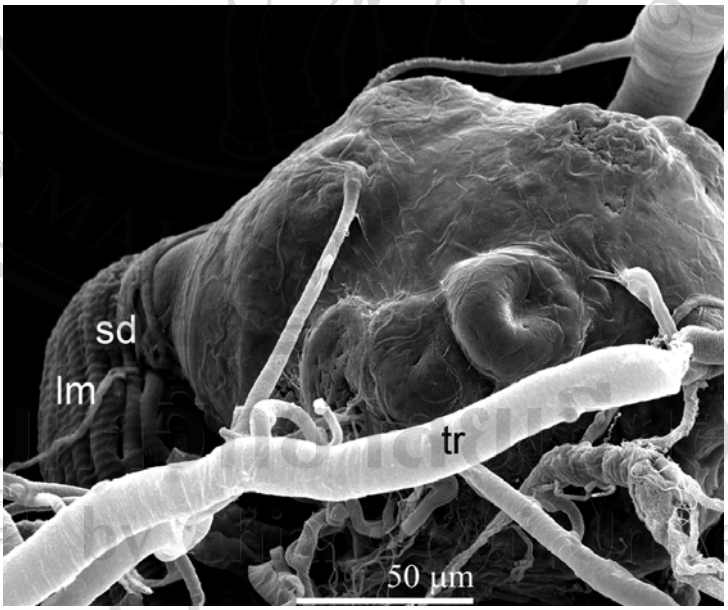

Figure 40 SEM micrograph of the spermathecae of *C. megacephala*. The basal region of a spermatheca showing the connection with the spermathecal duct (sd). Note the longitudinal muscle (lm) supporting the spermathecal duct. Tracheole (tr) inserted into the spermatheca.

Transmission electron microscopy of spermathecae of 3 day-old adult females: The cells of the spermathecae produce secretions that may aid in the maintenance of stored sperm and thus have many similarities in structure to the cells found in secretory gland structures. The spermathecal epithelial cells are characterized by the presence of a rounded nucleus, mitochondria, numerous vacuoles, rough endoplasmic reticulum and tracheoles (Figures 41A, 41B, 41C, 41D, 41E and 41F)). In the lumen of the cistern cells a fine fibrous material is present (Figures 41C, 41D), which is surrounded by rough endoplasmic reticulum, Golgi apparatus and dense droplets of secretion of different sizes (Figures 41C, 41D). In the cytoplasm of the epithelial cells numerous vacuoles of variable size are present which are penetrated by mitochondria. The mitochondria in the cytoplasm are of various shapes (Figures 41E, 41F). An important observation of the spermathecae of 3 day-old females was that they were not filled with spermatozoa.

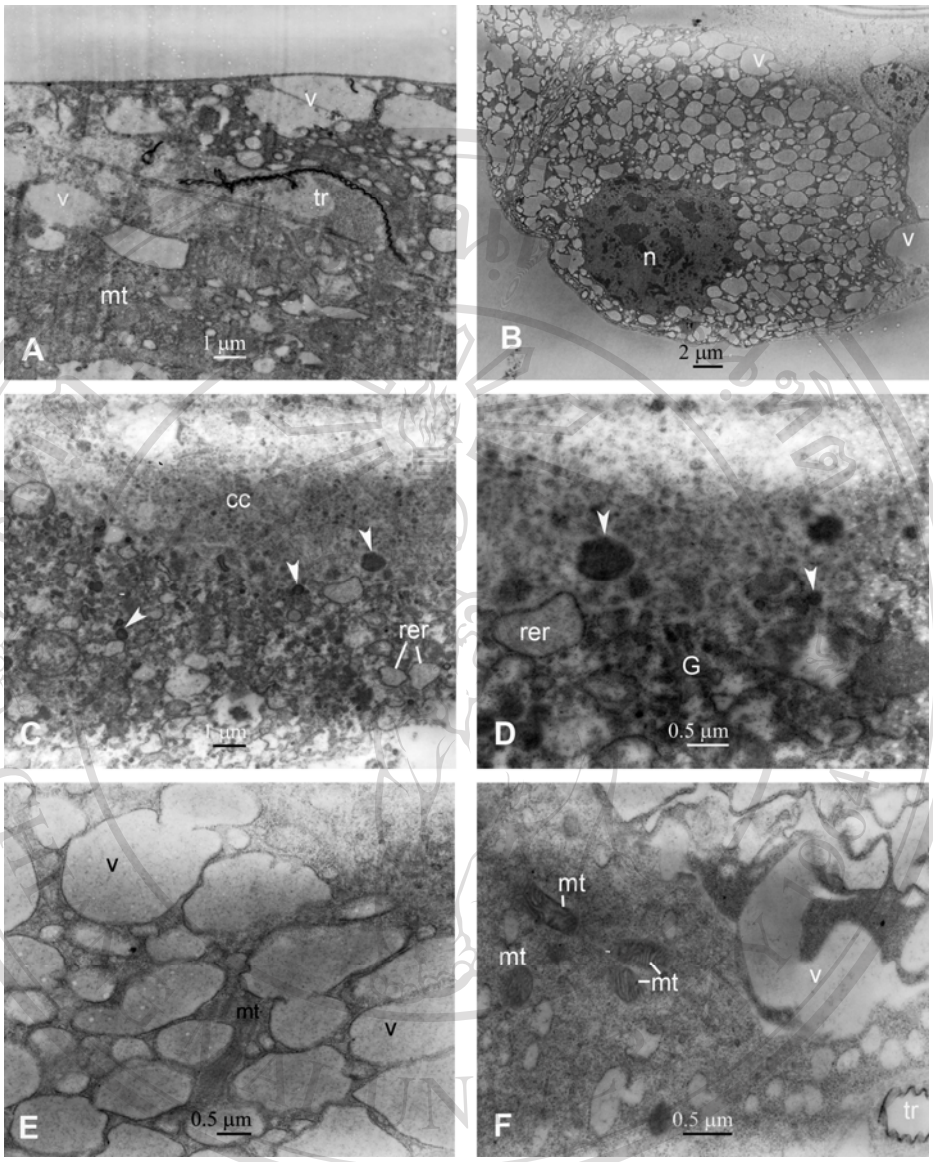

Figure 41 TEM micrographs of spermathecae of 3 day-old *C. megacephala*. (A-B) Transverse section through the epithelial cells of the spermathecae showing a rounded nucleus (n), mitochondria (mt), numerous vacuoles (v) and tracheoles (tr). (C-D) Transverse section of cistern cells (cc) containing rough endoplasmic reticulum (rer), Golgi apparatus (G) and secretion droplets (arrowheads). Note the spermatozoa are not present in the lumen of the cistern cell. (E-F) The cytoplasm of epithelial cells showing numerous vacuoles (v) of variable size interspersed by mitochondria (mt). Note mitochondria (mt) in the cytoplasm are varied in shape.

2.4 Genital chamber

Light microscopy: The genital chamber of *C. megacephala* is a single, long tubular organ. It is located in the posterior region of the abdomen of the female fly. It is the internal conduit for the reception of sperm and the passage of eggs. At its anterior end the genital chamber receives the common oviduct and the spermathecal ducts (Figure 26A). It is an invagination of the body wall and thus lined with cuticle.

Measurements of the length and width of genital chamber during early adult maturation are illustrated in Figures 42A, 42B. The median genital chamber length fluctuated but generally increased from about day three to its peak in length on about day seven (Figure 42A). Median genital chamber width also fluctuated but reached its maximal median value on day six (Figure 42B). Following day six the median width of the genital chamber declined, but maximum values were observed that were as high as the median obtained on day six (Figure 42B).

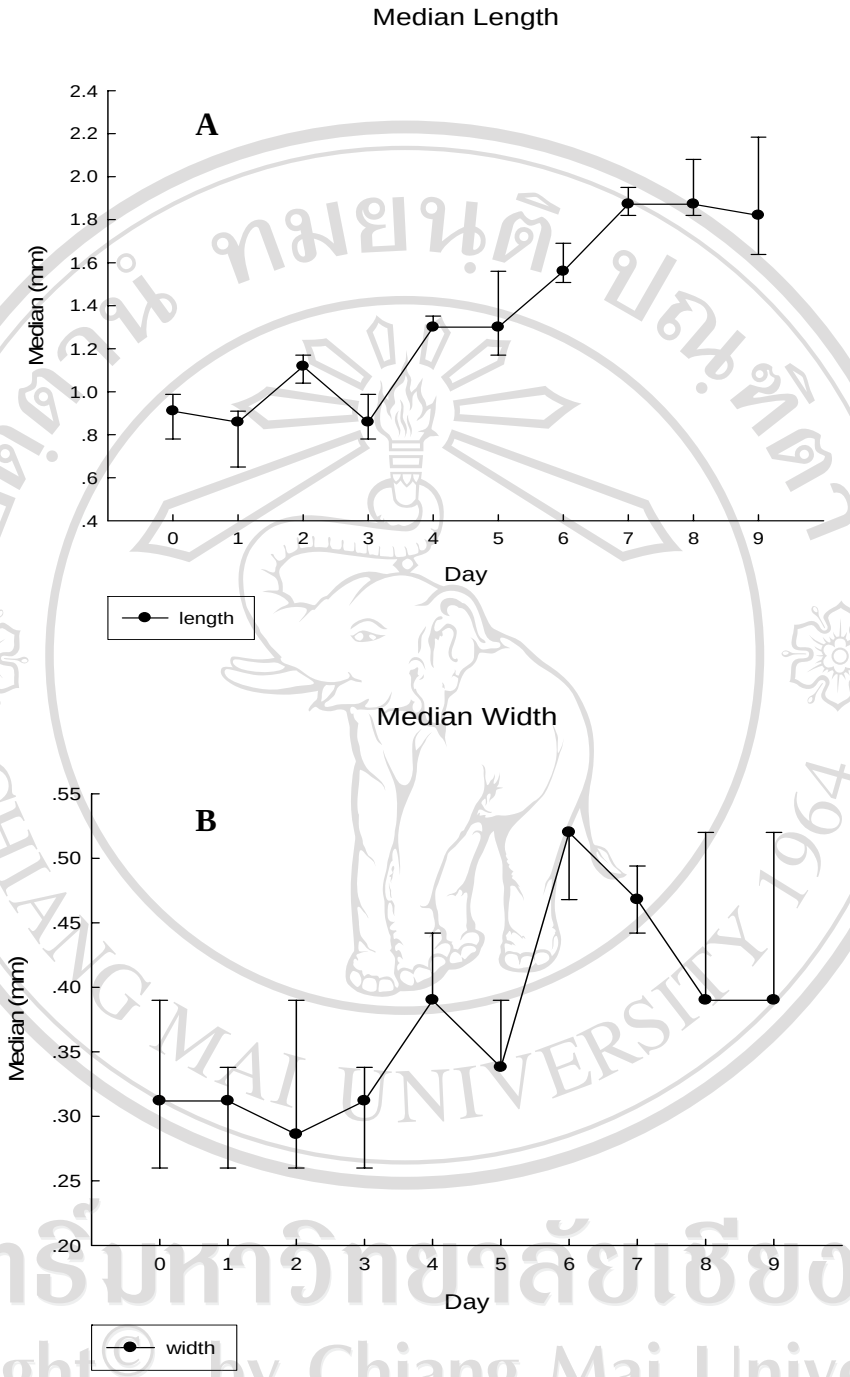

Figure 42 Median length (A) and width (B) (+/- maximum and minimum values) of the genital chamber of *C. megacephala* from just after emergence (day 0) until 9 days old illustrating the change in both length and width of the genital chamber during maturation.

Scanning electron microscopy: SEM images showed that the genital chamber is also occasionally penetrated by tracheoles (Figure 43A). When viewed with SEM, the genital chamber is a tubular shaped duct with an external surface that has a distinct pattern consistent with longitudinal muscle interfacing with circular muscle (Figure 43A). Detailed images of the circular muscle show tracheoles penetrating between muscle fibers into the spaces between the cell layers (Figure 43A). Neither of these features was observable under light microscopy.

Semi-thin section and transmission electron microscopy: The morphology of the genital chamber was observed in 3-day-old flies using semi-thin sections and TEM micrographs. Cross-sections of the genital chamber revealed that the chamber consists of a central lumen surrounded by five distinct cell layers as described in the following sections.

Genital chamber of 3 day-old adult females: Semi-thin cross sections through the tubular genital chamber demonstrated five columnar epithelial cell layers encircling the lumen (Figures 43B, 43D). A semi-thin section of the genital chamber lumen showed that a thin convoluted cuticle lines the lumen (Figure 43C). TEM images confirmed that the central lumen of the genital chamber is surrounded by five distinct cell layers. Moving from the center outward the first-layer is the cuticle-layer which projects into the lumen and the fifth-layer is the outermost layer. The fine structures of each layer are described by using TEM magnifications in the following sections.

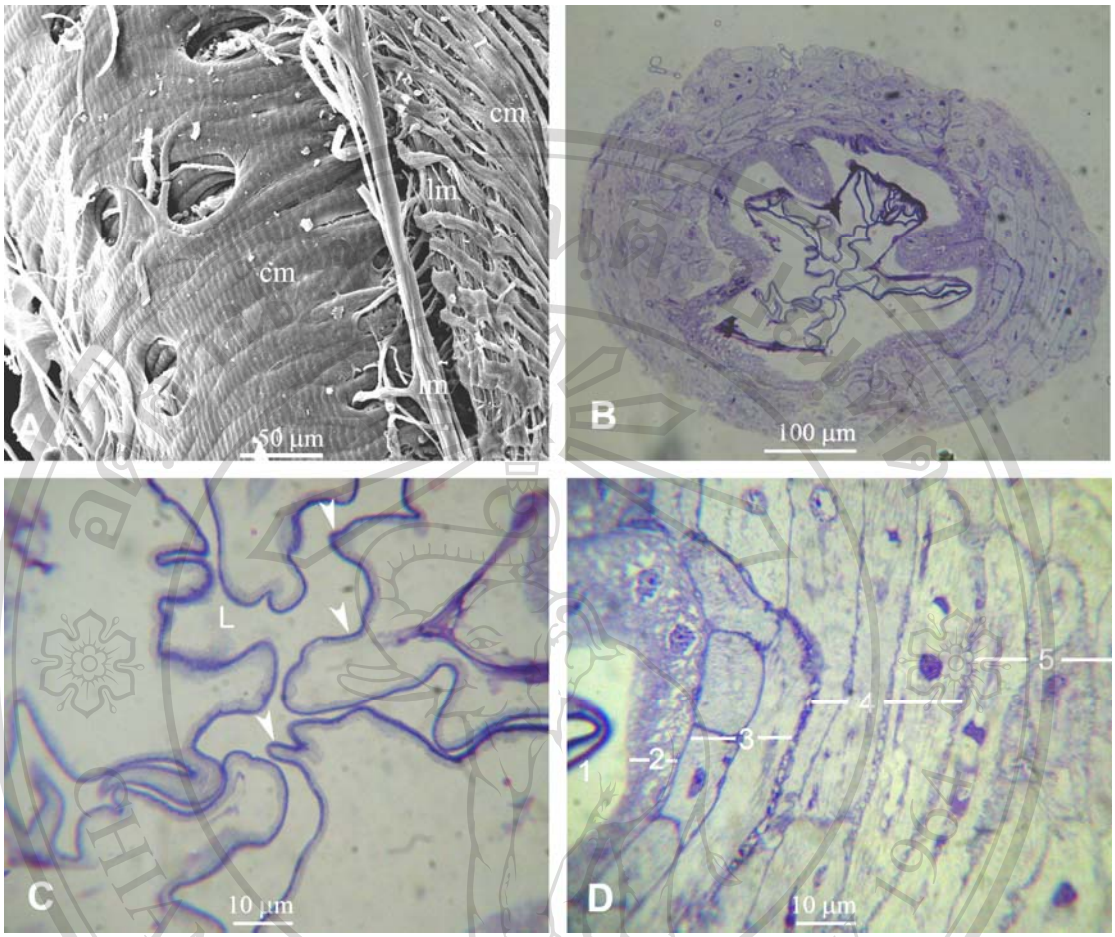

Figure 43 Micrographs of the genital chamber of female *C. megacephala*. (A) SEM micrograph showing longitudinal muscle (lm) interfacing with the circular muscle (cm). (B-D) Semi-thin cross sections of the tubular genital chamber of a 3 day-old *C. megacephala*. (B) Cross section illustrating the five epithelial cell layers encircling the lumen. (C) Cross section through the genital chamber lumen (L) showing the lining of thin cuticle (arrowheads). (D) Higher magnification cross section through the five layers of the genital chamber.

The first-layer of the genital chamber: A transverse section of the first-layer, located adjacent to the lumen, showed that it consists of the cuticle and the basement membrane of the cuticle (Figure 44A). The basement membrane is lined by a thin basal lamina (Figures 44A, 44B). Higher magnification reveals that the cuticle of the genital chamber is composed of an inner epicuticle and an overlying endocuticle (Figure 44C). The inner epicuticle is thin with an electron-dense layer and a double membrane, while the endocuticle is a less electron-dense layer but much thinner (Figure 44C). The secretion material perforated to lumen through the epicuticle layer (Figure 44C, arrowheads).

The second-layer of the genital chamber: A transverse section of the genital chamber showing the second- and third-layer revealed that a membrane junction exists between both layers as shown in Figure 45A. The membrane junction contains numerous tracheoles (Figure 45A). In the second-layer, numerous mitochondria of variable size and shape were visible as well as numerous vacuoles (Figure 45A). At the highest magnification, elongated mitochondria were observed (Figure 45B). Approximately 10-15 mitochondria were found in each cell in the second-layer (as counted under the transmission electron microscope).

The third-layer of the genital chamber: The one cellular chamber of the third-layer is surrounded by a cell membrane with tracheoles. The third-layer is rich in mitocondria with much higher densities in each cell (≈75-85 mitochondria/cell) than those found in the second-layer. Higher magnifications of the third-layer revealed mitochondria of variable shape including round mitochondria and elongate mitochondria. An elongate nucleus was also present with two visible nucleopores.

Next to the nucleus, a pack of ribosomes is visible and it is occasionally penetrated by mitochondria. Additionally, a muscle layer was visible in the third-layer.

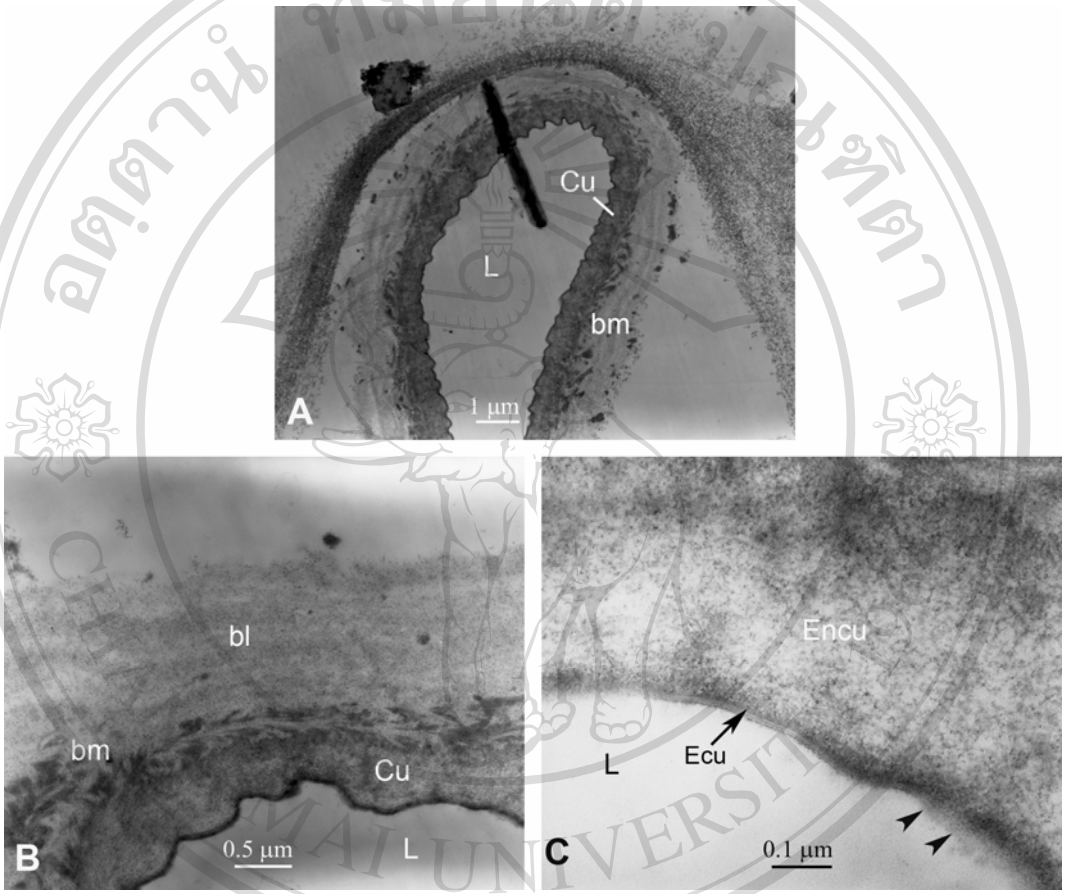

Figure 44 TEM micrographs of the tubular genital chamber of a 3 day-old *C. megacephala*. (A) Transverse section showing the first-layer located adjacent to the lumen (L) consists of cuticle (Cu) and basement membrane (bm). (B) Higher magnification showing the cuticle (Cu) surrounded by the basement membrane (bm) and lined by basal lamina (bl). (C) Higher magnification through the cuticle illustrating an inner epicuticle (Ecu) and thick endocuticle (Encu). The secretion material (arrowheads) perforates to lumen (L) through the epicuticle layer.

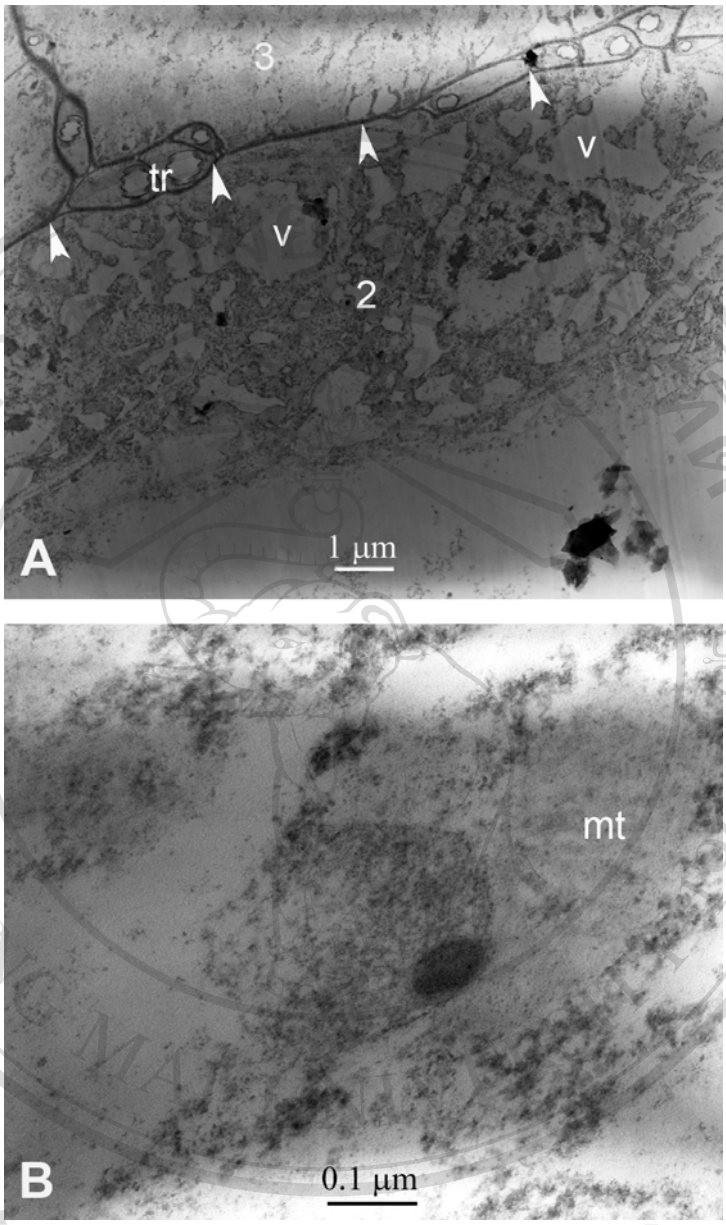

Figure 45 TEM micrographs of the tubular genital chamber of a 3 day-old *C. megacephala*. (A) Transverse section of the genital chamber showing the second-layer (2) and the third-layer (3). Tracheoles (tr) are present in the junction (arrows) between the second- and third-layer. Cells in the second layer display vacuolated (v) cytoplasm. (B) In the second-layer of the genital chamber elongate mitochondria (mt) are present.

The fourth-layer of the genital chamber: TEM images of the fourth-layer show that it consists of multi-layer cells, which each are elongate and contain numerous mitochondria. Approximately 100 mitochondria/cell were found in the cells of this layer (Figure 46A). Each layer of cells is separated by a membrane junction, shown as a line of electron- dense material with tracheoles (Figure 46B). Multi-layer cells of the fourth-layer of the genital chamber had two different types of nuclei, characterized by either compact chromatin in the nucleus or dispersed chromatin in the nucleus (Figure 46C). The compact chromatin nuclei have a compact homogeneous dense chromatin within the nucleus (Figure 46C, n1), whereas, the dispersed chromatin nuclei display a dispersed dense chromatin (Figure 46C, n2).

The fifth-layer of the genital chamber: The fifth-layer consists of columnar cells with bundles of ribosomes located centrally. Each columnar cell is separated by a cell membrane with tracheoles present in between the cell membranes (Figure 47A). The nuclei of the fifth-layer cells are elongate and with packs of chromatin peripherally located near the nuclear-membrane (Figure 47B). A bundle of ribosomes are visible at the pole of the nucleus of the cell imaged in Figure 47B, which is also penetrated by numerous mitochondria. The number of mitochondria in the cells of the fifth-layer is much higher than 100 mitochondria/cell of variable size which are located within the ribosome packs (Figure 47A).

The cytoplasm of the epithelial cells (the second-layer to the fifth-layer) is high in cellular organelles with most cells occupied largely by mitochondria. However, the muscle layer was only visible in the third-layer, and was not observed in any of the other layers of the genital chamber of *C. megacephala*.

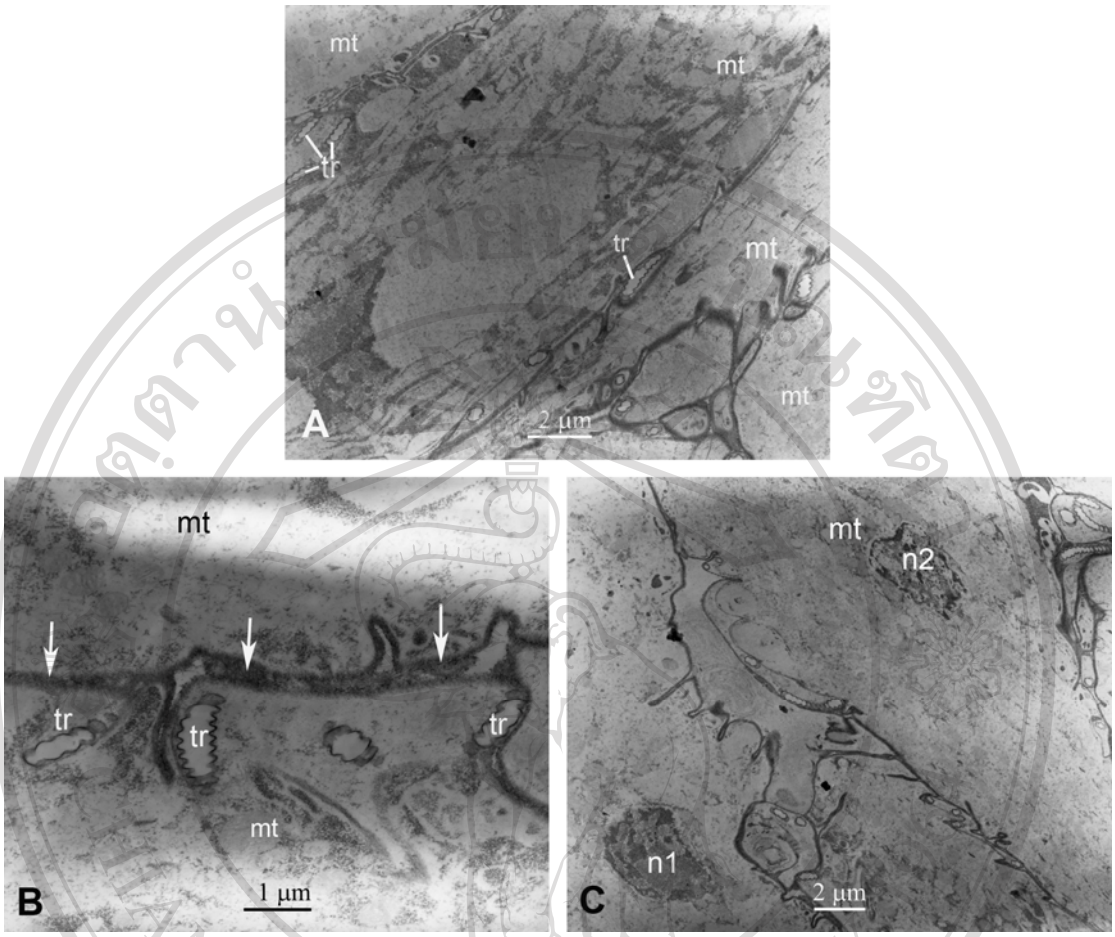

Figure 46 TEM micrographs of the tubular genital chamber of a 3 day-old *C. megacephala*. (A) Transverse section of the genital chamber showing the fourth-layer consisting of multi-layer cells which each are elongate and contain numerous mitochondria (mt). Tracheoles (tr) inserted between these cells. (B) The junction between each cell of the fourth-layer showing a line of electron dense material (arrows) and tracheoles (tr). Mitochondria (mt) are presented. (C) Multi-layer cells of the fourth-layer with two different types of nuclei, characterized by either compact chromatin in the nucleus (n1) or dispersed chromatin in the nucleus (n2). Mitochondria (mt) are presented in the cytoplasm.

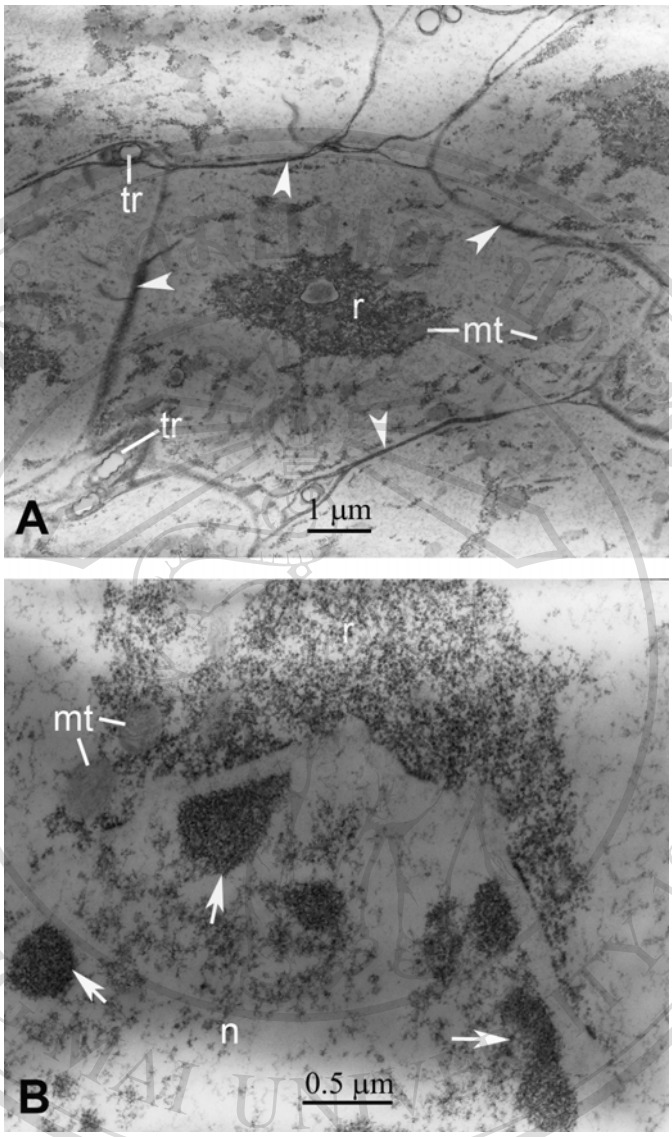

Figure 47 TEM micrographs of the tubular genital chamber of a 3 day-old *C. megacephala*. (A) Transverse section of the genital chamber showing the fifth layer consisting of cells with a bundle of ribosomes (r) located in the center of the cell. Each cell is separated by a cell membrane (arrowhead) with tracheoles (tr). (B). Nucleus of the fifth-layer illustrating that packs of chromatin (arrows) are present peripherally around the nuclear-membrane. Near the nucleus (n), mitochondria (mt) are evident.

3. The external genitalia: male and female of *Chrysomya. megacephala*

The male genitalia of *C. megacephala* reveal characters of the cercus, surstylus, epandrium, phallus, ejaculatory apodeme, and aedeagal apodeme (Figure 48A). The epandrium is broad structure similar to a crescent shape. The ejaculatory apodeme and aedeagal apodeme are resembled in their lengths. The cercus is significantly longer than the surstylus (Figures 48B, 48D). The apical end of the cercus is more or less rounded laterally (Figure 48C). The external portion of the cercus is endowed along the lower half area with long bristles; the sensilla chaetica and sensilla trichodea (Figure 48B), and the short bristles, sensilla basiconica, exist at the proximal area (Figure 48C). The cercus is enlargement at the upper half, united at its base, and separates distally into two arms, which gradually taper at the lower half with the terminal end of each being abruptly truncate (Figure 48D). The surstylus is stoutly triangular shaped, and the proximal half is much covered with sensilla chaetica and sensilla trichodea (Figure 48E).

The aedeagus is a clavate shape formed by two main parts, the base theca and the elongated phallus (Figure 49A). The theca expands proximally, forming a slight central ridge. The distal end of the theca is connected to the proximal part of the phallus called the corpus (Figure 49B). The corpus is elongated part of the structure, having gently curved microtrichia inside (Figures 49A, 49B). The end of the phallus is bilobed, smooth-surfaced vesica (Figure 49C). Lying medially between the vesica is the juxta. The outer edge of both the juxta and the juxta process are covered with many rows of strong spines (Figure 49C). Lying between the juxta processes is the stylus formed by a membranous tube. Connected to the corpus is the harpe, which is recurved anteriorly, and distally pointed (Figures 49D, 49E, 49F). Some specimens

revealed that the anterior end of the harpe is either inserted between the juxta and the vesica (Figure 49C) or beneath the vesica (Figures 49E, 49F). The juxta is found to either lie between the vesica (Figure 49D) or to be movable beneath the vesica (Figure 49E).

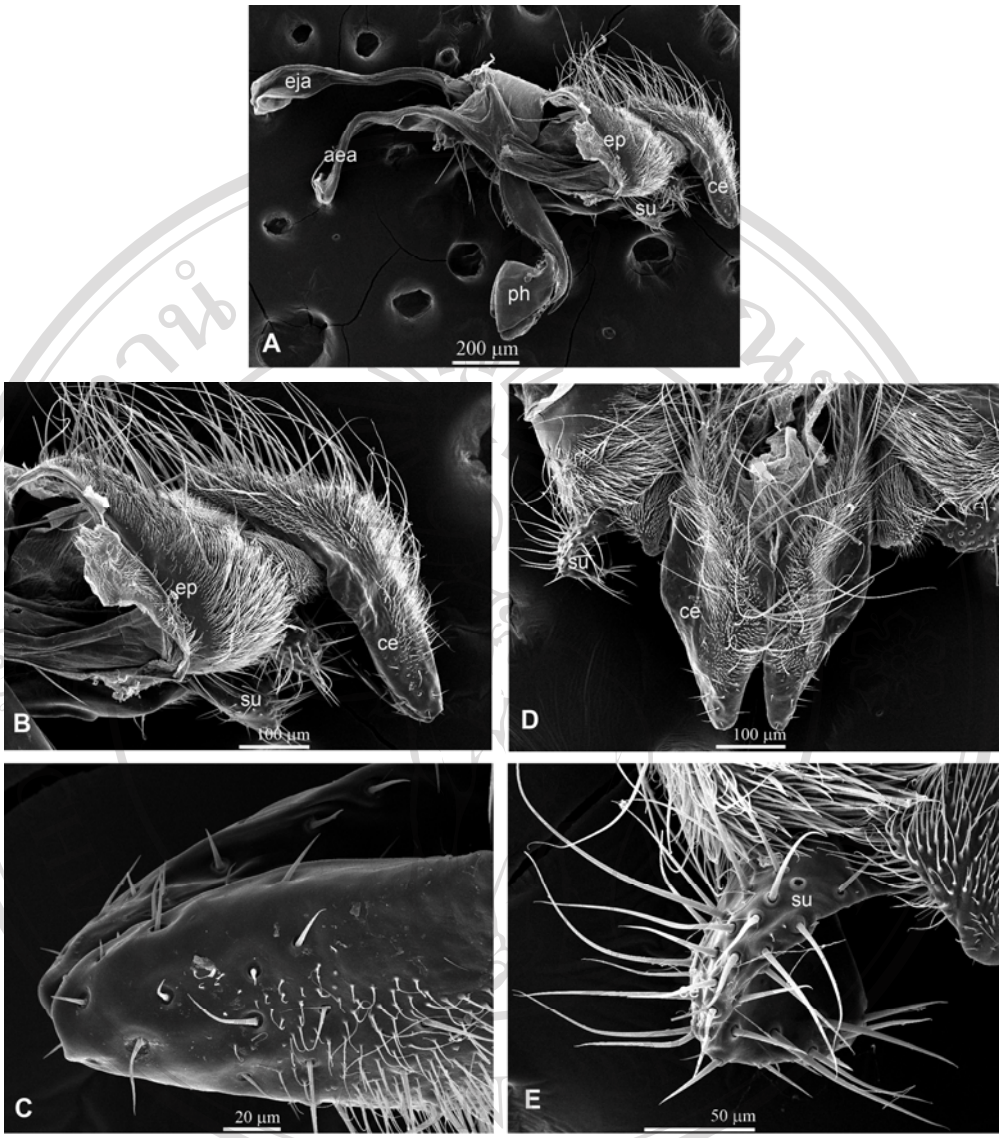

Figure 48 SEM micrograph of the male genitalia of *C. megacephala*. (A) The entire genitalia showing the cercus (ce), surstylus (su), epandrium (ep), phallus (ph), ejaculatory apodeme (eja), and aedeagal apodeme (aea). (B) The lower part of the male genitalia pointing at longer cercus (ce) compared to the surstylus (su), which lies underneath the base of epandrium (ep). (C) The cercus. (D) The enlargement at the upper half of the cercus (ce), laterally lying with the surstylus (su). (E) The surstylus (su) endowed with sensilla chaetica and sensilla trichodea.

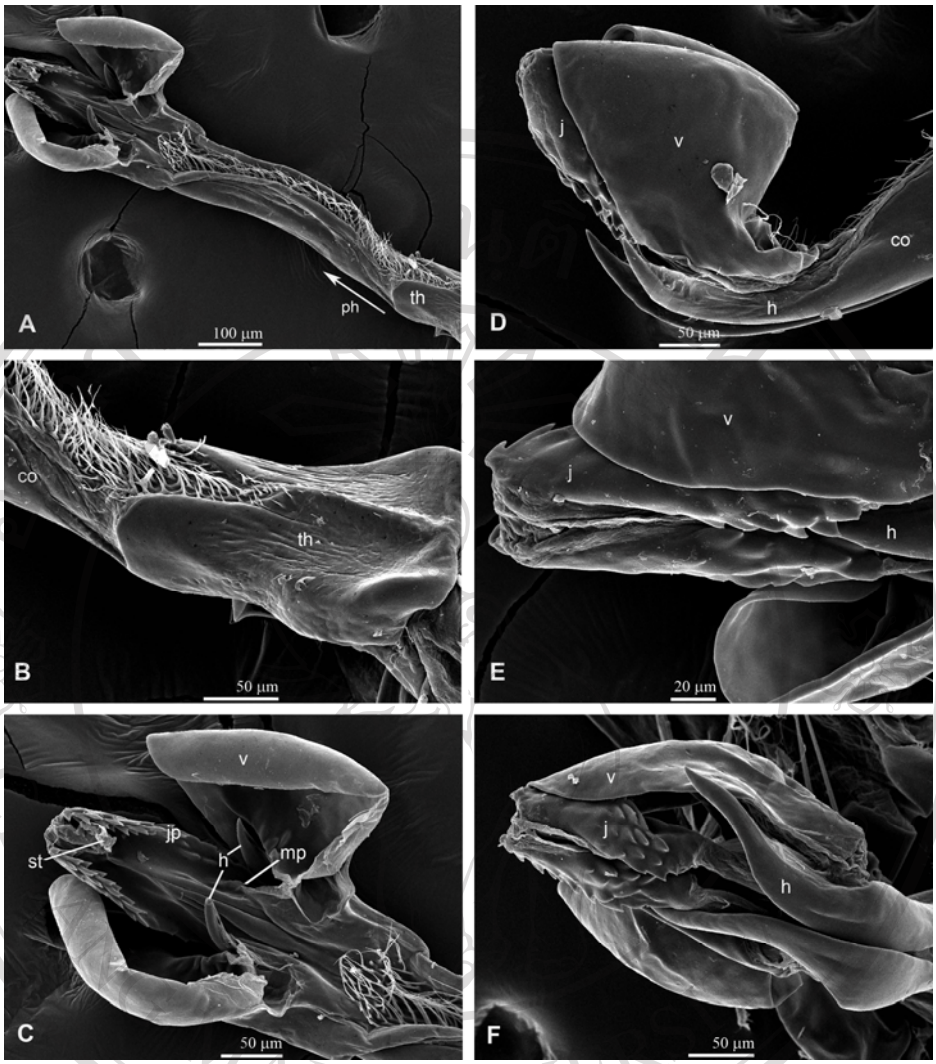

Figure 49 SEM micrograph of the aedeagus of male *C. megacephala*. (A) Aedeagus appearing as a clavate shape formed by the base theca (th) and the phallus (ph). (B) Theca (th) connected to the corpus (co). (C) The apical end of the phallus showing bilobed vesica (v), juxta process (jp), stylus (st), and harpe (h) recurved between the median processes (mp). (D) Aedeagus with elongated corpus (co), harpe (h), juxta (j), and vesica (v). (E) Apical aedeagus showing vesica (v), harpe (h), and juxta (j). (F) Ventral view of the apical aedeagus showing vesica (v), harpe (h), and juxta (j).

Female genitalia or ovipositor of *C. megacephala* are presented in Figures 50A, 50B, 50C, 50D, 50E, 51A, 51B, 51C, 51D, 52A, 52B, 52C and 52D. The ovipositor extends from the last pre-abdominal segment and is comprised of four visible segments (Figures 50A, 50D). The dorsum of the last segment “supraanal plate” or “epiproct” is more or less triangular-shaped (Figure 50B). Both the supraanal plate and cercus have a scarcity of sensilla (Figure 50B). Short sensilla basiconica (Figure 50C) and sensilla placodea were found on the supraanal plate.

The ventral view of the female genitalia revealed elongated ovipositor (Figure 50D). The subanal plate or paraproct is a stout maple leaf, whereas the cercus is a clavate shape (Figure 50E). Different sensillum types were observed on the ventrolateral cercus. The sensilla trichodea bears longitudinal grooves externally, which are of variable lengths (arrows in Figure 50E). Based on Zacharuk (1985), sensilla basiconica is short hair shafts bearing longitudinal grooves (Figure 51A) or having smooth surfaces (Figures 52B, 51C) inserted into cuticular sockets. Stout hair shafts at the base (Figure 51D) or subapical region (Figure 52A) of the sensilla basiconica were also observed (Figure 51D). Globular sensilla that were probably sensilla styloconica were also observed. This sensillum reveals as a ball-shaped peg, with a rugose surface (Figure 52B). The sensilla placodea appears as a plate-like cuticle that is usually recessed in a shallow pit with a characteristic central pore (Figures 52C, 52D).

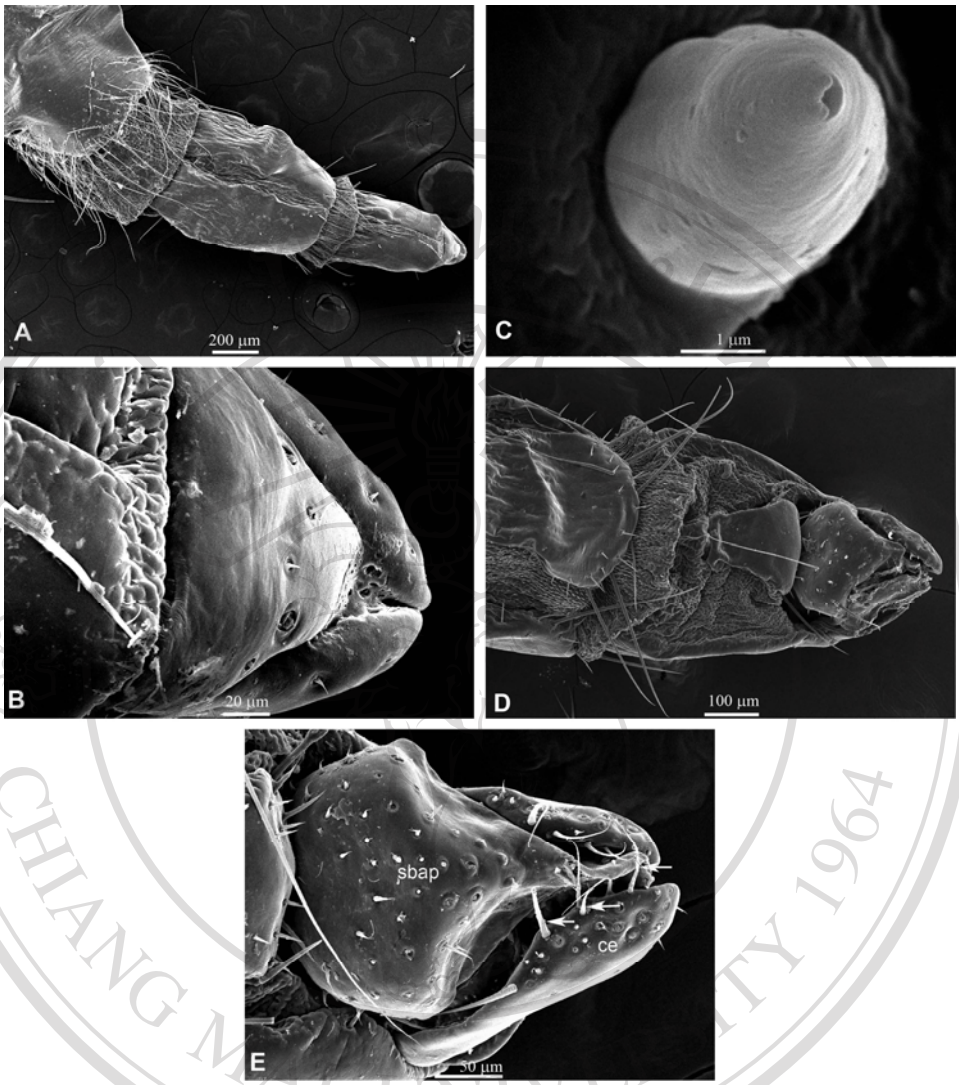

Figure 50 SEM micrograph of the female genitalia of *C. megacephala*. (A) Ovipositor in dorsal view. (B) Ovipositor showing supraanal plate lying between short cerci. (C) The sensilla basiconica with short length observed at the supraanal plate. (D) Ventral view of the ovipositor. (E) Enlargement of the ventral view showing the subanal plate (sbap) and cercus (ce).

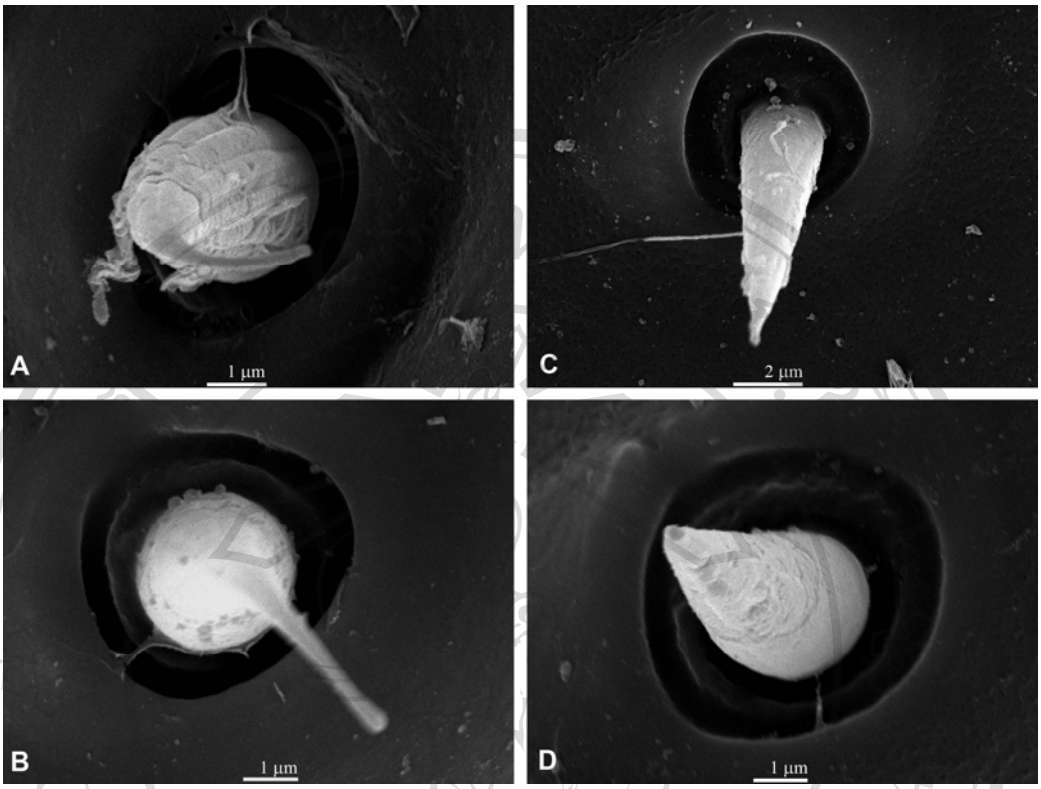

Figure 51 SEM micrograph of the sensilla found on the cercus of the female genitalia of *C. megacephala*. (A) Sensilla basiconica with short hair shafts having longitudinal grooves. (B) Sensilla basiconica with short hair shafts bearing a smooth surface. (C) Sensilla basiconica with short hair shafts bearing a smooth surface. (D) Sensilla basiconica with stout hair shaft.

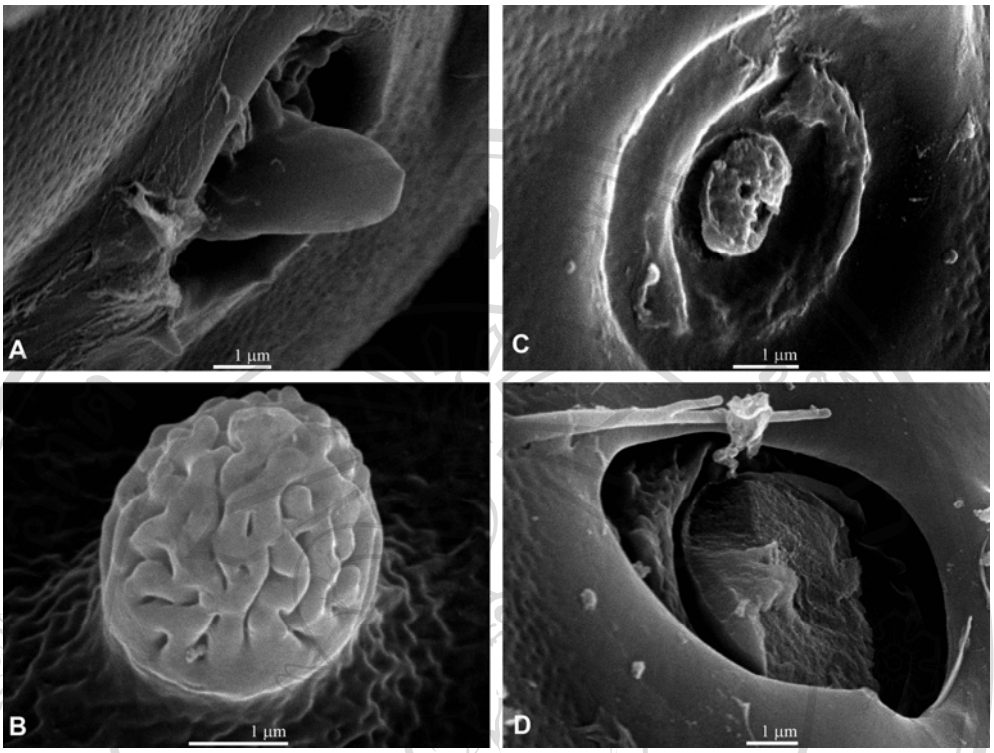

Figure 52 SEM micrograph of the sensilla observed on the cercus of the female genitalia of *C. megacephala*. (A) Sensilla basiconica. (B) Sensilla styloconica. (C) Sensilla placodea. (D) Sensilla placodea.

4. Efficacy of the human contraceptive on the male and female reproductive system of *Chrysomya. megacephala*

4.1 Effect of human contraceptive fed to male *C. megacephala* (Experiment I)

Seven days after male flies were treated with 0.036 mg/ml HC and 0.072 mg/ml HC, ten flies from each female group were randomly collected and dissected in order to obtain their ovaries. The ovarian follicles were classified after spreading them on a slide at 400× magnification using a compound microscope. Changes in the ovarioles were classified according to the descriptions obtained through observations during egg development as previously described in part 2.1.2. In the parental flies all females were gravid in all groups, which in this experiment were not treated with human contraceptive (Figure 53). However, following crossing of male treatment groups with untreated females and inbreeding through two subsequent generations (i.e., the first, second and third generations) there was a reduction in the number of females that were observed to be gravid at seven days of age. This suggests that there was a delay in egg maturation in those females that had been crossed with males fed both 0.036 mg/ml HC and 0.072 mg/ml HC. In the control group 100% of the female flies presented with mature eggs (Figure 53).

Table 4 shows the median number of ovarioles per female *C. megacephala* for the parental flies when males were treated with each concentration of human contraceptive. It also displays the median number of ovarioles for the following three generations after crossing and inbreeding. No significant difference (P>0.05) was observed among control, 0.036 mg/ml HC or 0.072 mg/ml HC in the median number

of ovarioles in the ovaries of parental females using a Kruskal-Wallis ANOVA based on ranks.

In the first, second and third generations the Kruskal-Wallis ANOVA revealed a significant difference among the treatment groups thus Mann-Whitney *U* tests were used to determine which groups differed from each other. When compared with the control the median number of ovarioles in the ovaries from the 0.072 mg/ml HC treatment group was significantly ($P<0.05$) lower in the first, second and third generations (Table 4). Additionally, in the first and second generations the median number of ovarioles from the 0.072 mg/ml HC treated male groups were significantly ($P<0.05$) lower than the 0.036 mg/ml HC treatment group, but this effect was not observed in the third generation (Table 4). However, the median number of ovarioles from 0.036 mg/ml HC treated male groups was significantly ($P< 0.05$) decreased from the control only in the third generation.

Table 5 illustrated the number of females in each ovarian stage for each treatment group in each generation (the parental, the first, second and third generations). During the first generation all the ovarioles observed in each treatment were in the final stage of maturity. In the subsequent generations the percentage of females in each stage for both the high and low treatments differed from the control. In the first generation the least mature ovarioles were at stage III in the 0.072 mg/ml HC while those in the 0.036 mg/ml HC treatment were least mature at stage V. The number of fully mature ovarioles declined over generations in the 0.036 mg/ml HC treatment group. Approximately half of the females had fully mature ovarioles in the 0.072 mg/ml HC group for the first, second and third generations.

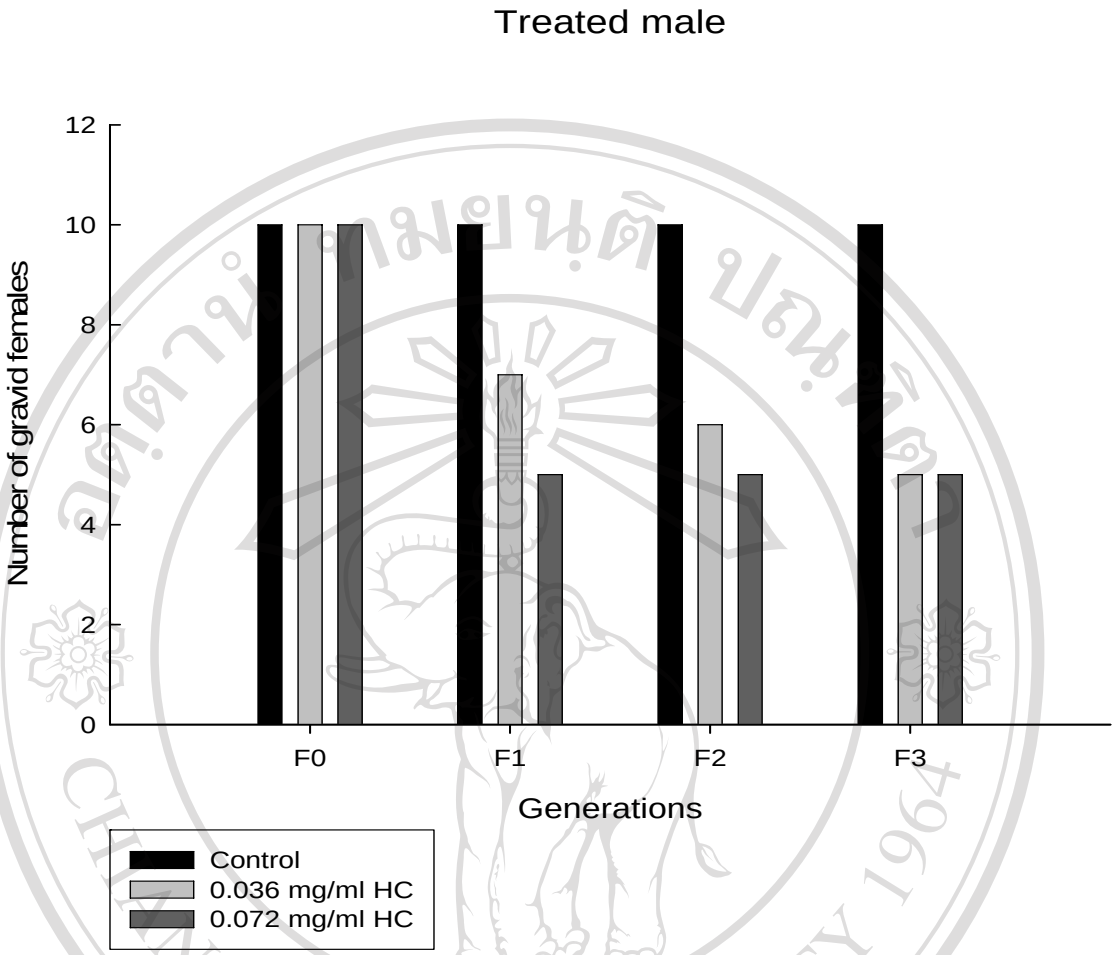

Figure 53 The number of gravid female *C. megacephala* out of the ten dissected and observed at 7 days after emergence in each treatment group for four generations. Only parental males (F0) were treated with human contraceptive, crossed with females (F1 or first generation) then inbred for the second (F2) and third generations (F3).

Table 4 Number of ovarioles in untreated female *C. megacephala* after crossing with male treated with human contraceptive[1].

Treatment[2]	Median of number of ovarioles (range)[3]			
	Parental flies	First generation	Second generation	Third generation
Control	262.0 (226-304)[a]	297.3 (257-335)[a]	291.3 (249-336)[a]	329.0 (305-365)[a]
0.036 mg/ml HC	255.9 (223-288)[a]	286.1 (226-304)[a]	272.8 (251-301)[a]	286.0 (266-306)[b]
0.072 mg/ml HC	260.5 (226-301)[a]	252.0 (223-282)[b]	251.9 (214-308)[b]	287.6 (272-299)[b]

[1]Human contraceptive: Microgest®, each beige tablet contained levonorgestrel at 0.15 mg and ethinylestradiol at 0.03 mg.

[2]Each treatment involved 10 female flies.

[2]Control = Male $_{(10\%\ \text{sucrose solution})}$ × Female $_{(10\%\ \text{sucrose solution})}$

[2]0.036 mg/ml HC= Male $_{(0.036\ \text{mg/ml HC})}$ × Female $_{(10\%\ \text{sucrose solution})}$

[2]0.072 mg/ml HC = Male $_{(0.072\ \text{mg/ml HC})}$ × Female $_{(10\%\ \text{sucrose solution})}$

[3]Median followed by the different letters indicate significant differences at the $P<0.05$ level within each column (generation) as determined by the Mann-Whitney *U* test.

Table 5 Percentage of total females in each ovarian stage for each treatment of the parental flies, first, second and third generations when males were treated with human contraceptive.

Treatment[1]	Ovarian stages[2]							
	I	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**
Parental flies								
Control	0	0	0	0	0	0	0	100
0.036 mg/ml HC	0	0	0	0	0	0	0	100
0.072 mg/ml HC	0	0	0	0	0	0	0	100
First generation								
Control	0	0	0	0	0	0	0	100
0.036 mg/ml HC	0	0	0	0	20	10	0	70
0.072 mg/ml HC	0	0	10	10	30	0	0	50
Second generation								
Control	0	0	0	0	0	0	0	100
0.036 mg/ml HC	0	0	0	10	20	10	0	60
0.072 mg/ml HC	0	0	0	20	30	10	0	40
Third generation								
Control	0	0	0	0	0	0	0	100
0.036 mg/ml HC	0	0	0	20	20	10	0	50
0.072 mg/ml HC	0	0	0	10	30	10	0	50

[1]control: 0 mg/ml HC, 0.036 mg/ml HC, 0.072 mg/ml HC

[2]Ovarial stage from the same row involved 10 female flies.

4.2 Effect of human contraceptive fed to female *C. megacephala* (Experiment II)

Seven days after female flies were treated with 0.036 mg/ml HC and 0.072 mg/ml HC, parental females also revealed mature eggs in all treatment groups (Figure 54). However, following crossing and inbreeding to maintain the colonies over the next three generations (F_1, F_2 and F_3) there was delayed egg maturation in both the 0.036 mg/ml HC and 0.072 mg/ml HC treatment groups. Females in the control group all contained mature eggs (Figure 54).

Table 6 displays the median number of ovarioles per female *C. megacephala* of the parental flies treated with human contraceptive crossed and inbred females for the subsequent first, second and third generations. The Kruskal-Wallis ANOVA detected no significant ($P>0.05$) difference among any of the treatment groups in the number of ovarioles in the ovaries from the parental generation.

In the subsequent generations the Kruskal-Wallis ANOVA detected significant differences due to a treatment effect. Mann-Whitney *U* tests showed that when compared with the control, the median number of ovarioles in the ovaries of 0.072 mg/ml HC treatment group females was significantly ($P<0.05$) lower in the first, second and third generations (Table 6). The median number of ovarioles from females in the 0.036 mg/ml HC treatment group was also significantly ($P<0.05$) lower in the first, second and third generations when compared with the control (Table 5). The Mann-Whitney *U* test comparison of the median number of ovarioles from females in the 0.036 mg/ml HC and 0.072 mg/ml HC treatment groups showed a significant difference ($P<0.05$) with a lower mean observed in the 0.072 mg/ml HC group in the first generation. However, no significant ($P> 0.05$) difference was observed between

the 0.036 mg/ml HC and 0.072 mg/ml HC groups in the second and third generations (Table 6).

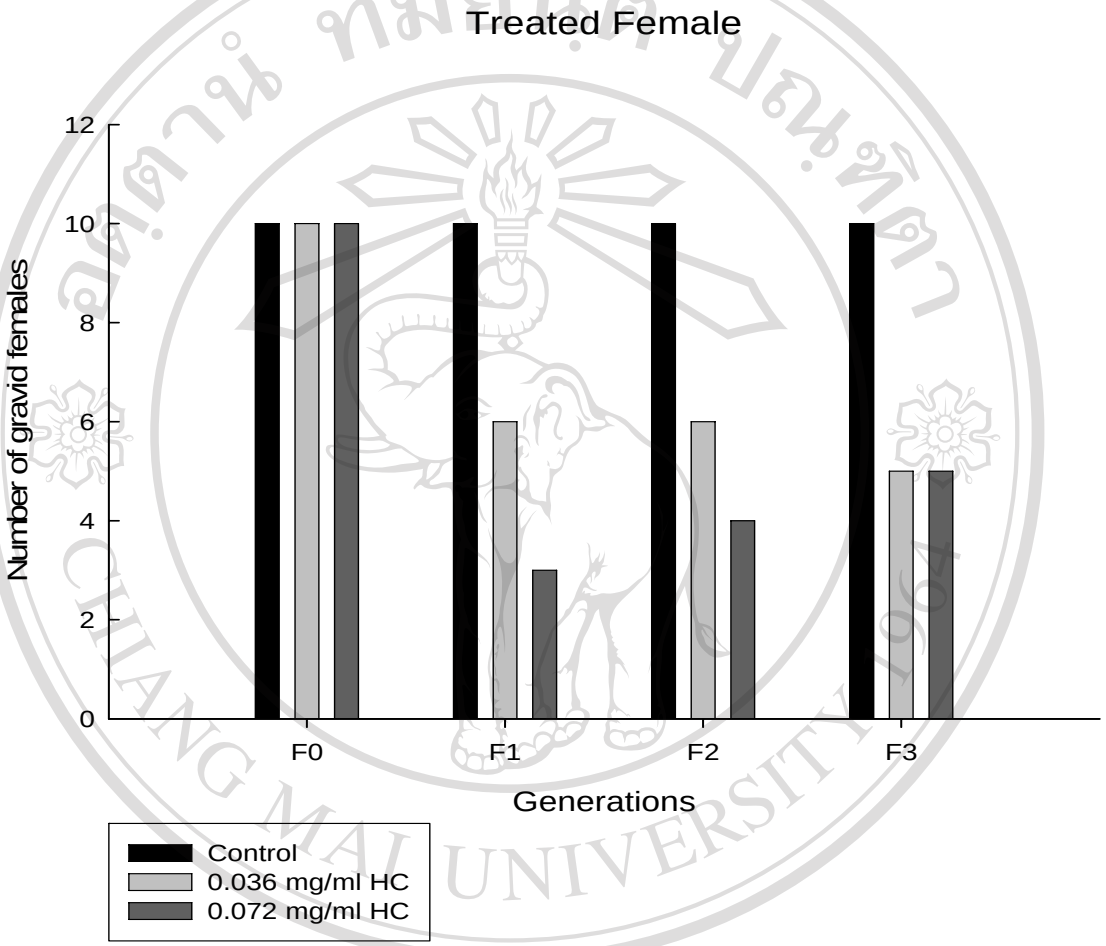

Figure 54 The number of gravid female *C. megacephala* out of the ten dissected and observed at 7 days after emergence in each treatment group. Only parental females (F0) were treated with human contraceptive, crossed with males (F1 or the first generation) then inbred for the second (F2) and third generations (F3).

Table 6 Number of ovarioles in female *C. megacephala* treated with human contraceptive[1] after crossing with untreated male.

Treatment[2]	Median of number of ovarioles (range)[3]			
	Parental flies	First generation	Second generation	Third generation
Control	262.0 (226-304)[a]	297.3 (257-335)[a]	291.3 (249-336)[a]	329.0 (305-365)[a]
0.036 mg/ml HC	256.5 (230-289)[a]	271.0 (242-296)[b]	258.9 (238-275)[b]	271.0 (238-293)[b]
0.072 mg/ml HC	257.3 (220-306)[a]	247.2 (215-276)[c]	248.1 (218-312)[b]	287.5 (255-347)[b]

[1]Human contraceptive: Microgest®, each beige tablet contained levonorgestrel at 0.15 mg and ethinylestradiol at 0.03 mg.

[2]Each treatment involved 10 female flies.

[2]Control = Female $_{(10\%\ \text{sucrose solution})}$ × Male $_{(10\%\ \text{sucrose solution})}$

[2]0.036 mg/ml HC = Female $_{(0.036\ \text{mg/ml HC})}$ × Male $_{(10\%\ \text{sucrose solution})}$

[2]0.072 mg/ml HC = Female $_{(0.072\ \text{mg/ml HC})}$ × Male $_{(10\%\ \text{sucrose solution})}$

[3]Median followed by the different letters indicate significant differences at the $P<0.05$ level within each column (generation) as determined by the Mann-Whitney *U* test.

Table 7 illustrates the number of females in each ovarian stage for each treatment group in each generation (parental, the first, second and third generations). During the first generation all the ovarioles observed in each treatment were also in the final stage of maturity. In the subsequent generations the percentage of females in each stage for both the high and low treatments differed from the control. In the first generation the least mature ovarioles were at stage III in the 0.072 mg/ml HC while those in the 0.036 mg/ml HC treatment were least mature at stage IV. The number of fully mature ovarioles declined from 60% in the first and second generations to 50% in the third generation in the 0.036 mg/ml HC treatment group. In the 0.072 mg/ml HC group only 30% of females had fully mature ovarioles for the first generation, but 50% were fully mature in the second and third generations.

Table 7 Percentage of total females in each ovarian stage for each treatment of parental flies, first, second and third generations after females were treated with human contraceptive.

Treatment[1]	Ovarian stages[2]							
	I	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**
Parental flies								
Control	0	0	0	0	0	0	0	100
0.036 mg/ml HC	0	0	0	0	0	0	0	100
0.072 mg/ml HC	0	0	0	0	0	0	0	100
First generation								
Control	0	0	0	0	0	0	0	100
0.036 mg/ml HC	0	0	0	20	20	0	0	60
0.072 mg/ml HC	0	0	20	20	30	0	0	30
Second generation								
Control	0	0	0	0	0	0	0	100
0.036 mg/ml HC	0	0	10	20	30	0	0	60
0.072 mg/ml HC	0	0	0	30	20	0	0	50
Third generation								
Control	0	0	0	0	0	0	0	100
0.036 mg/ml HC	0	0	0	10	30	10	0	50
0.072 mg/ml HC	0	0	0	30	20	0	0	50

[1]control: 0 mg/ml HC, 0.036 mg/ml HC, 0.072 mg/ml HC

[2]Ovarial stage from the same row involved 10 female flies.

4.3 Effect of human contraceptive fed to male and female *C. megacephala* (Experiment III)

Seven days after both male and female flies were treated with 0.036 mg/ml HC and 0.072 mg/ml HC, all parental females contained mature eggs in all treatment groups as in the other experiments (Figure 55). As in the previous experiments in the subsequent generations following crossing and inbreeding for the first, second and third generations, there was a reduction in the number of females with mature eggs at seven days of age in both the 0.036 mg/ml HC and 0.072 mg/ml HC groups, while in the control group all female flies contained mature eggs (Figure 55).

Table 8 displays the median number of ovarioles in female *C. megacephala* when both male and female parental flies were treated with human contraceptive and followed through the first, second and third generations. As in the previous experiments the Kruskal-Wallis ANOVA revealed no significant (P>0.05) difference among the treatments in the parental flies in the median number of ovarioles obtained from females. In this experiment the median number of ovarioles from females derived from the 0.072 mg/ml HC treatment group when both males and females were treated was significantly lower (P<0.05) in the first and third generations when compared with the control. Whereas, the number median of ovarioles derived from the 0.036 mg/ml HC treatment group was significantly lower (P<0.05) only in the third generation when compared with the control. No significant difference (P>0.05) was observed between 0.036 mg/ml HC and 0.072 mg/ml HC groups in the first, second and third generations. Interestingly, no significant difference (P>0.05) was observed among the treatments at any level for the number of ovarioles in the ovaries of females from the second generation.

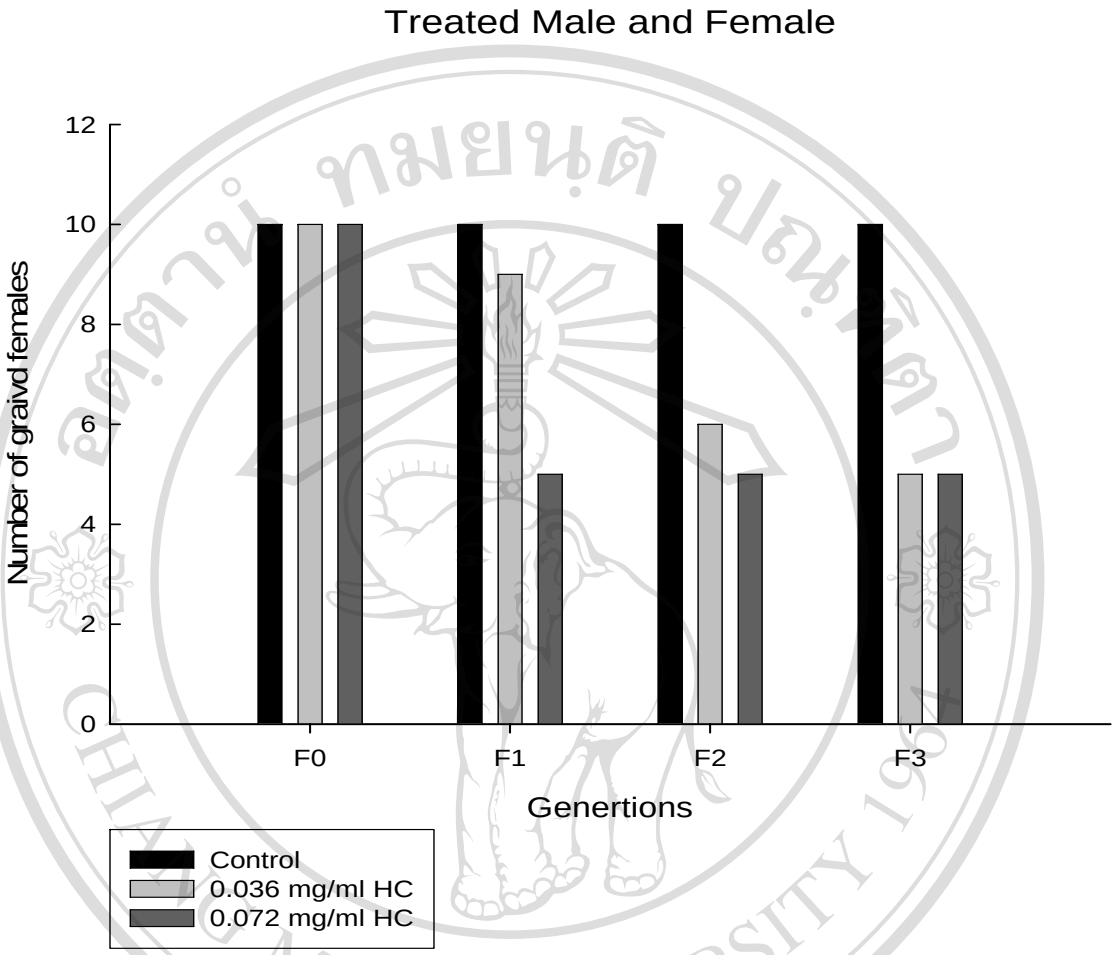

Figure 55 The number of gravid female *C. megacephala* out of the ten dissected and observed at 7 days after emergence in each treatment group. Both parental males and females (F0) were treated with human contraceptive, crossed (F1 or the first geneation) and then inbred for the second (F2) and third generations (F3).

Table 8 Number of ovarioles in female *C. megacephala* treated with human contraceptive after crossing with males treated with human contraceptive[1].

Treatment[2]	Median of number of ovarioles (range)[3]			
	Parental flies	First generation	Second generation	Third generation
Control	205.8 (180-244)[a]	283.2 (243-314)[a]	221.6 (207-236)[a]	306.0 (260-363)[a]
0.036 mg/ml HC	205.2 (184-222)[a]	263.2 (225-302)[a]	212.0 (185-254)[a]	208.8 (189-243)[b]
0.072 mg/ml HC	183.0 (162-333)[a]	243.8 (228-2532)[b]	203.0 (173-242)[a]	220.8 (186-244)[b]

[1]Human contraceptive: Microgest®, each beige tablet contained levonorgestrel at 0.15 mg and ethinylestradiol at 0.03 mg.

[2]Each treatment involved 10 female flies.

[2]Control = Female $_{(10\% \text{ sucrose solution})}$ × Male $_{(10\% \text{ sucrose solution})}$

[2]0.036 mg/ml HC= Female $_{(0.036 \text{ mg/ml HC})}$ × Male $_{(0.036 \text{ mg/ml HC})}$

[2]0.072 mg/ml HC = Female $_{(0.072 \text{ mg/ml HC})}$ × Male $_{(0.072 \text{ mg/ml HC})}$

[3]Median followed by the different letters indicate significant differences at the $P<0.05$ level within each column (generation) as determined by the Mann-Whitney *U* test.

Table 9 illustrates the number of females in each ovarian stage for each treatment group in each generation (parental, first, second and third generations). During the first generation all the ovarioles observed in each treatment were also in the final stage of maturity. In the subsequent generations the percentage of females in each stage for both the high and low treatments differed from the control. In the first generation the least mature ovarioles were at stage IV in both 0.072 mg/ml HC and 0.036 mg/ml HC IV. In the 0.036 mg/ml HC treatment group the number of females with fully mature ovarioles in the first generation was 90% but declined only 50% over next two generations. Half of the females had fully mature ovarioles in the 0.072 mg/ml HC group for the first, second and third generations.

Table 9 Percentage of total females in each ovarian stage for each treatment of the parental flies, first, second and third generations after males and females were treated with human contraceptive.

Treatment[1]	Ovarian stages[2]							
	I	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**
Parental flies								
Control	0	0	0	0	0	0	0	100
0.036 mg/ml HC	0	0	0	0	0	0	0	100
0.072 mg/ml HC	0	0	0	0	0	0	0	100
First generation								
Control	0	0	0	0	0	0	0	100
0.036 mg/ml HC	0	0	0	10	0	0	0	90
0.072 mg/ml HC	0	0	0	40	10	0	0	50
Second generation								
Control	0	0	0	0	0	0	0	100
0.036 mg/ml HC	0	0	0	20	20	0	0	60
0.072 mg/ml HC	0	0	0	10	30	10	0	50
Third generation								
Control	0	0	0	0	0	0	0	100
0.036 mg/ml HC	0	0	0	30	10	10	0	50
0.072 mg/ml HC	0	0	0	30	0	20	0	50

[1]control: 0 mg/ml HC, 0.036 mg/ml HC, 0.072 mg/ml HC

[2]Ovarial stage from the same row involved 10 female flies.

4.4 Morphological changes in the internal reproductive organs of treated male and female *C. megacephala* with 0.072 mg/ml HC using SEM

Male internal reproductive organs: SEM images were taken of the male internal reproductive organs of *C. megacephala* from the control group (Figure 56A) and the treatment group in which males were treated with 0.072 mg/ml HC (Figure 56B) in the parental fly generation, and the subsequent first generation in which no treatment was applied but observations were made (Figure 56C). There was no noticeable difference in the morphological characteristics of the testes, vas deferens, accessory glands or ejaculatory duct between the control or treatment groups of the parental flies or the first generation (Figures 56A, 56B and 56C).

Female internal reproductive organs: Scanning electron micrographs of the female reproductive system were only observed for morphological changes in the ovary. The results reported here document the morphology of the ovaries obtained from control females (Figure 57A) and females treated with 0.072 mg/ml HC (Figure 57B) in the parental flies and female flies of subsequent first generation (Figure 57C). There were no noticeable morphological differences observed between the control and treated females in the first generation flies. Morphological changes in the ovaries were observed, however, in female flies of the first generation (Figure 57C). SEM micrographs showed that the ovarioles in the ovaries of females in the first generation had cracks in the surface of the ovarioles (Figure 57C, arrowhead) and in some instances the ovariole had completely worn away to reveal the chorion of the developing egg (Figure 57C, arrow). Moreover, the ovarian envelope is thicker and penetrated by many tracheoles. An immature egg (Figure 57C, asterisk) is also visible in the ovary obtained from a female from the first generation.

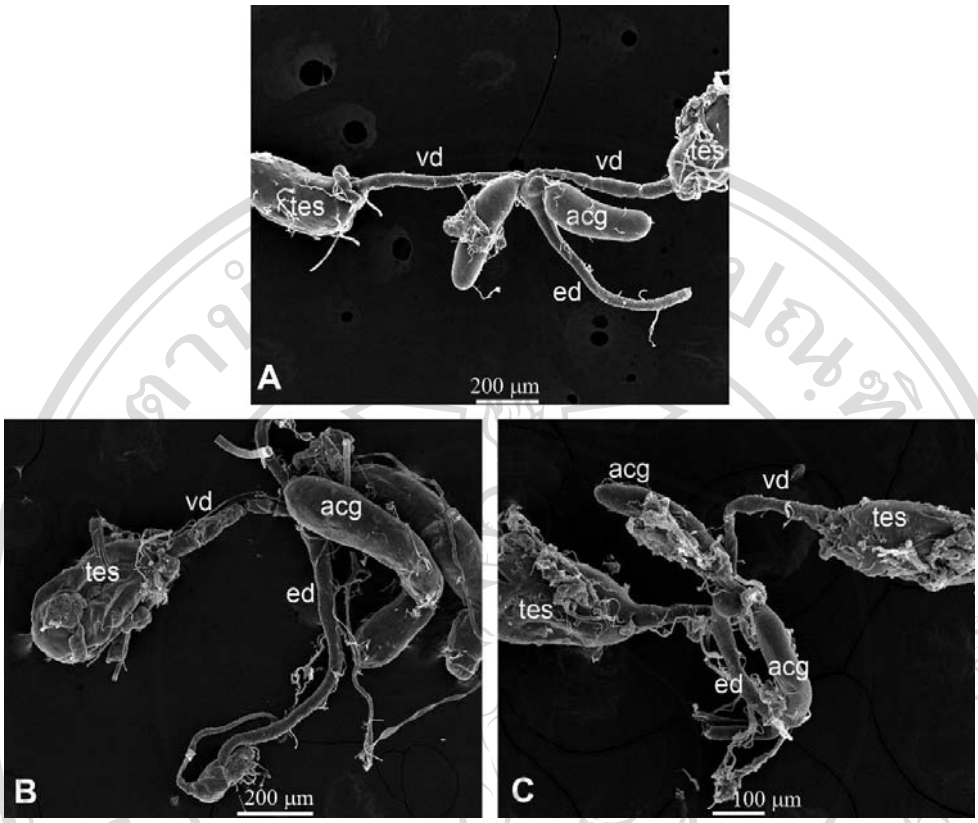

Figure 56 SEM micrographs of the internal male reproductive organs of *C. megacephala* during a study examining the effect of human contraceptive on the morphology of the reproductive system. (A) Internal male reproductive organs from the control group (parental flies). (B) Internal male reproductive organs obtained from males treated with 0.072 mg/ml HC (parental flies). (C) Internal male reproductive organs obtained from the first generation in which the parental generation was treated with a 0.072 mg/ml HC. Abbreviation; acg, accessory gland; ed, ejaculatory duct; tes, testes; vd, vas deferens.

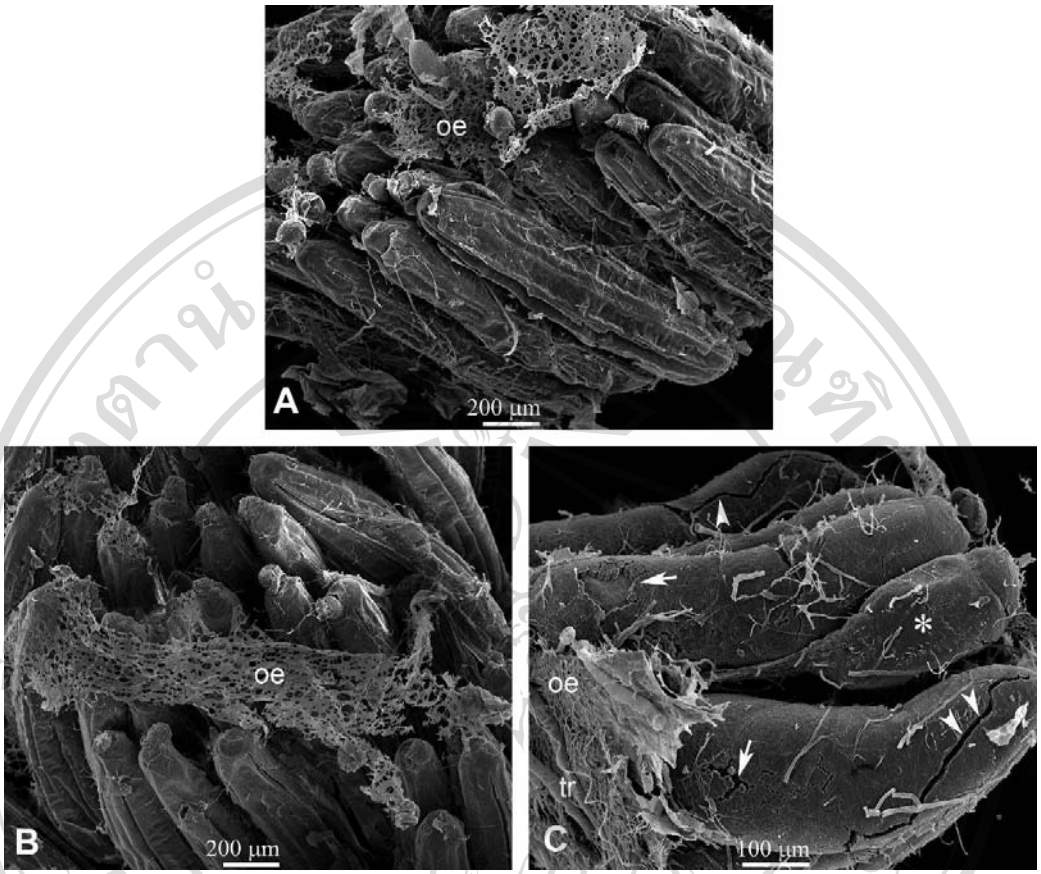

Figure 57 SEM micrographs of ovarioles of female *C. megacephala* during a study examining the effect of human contraceptive on the reproductive system morphology. (A) The ovary of a female from the control (parental flies). (B) Ovarioles obtained from a female treated with 0.072 mg/ml HC (parental flies). (C) Ovarioles obtained from a female from the first generation of flies after the parental generation was treated with a 0.072 mg/ml HC. The first generation fly shows obvious cracks in the ovarioles (arrowheads) and the ovarian envelope has completely worn away revealing the chorion of the developing egg (arrows), the ovarian envelope is thicker than control or parental treatment group fly and is penetrated by many tracheoles (tr), an immature egg (asterisk) is also visible. Abbreviation; oe, ovarian envelope.

4.5 Cellular changes in the testes and ovarioles of male and female *C. megacephala* treated with 0.072 mg/ml HC, using TEM

Seven days after both male and female flies were treated with a 0.072 mg/ml HC of human contraceptive (0.072 mg/ml), parental flies were dissected to obtain the testes and ovarioles to observe possible cellular changes using TEM and these features were followed in inbred mated males and females in the first, second and third generations. The cellular changes in the testes and ovarioles of each generation are described in the following sections.

4.5.1 Cellular changes in the testis and ovarioles of the parental flies

A comparison of cellular morphology in the testis wall of control and treated males revealed that the muscular layer of the treated males was densely packed and thinner than in the control (Figures 58A, 58B). An unknown organelle was also observed penetrating the peritoneal sheath (Figure 58B, asterisk) which resembles those in the muscle layer. A TEM image of a testis of a control fly shows the bundled nuclei and the flagella of the spermatozoa (Figure 58C). The bundled nuclei visible in the treated males appear to be degenerated and the flagella are not visible (Figure 58D).

A TEM image of an ovariole of a control female illustrates the oocyte, which is filled with the yolk body (Figure 59A). The cellular morphology of the ovarioles obtained from treated females showed that nurse cells and vacuoles were visible in the oocyte (Figure 59B) which is illustrative of an earlier ovarian stage then that observed in the control.

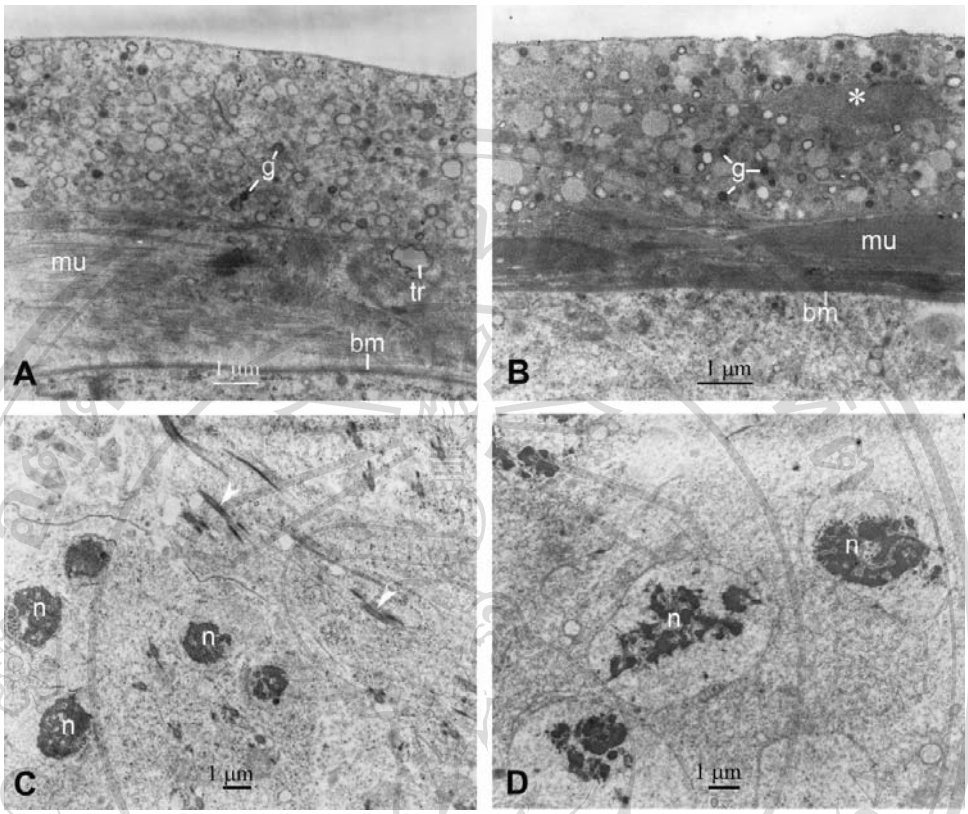

Figure 58 TEM micrographs of the testes of parental male *C. megacephala* in a study examining the effect of human contraceptive on the morphology of the reproductive system. (A) Testis wall of a control fly showing pigment granules (g) in the peritoneal sheath, the muscular layer (mu), the basement membrane (bm) and tracheoles (tr). (B) Testis wall of a male treated with 0.072 mg/ml HC showing pigment granules (g), an unknown organelle similar in appearance to those observed in the muscle layer (asterisk) and the densely packed muscular layer (mu) overlay the basement membrane (bm). (C) Testis of a control fly showing the bundled nuclei (n) and the flagella (arrowheads) of the spermatozoa. (D) Testis of a male treated with 0.072 mg/ml HC showing the degenerated nuclei (n), the flagella are not visible.

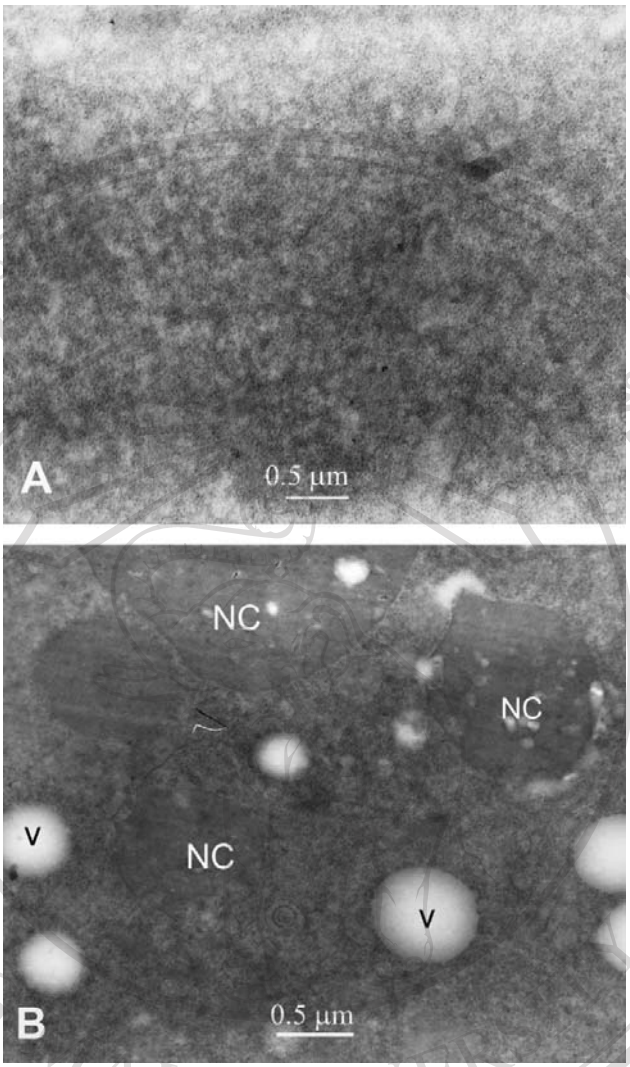

Figure 59 TEM micrographs of the ovariole of parental female *C. megacephala* in a study examining the effect of human contraceptive on the morphology of the reproductive system. (A) Oocyte of a control female showing the normal yolk body. (B) Oocyte obtained from a female treated with 0.072 mg/ml HC illustrating the nurse cells (NC) and vacuoles (V).

4.5.2 Cellular changes in the testes and ovarioles of the first generation

The cellular morphology of the testis wall of male flies of the first generaton that was descended from parental flies treated with 0.072 mg/ml HC was observed (Figure 60B). The peritoneal sheath of a fly from the treated group appears thinner than in the control (Figures 60A, 60B). A TEM image of the testis of a control fly shows the bundled spermatozoa (Figure 60C), while the testis of the first generation fly shows degenerated cells and vacuolated nuclei (Figure 60D).

In the first generation female *C. megacephala* from the control group had oocytes containing the yolk body (Figure 61A) as in the parental generation. A deformed yolk body was observed in the oocyte of an ovariole from the first generation when the parental flies were treated with 0.072 mg/ml HC (Figure 61B).

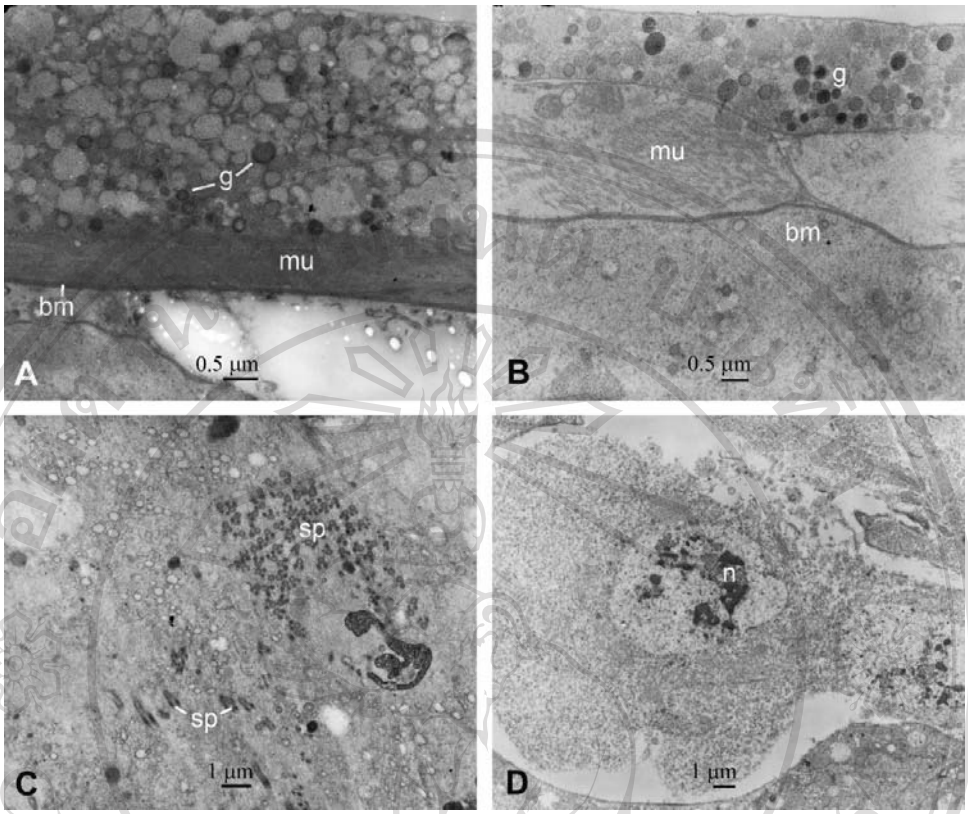

Figure 60 TEM micrographs of the testes of first generation male *C. megacephala* in a study examining the effect of human contraceptive on the morphology of the reproductive system. (A) Testis wall of a control fly (first generation) showing pigment granules (g), the muscular layer (mu) and the basement membrane (bm). (B) Testis wall of the first generation flies after males and females were treated with a 0.072 mg/ml HC showing that the peritoneal sheath is thinner than in the control and the pigment granules (g) are still distinguishable, however the muscular layer (mu) is largely transparent and electron-dense and is now thicker than the control. (C) Testis from a control fly showing the bundled spermatozoa (sp). (D) Testis of the first generation flies following parental treatment, showing the degenerated cells as well as the vacuolated nuclei (n).

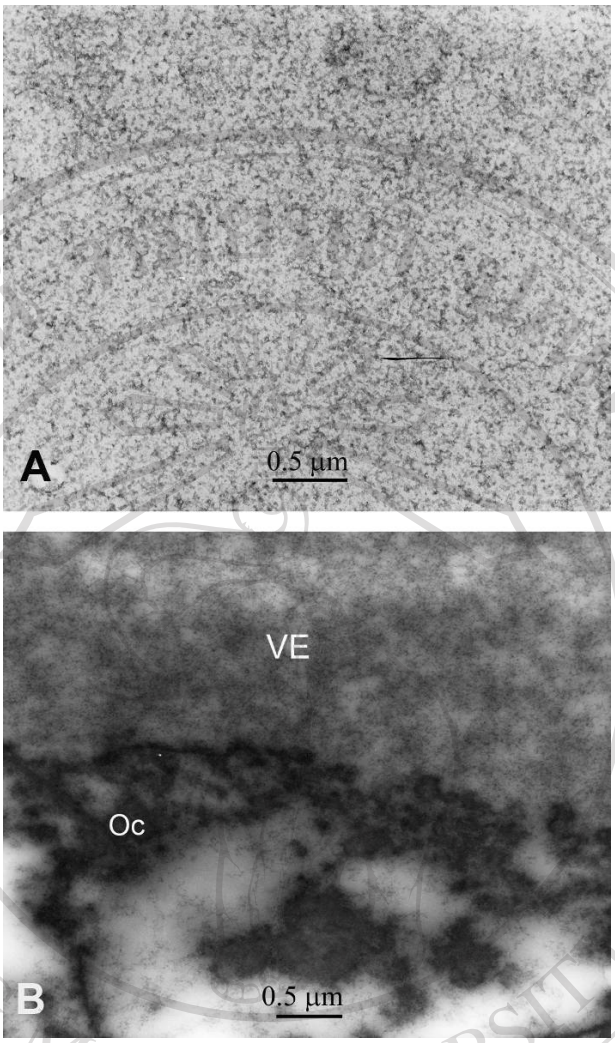

Figure 61 TEM micrographs of ovarioles of the first generation *C. megacephala* in a study examining the effect of human contraceptive on the morphology of the reproductive system. (A) Oocyte from the control group in the first generation illustrating the uniform yolk body. (B) Ovarioles of the first generation after the parental flies, were treated with 0.072 mg/ml HC showing the deformed yolk body in the oocyte (Oc).

4.5.3 Cellular changes in the testis and ovariole of the second generation

A TEM image of the testis of a control group male (the second generation) showed visible bundles of spermatozoa (Figure 62A) and flagellae (Figure 62A, arrowhead). The spermatozoa in the second generation of treated male were apparently reduced when compared to the control (Figure, 62B). Moreover, vacuolated nuclei are visible in the testis of the treated male.

Observations of the ovarioles of the second generation of females revealed a similar trend in the apparent cellular changes between the treatments as shown in Figures 63A and 63B. The imaged oocyte from the ovariole of a control group of the second generation female shows a uniform yolk body (Figure 63B) as in the previous control group generations. In contrast to the control female the ovarioles of the treated the second generation female contain nurse cells and have the appearance of wrinkles (Figure 63A, arrowhead) in the oocyte. The presence of nurse cells is indicative of an earlier ovarian stage than that observed in the control group female ovarioles.

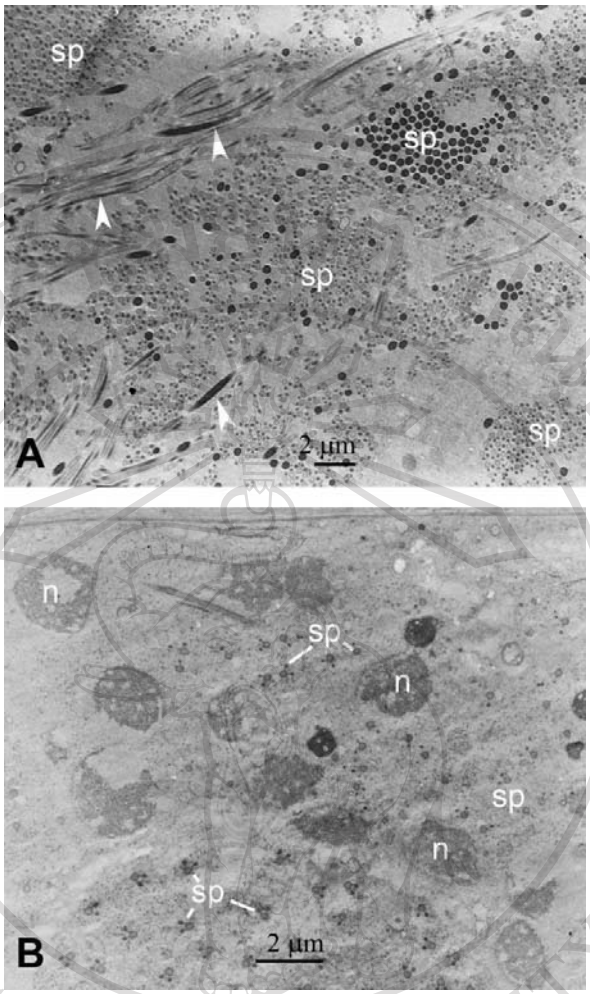

Figure 62 TEM micrographs of the testes from second generation of male *C. megacephala* in a study examining the effect of human contraceptive on the morphology of the reproductive system. (A) Testis of a male from the control group showing bundles spermatozoa (sp) and flagella (arrowheads). (B) Testis of the second generation male from the treatment group (parental generation treated with 0.072 mg/ml of human contraceptive) illustrating the apparently reduced number of spermatozoa (sp) and vacuolated nuclei (n).

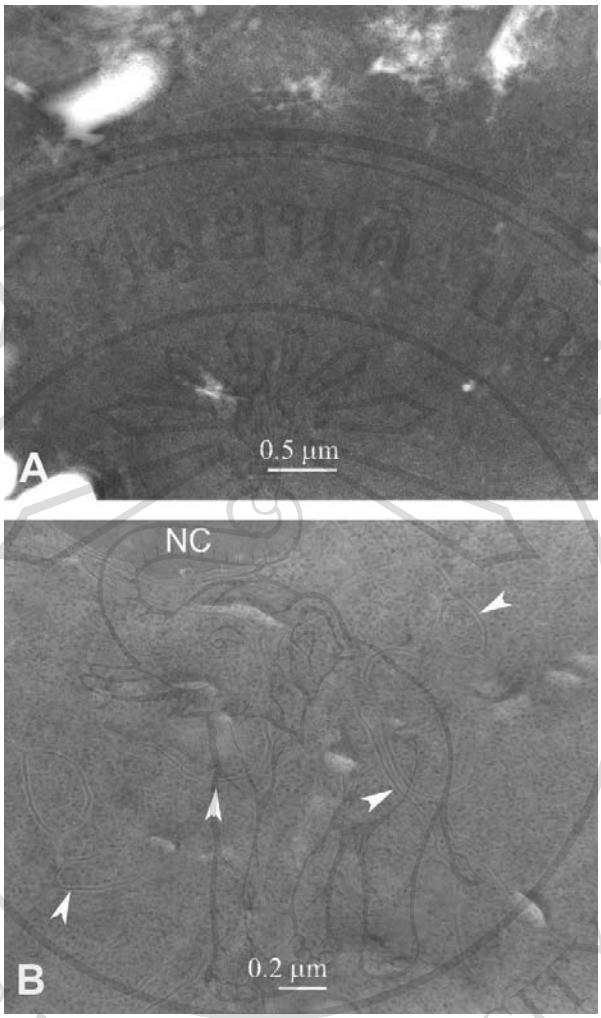

Figure 63 TEM micrographs of ovarioles of the second generation of female *C. megacephala* in a study examining the effect of human contraceptive on the morphology of the reproductive system. (A) Oocyte of a control group female showing the uniform yolk body. (B) Oocyte from the second generation female derived from the treatment group (parental generation treated with 0.072 mg/ml of human contraceptive) shows the presence of nurse cells (NC) and the wrinkled (arrowheads) appearance of the oocyte.

4.5.4 Cellular changes in the testes and ovarioles of the third generation

The cellular changes of the testes of both control and treated males were observed until the third generation (Figure 64B). In the testis of a control group male flagellae are visible (Figure 64A, arrowheads). Flagellae in the testis of a third generation treated male (Figure 64B, arrowheads) are visible, however in lower numbers than in the control (Figure 64A). Moreover, the cross section shows that the testes of treated males are penetrated by vacuoles (Figure 64B).

The cellular changes in the ovarioles of the third generation female *C. megacephala* are presented in Figures 65A and 65B. The oocyte of the control female showed a uniform yolk body (Figure 65A) as in all the previous generations. When compared with the control, the TEM image of the oocyte of the third generation female treated with 0.072 mg/ml HC in the parental generation demonstrated a large number of vacuoles (Figure 65B).

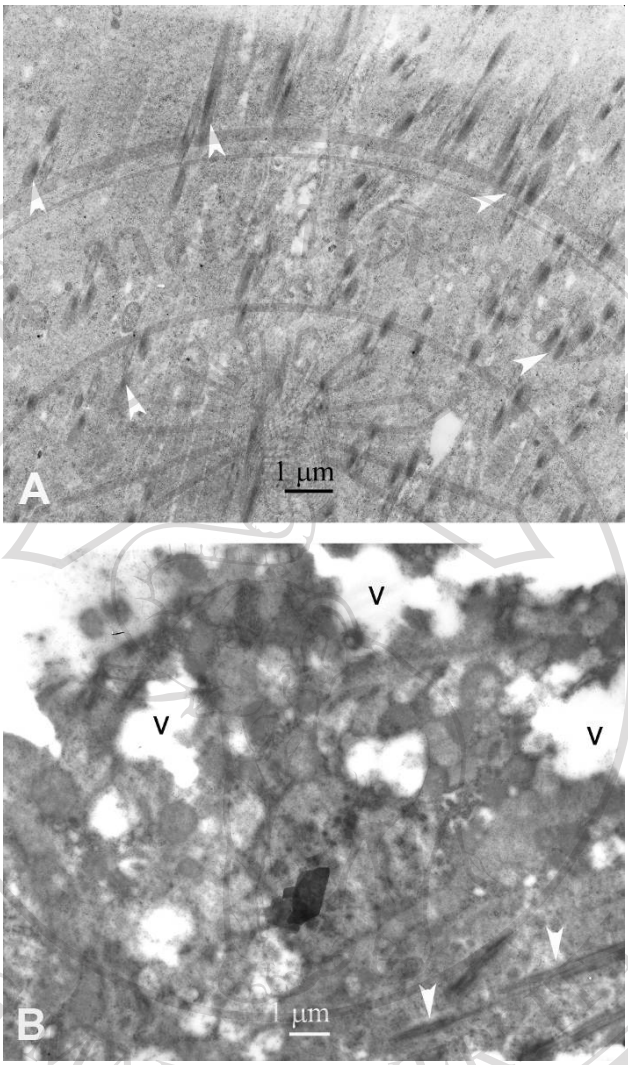

Figure 64 TEM micrographs of the testes of the third generation *C. megacephala* in a study examining the effect of human contraceptive on the morphology of the reproductive system. (A) Testis from the third generation control male displaying flagellae (arrowheads) of the spermatozoa. (B) Testis from the third generation treat group male (parental generation was treated with 0.072 mg/ml of human contraceptive) showing the abundance and penetration of vacuoles (v) and visible flagellae (arrowheads).

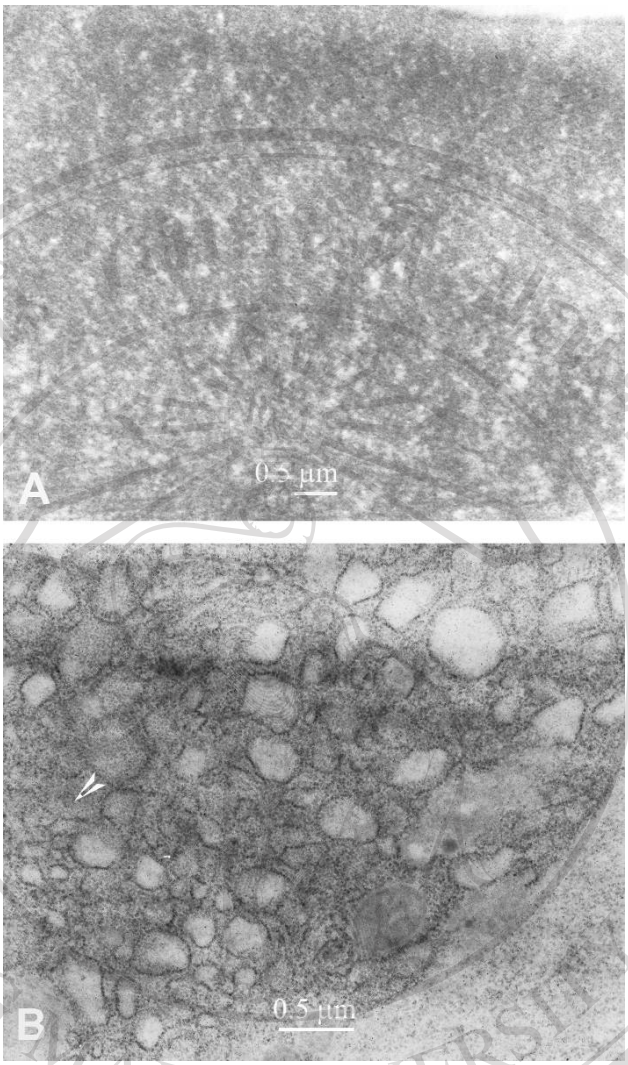

Figure 65 TEM micrographs of ovarioles from the third generation female *C. megacephala* in a study examining the effect of human contraceptive on the morphology of the reproductive system. (A) Oocyte from a control group female illustrating the uniform yolk body. (B) Oocyte from the third generation treated female (parental generation treated with 0.072 mg/ml of human contraceptive) illustrating the vacuolated oocyte.