CHAPTER V

CONCLUSION

The ultrastructure of the reproductive organs of the male and female *Chrysomya megacephala* using SEM and TEM

Male reproductive organs of C. 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. 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.

The testes of *C. megacephala* are oval-shaped, the color of testes from flies just after emergence is a pale orange. The color changes to become a reddish orange in 1 old-day flies, and continuously from reddish orange to brown or to fuscous in older males. The SEM investigation of testes shows a smooth surface. TEM micrographs of the testis wall show the outer epithelium is full of the rounded grains containing the pigment, which give the organ its characteristic color. The testes of 3 day-old flies revealed that the developing axoneme pattern had yet to be transformed into the typical 9+2+2 microtubule pattern. Changes in the color and shape of the testes are clearly visualized during the adult stages with light microscopy, SEM, and

TEM. These changes correlate closely with the age of the male fly. Daily light microscopy measurements showed a progression of testis development.

The male 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. SEM image exhibits a simple type, characterized by a slender tubule, elongated and sac-like, with an apical rounded end. TEM analysis revealed the presence of numerous RER and primary and secondary secretory granules in 3-day-old glandular cells.

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. The ultrastructure of the vas deferens of this blowfly consisted of three layers including the epithelial cell layer with muscle cells, a connective tissue layer and a plasma membrane layer adjacent to the duct lumen.

C. megacephala has short form of ejaculatory duct. SEM micrographs showed a pattern consistent with longitudinal muscle on the surface of the ejaculatory duct. The current study also demonstrated the presence of secretory cells in the plasma membrane of the vas deferens and the epithelial cells of ejaculatory duct of *C. megacephala*. Secretory cells are usually associated with the production of secretions that support the functions of accessory reproductive glands, such as spermatopore production, mating plug formation, and sperm activation.

Female reproductive organs of C. 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. The round ovaries are

placed dorsolaterally to the alimentary canal, enclosed in a peritoneal sheath. 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. A paired of long tubular accessory glands, opened into the dilated anterior genital chamber or vagina.

The results of this study present a method for age determination of female flies that can be easily adapted for field studies as it is based on practical light microscopy methods. TEM images of the developing embryonic chorion in this fly which provide interesting insight into the development of the chorion as the egg matures. Measurements of the ovaries also detected significant increases in the size of the ovaries during the period of study, both in length and width based on measurement analyses, which also have potential utility in age determination of female *C. megacephala*. The morphological analysis of the ovaries confirmed that it can be used to differentiate the age of females in this blow fly species. Young blow flies are characterized by a tightly wound and well developed tracheal system surrounding the ovaries and SEM images revealed a thick outer epithelial sheath. In contrast there is a reduction in the extent of the tracheal system in older flies and SEM images revealed a thin ovarian envelope with many holes. In this investigation, *C. megacephala* females completed egg development in eight stages and about ten days under ambient temperature (18-27°C). The development of follicular epithelium of the 3- and 7-day-old blowfly passes through several distinct stages of oognesis. TEM micrographs illustrated the differentiation of the developing chorion layers in young and old adult females. Hence the stages of ovarian development in *C. megacephala* can be a useful tool for determining the age and approximate generation time and population potential

of this blow fly. The light microscopy based age grading system presented herein can also be used to determine the age of flies collected from the field. In addition, the basic information provided by this study can be used to understand the ovarian development and fine structure of the ovaries and ovarioles of *C. megacephala* which might prove to useful in developing new methods to control this blowfly in the future.

The female accessory glands of *C. megacephala* are paired long and tubular glands that open into the dilated anterior genital chamber. Each gland is composed of two distinct regions including an apical bulb and a tubular gland duct which differentiate during maturation of the fly as evidenced by TEM micrographs of 3-day-old flies. SEM micrographs show that the surface of the female accessory glands of this blowfly are covered in papillae and occasionally penetrated by tracheoles. The structure of the epithelial cells of the female accessory gland consisted of both secretory cells and duct forming cells. Cellular level on the morphology of the 3-day-old female accessory glands revealed that the cistern cells contained transparent electron dense material, where large amounts of secretions are normally stored, suggesting that was missing or had not yet formed. The tubular duct portion of the gland did have secretions present in the cistern cells. A low volume of secretions was present in the central lumen.

C. megacephala has three spermathecae, which are arranged in the 1:2 configuration, with the two of one side loosely bound together and the one on the other side unattached. SEM images showed that tubercles cover the surface of all three of the spermathecae and that each is penetrated by tracheoles. The basal region of spermathecae is connected via a spermathecal duct and longitudinal muscle is clearly visible on surface of the spermathecal duct. TEM images of the epithelial cells

showed that cistern cells are present which store secretions and spermatozoa. The epithelial cells of 3 day-old flies, rarely showed cells with extracellular cisterns and the lumens these cells did not contain spermatozoa, probably suggesting that 3 day-old *C. megacephala* may not yet be inseminated. The ultrastructure of the spermathecae of *C. megacephala* could be useful for determining the physiological mechanisms responsible for changes in behavior occurring before and during mating and oviposition as well as how fertilization of the eggs occurs in this blow fly.

The genital chamber of *C. megacephala* is a single long tubular organ. Imaging revealed that the genital chamber consists of a central lumen wrapped by five distinct cell layers, with each layer characterized by a difference in structure and cellular organelles. TEM analyses showed a muscle layer that was only visible in the third-layer, while longitudinal and circular muscles were visible on the surface in the SEM micrographs. Measurements of the length and width of the genital chamber showed a peak in length on about day seven and a peak in width on day six. The ultrastructural features of the genital chamber of this fly can be useful to clarify morphology and to understand the function of the reproductive system components.

The external genitalia: male and female: The male genitalia of *C. megacephala* consist of the cercus, surstylus, epandrium, phallus, ejaculatory apodeme, and aedeagal apodeme. The epandrium is a broad organ, resembling a crescent shape; while the ejaculatory apodeme and aedeagal apodeme show similarity in their lengths. The cercus is significantly longer than the surstylus, with its apical end being more or less rounded. Long bristles, morphologically similar to the sensilla chaetica and sensilla trichodea, were densely found along the lower half of the cercus. The surstylus is a stout triangular shape, and the proximal half is greatly endowed

with probable sensilla chaetica and sensilla trichodea. The aedeagus *per se* is prominent, appearing as a clavate shape formed by the base theca and elongated phallus. The ventralia of the phallus exhibits a smooth bilobed, which is curved inward. The juxta and juxta process are armored with many rows of strong spines, resembling thorns in appearance. The harpe is slender, recurved anteriorly and distally pointed like a sickle.

Regarding the female genitalia, the eversible ovipositor extends from the last preabdominal segment and is comprised of four visible segments. The supra-anal plate revealed it to be sclerotized and more or less triangular-shaped, lying between a pair of short cerci. Several types of sensilla were found on the supra-anal plate, sub-anal plate and cercus, including the sensilla trichodea, sensilla basiconica, sensilla placodea and sensilla styloconica. The sensilla trichodea bears longitudinal grooves externally, which are of variable lengths and distributed specifically along the ventrolateral margin of the structure. The sensilla basiconica appear as short hair shafts bearing longitudinal grooves or having smooth surfaces inserted into cuticular sockets. The sensilla placodea appears as a plate-like cuticle that is usually recessed in a shallow pit with a characteristic central pore. The sensilla styloconica presents as a ball-shaped peg with a rugose surface throughout.

Efficacy of the human contraceptive on the reproductive system of *Chrysomya. megacephala*

This study presents the effects of a human contraceptive, which containing levonorgestrel and ethinylestradiol on the development of eggs and the morphology of the reproductive structures themselves in *C. megacephala*. A reduction in egg production and delayed egg maturation were evident in females treated with human contraceptive. These effects were only observed in the first, second and third generations but not in the parental generation. Additionally, delayed egg maturation was found in adult *C. megacephala* treated with human contraceptive.

Ultrastructural studies of the treated flies with human contraceptive revealed the cellular alteration of both ovary and testis of flies in the subsequent generations. The alterations observed under SEM in the ovaries of the first generation females were thin ovarian envelope, cracks in the surface of the ovarioles, a wearing away of the ovarian envelope to reveal the chorion of the developing egg, as well as the presence of immature eggs. The effect of the human contraceptive on female *C. megacephala* was shown to cause a decrease in egg production and delayed egg maturation which was confirmed with observations of cellular changes in the ovarioles using electron microscopy techniques. Cellular alteration was also observed in the testes of treated males, indicating by the presence of the degenerated nuclei of the spermatozoa during spermatogenesis. The data presented here suggest that the human contraceptive could be used as part of an integrated control program to reduce reproduction of this blow fly species.