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Effects of human contraceptive on reproduction and offspring in *Chrysomya megacephala*

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ABSTRACT

Objective: To investigate the effect of human contraceptive (HC) as ability to suppress the reproductive success of blow fly, *Chrysomya megacephala* (Fabricius) *(C. megacephala)* and offspring under controlled laboratory conditions. **Methods:** Adult *C. megacephala* were fed with low (0.036 mg/mL) and high dose (0.072 mg/mL) HC (Microgest®, Thailand), containing levonorgestrel and ethinyl estradiol, in their drinking water for 7 days. Three experiments were set; experiment I with fed only in parental males, experiment II with fed only in parental females and experiment III with fed in both males and females. All experiments were then maintained for 3 generations after crossing and inbreeding. **Results:** A lower ovariole production and less fully mature ovarioles were evident in F1, F2 and F3 than control when parent males, females and both had been fed with high dose HC. Cellular changes during spermatogenesis in F1, F2 and F3 testes was confirmed using transmission electron microscopy (TEM), showing the low level of condensed chromatin, necrotic chromatin, irregularities and degenerated nuclear envelope in the nucleus. In the cytoplasm, mitochondrial swelling, rough endoplasmic reticulum swelling as well as vacuolated cytoplasm were noticed. As for the sperm *per se*, we found the degenerated nuclei and/or incomplete mitochondrial derivative, axoneme and vacuolated flagella. Regarding deformity in F1, F2 and F3 ovariole, ultrastructural alteration observed by scanning electron microscopy (SEM) included malformations involving fragile enveloping peritoneal sheath, cracked ovarioles, peel away chorion, crumbled eggshell and incomplete development; whereas TEM presented malformed and disorganized mass of cells, proteic yolk granules and vacuolated vesicles. **Conclusions:** Administer of HC to adult *C. megacephala* caused ovariole reduction, less matured ovariole and affected cellular changes in testes and ovariole of offspring up to F3.

1. Introduction

Chrysomya megacephala (Fabricius, 1794) *(C. megacephala)* is a medically important blow fly species worldwide. Adult flies not only cause annoyance to humans and domestic animals, but being a mechanical carrier of numerous pathogens infectious to humans, whereas the larvae can produce myiasis. Problems arising from *C. megacephala* are results of its rapid development capability under warm temperatures as well as the wide variety of filth sources that this fly can take advantage of as breeding sites. Therefore, maintenance of populations of this species below disease transmission thresholds is of critical importance.

Control strategies for pest populations currently rely heavily on insecticides and some problems have been encountered, including environmental pollution, insecticide resistance and increased pesticide costs. With the current movement towards using decreased amounts of any kind of insecticide, alternative control methods that can be included in an integrated pest control program should be developed and tested for further application. Besides the natural products derived from plants that have been extensively evaluated against insects, such

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other kinds as the vertebrate steroid hormones have also been investigated due to the evidence that the vertebrate steroid hormones are present in insects[1]. In humans, the major endocrine products of follicles are estrogens, which are the steroids that produce female characteristics. There are three physiologically significant estrogens: estrone, estradiol and estriol. Estradiol has been identified in the ovary of the insects, such as silkworm, *Bombyx mori* (Lepidoptera: Bombycidae)[1]. Investigations pertaining to the effects of steroid hormone on development of insects have been recorded by many researchers. Examples of this was provided by Lee and Choi[2] who reported the effects of bisphenol A and ethynyl estradiol on growth and development of the aquatic midge, *Chironomus riparius* (Diptera: Chironomidae). Despite the investigations of vertebrate steroid hormone in insects, only a few studies have been performed on the effects of these compounds on the reproduction and offspring of *C. megacephala*, which is the medically important blow fly species in Thailand and the Oriental region. Here, we reported the effects of female human contraceptive (HC), estrogen, on the reproduction and offspring of *C. megacephala*. This HC was chosen because of its close structural similarity to ecdysone which is naturally produced in the insect and is an important hormone involved in development and reproduction. Observation on the effects of HC against *C. megacephala* was monitored in flies directly affected parental, F1, F2 and F3 to determine any reduction in egg production and possible deformity in the reproductive organs. Such information provided scientific knowledge that may be applied in fly control strategy in the future.

2. Materials and methods

2.1. Fly maintenance

The adult male and female *C. megacephala* used in this study were obtained from a laboratory colony and reared in the Department of Parasitology, Faculty of Medicine, Chiang Mai University, Chiang Mai (at 17-21 °N, 98-99 °E), Thailand. Laboratory colonies were maintained as described by Sukontason *et al*[3].

2.2. Feeding bioassay

The HC from Microgest®, Thailand was used. Each beige tablet contained 0.15 mg of levonorgestrel and 0.03 mg of ethinyl estradiol. Two different doses of HC were examined and the concentrations were prepared as follows: (1) Low dose: 10 beige tablets were homogenized with 50 mL of 10% (w/v) sucrose solution; giving a concentration of 0.036 mg/mL; (2) High dose: high dose group was prepared using 20 beige tablets; giving a concentration of 0.076 mg/mL. Control was used 10% (w/v) sucrose solution only. The overall study design consisted of a series of 3 experiments which examined the effects of feeding HC on males only (Experiment I), females only (Experiment II), and to both males and females (Experiment III).

2.2.1. Experiment I

Effect of HC fed to males: One-day old flies were divided into 6 groups (100 flies/group) which were subsequently provided the food treatments, as shown in Table 1. This food was provided for 7 days. After 7 days, 10 flies of each group were randomly collected and dissected the reproductive organs for investigations using scanning electron microscope (SEM) and transmission electron microscopy (TEM). In addition, 10 flies from each female group were collected and dissected to examine the number of eggs in the ovaries. The reason that the dissection and enumeration of ovarioles was chosen to determine fecundity was that this was considered a more accurate method when compared to enumerating eggs laid due to the difficulty involved in defining a blowfly egg mass. Changes in the ovarioles were classified according to the descriptions obtained through observations during egg development.

In order to determine direct effects of HC, matings were conducted between the treatment groups to determine female fecundity. Three groups of males and females were paired: males (group 1) + females (group 4), treated males with low dose (group 2) + untreated females (group 5), and treated males with high dose (group 3) + untreated females (group 6). Each combined group was provided with 50 mL of sucrose solution and a piece of fresh pork liver, until eggs were found. After that, the eggs of each combined group were reared separately.

Moreover, developmental changes were observed for assessing the possible effects of HC, including failure of larvae to molt normally, failure of larvae to form pupae, and inability of flies to complete adult eclosion. Any developmental changes observed in each stage were morphologically examined using SEM.

2.2.2. Experiment II

Effect of HC fed to females: 6 groups of flies were supplied food as shown in Table 1. This resulted in the formation of combined treatment groups as follows: males (group 1) + females (group 4), untreated males (group 2) + treated females with low dose (group 5), and untreated males (group 3) + treated females with high dose (group 6).

2.2.3. Experiment III

Effect of HC fed to males and females: 6 groups of flies were offered food as shown in Table 1, as following: untreated males (group 1) + untreated females (group 4), treated males with low dose (group 2) + treated females with low dose (group 5), and treated males with high dose (group 3) + treated females with high dose (group 6).

Following adult eclosion, the first progeny of each combined group were provided reared further. Subsequently, 30 (5 to 7-day-old) males and females (F1 progeny of each combined group) were dissected for observation of their reproductive organs using SEM. The testes and ovaries of the F1 were examined using TEM.

2.3. Scanning electron microscopy (SEM) investigations

Adults (n=5-10 for each sex) were dissected and the reproductive tract was processed for SEM by transferring them into a 2.5% glutaraldehyde mixture in a phosphate-buffered saline (PBS), pH 7.4 at 4 ℃ for 24 h. They were rinsed twice with PBS at 10 min intervals, and postfixed with 1% osmium tetroxide at room temperature for 3 days. The specimens were then rinsed twice with PBS and dehydrated with alcohol. The dehydration process was performed by subjecting to increase alcohol concentrations, and they were placed in absolute alcohol for two 12 h periods followed by treatment in acetone for two 12 h periods. Finally, they were

Table 1
Conditions for treatment of adult *C. megacephala* in experiments I, II and III: effect of feeding human contraceptive.

Group		Caged flies	Treatment	Adult diet
Experiment I (treated in males)	1	100 males	Untreated	Sucrose + fresh pork liver
	2	100 males	Treated	Low dose + sucrose + fresh pork liver
	3	100 males	Treated	High dose + sucrose + fresh pork liver
	4	100 females	Untreated	Sucrose + fresh pork liver
	5	100 females	Untreated	Sucrose + fresh pork liver
	6	100 females	Untreated	Sucrose + fresh pork liver
Experiment II (treated in females)	1	100 males	Untreated	Sucrose + fresh pork liver
	2	100 males	Untreated	Sucrose + fresh pork liver
	3	100 males	Untreated	Sucrose + fresh pork liver
	4	100 females	Untreated	Sucrose + fresh pork liver
	5	100 females	Treated	Low dose + sucrose + fresh pork liver
	6	100 females	Treated	High dose + sucrose + fresh pork liver
Experiment III (treated in males and females)	1	100 males	Untreated	Sucrose + fresh pork liver
	2	100 males	Treated	Low dose + sucrose + fresh pork liver
	3	100 males	Treated	High dose + sucrose + fresh pork liver
	4	100 females	Untreated	Sucrose + fresh pork liver
	5	100 females	Treated	Low dose + sucrose + fresh pork liver
	6	100 females	Treated	High dose + sucrose + fresh pork liver

Low dose: 0.036 mg/mL, high dose: 0.076 mg/mL.

subjected to critical point drying, attached to double-stick tape on aluminum stubs, coated with gold and viewed under a JEOL JSM-5910LV SEM (Tokyo, Japan).

2.4. TEM investigations

For TEM, testes and ovary dissected from 7-day-old adult of parental and offspring up to F3 were processed as described for the SEM. After post-fixation, they were dehydrated as previously described and placed in acetone for 2 h before transferring to resin and a acetone mixture at a ratio of 1:3 for 24 h, 1:1 for 24 h and 3:1 for 24 h to replace the acetone in the specimens with resin and ensure complete embedding. This process was repeated for each specimen twice in 100% resin for 3 h. Specimens were embedded in Spurr's resin by placing them into a plastic block template, and incubating at 70 ℃ for 24 h. Thick sections (0.5 μm) were cross-sectional cut, while the ultrathin sections (90 nm) were prepared. Sections were post-stained with uranyl acetate and lead citrate before examination by a Hitachi H700 transmission electron microscope (Japan) operated at 100 kV.

2.5. Statistical analysis

Analysis of fecundity data were expressed as median (range) and statistical analyzed using the Kruskal-Wallis analysis of variance due to the lower sample sizes used. If significant differences were observed, a Mann-Whitney U test was used to determine which groups differed from each other. For all experiments, the significance was defined as $P<0.05$, and statistical tests were performed using SPSS version 14.0.1.

3. Results

3.1. Experiment I

Table 2 shows the median number of ovarioles per female *C. megacephala* produced for the parental when males were treated with each concentration of HC, as well as the median number of ovarioles for the following 3 generations after crossing and inbreeding. No significant difference ($P>0.05$) was observed among control, low dose or high dose in the median number of ovarioles in the ovaries of parental females using a Kruskal-Wallis ANOVA based on ranks. In F1, F2 and F3, 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 from high dose HC group was significantly ($P<0.05$) lower in the F1, F2 and F3 (Table 2). Additionally, in F1 and F2 the median number of ovarioles from the high dose treated male groups were significantly ($P<0.05$) lower than the low dose treatment group, but this effect was not observed in F3 (Table 2). However, the median number of ovarioles from low dose treated male groups was significantly ($P<0.05$) decreased from the control only in F3.

The number of females in each ovarian stage for each treatment group in each generation (parental, F1, F2 and F3) was not shown. The number of fully mature ovarioles declined over generations in the low dose treatment group. With further increase dose to be high, an increase effect was noticed, approximately half of females had fully mature ovarioles in F1, F2 and F3.

3.2. Experiment II

Table 3 displays the median number of ovarioles per female *C. megacephala* of the parental flies treated with HC crossed and inbred females for the subsequent F1, F2 and F3. The Kruskal-Wallis ANOVA detected no significant ($P>0.05$) difference among any of the treatment groups in the number of ovarioles from the parental. In F1, F2 and F3, the Kruskal-Wallis ANOVA detected significant differences due to a treatment effect. Compared with the control, the median number of ovarioles of high dose treatment group females was significantly lower in F1, F2 and F3 (Mann-Whitney U

tests; $P<0.05$). The median number of ovarioles from females in the low dose treatment group was also significantly ($P<0.05$) lower in F1, F2 and F3 when compared with the control.

The number of females in each ovarian stage for each treatment group in each generation (parental, F1, F2, F3) was not shown. Ovarioles of control all reached the final stage of maturity. The number of fully mature ovarioles declined from 60% in F1 and F2 to 50% in F3 in the low dose treatment group. In the high dose group only 30% of females had fully mature ovarioles for F1 but 50% were fully mature in F2 and F3.

3.3. Experiment III

Tables 4 displayed the effect of HC fed to parental males and females *C. megacephala* (Experiment III). As in the previous experiments, no significant difference among the treatments in the parental flies in the median number of ovarioles obtained from females (Kruskal-Wallis ANOVA; $P>0.05$). Median number of ovarioles derived from exposing high dose treatment was significantly lower ($P<0.05$) in F1 and F3 when compared with the control. No significant difference ($P>0.05$) was observed between low dose and high dose groups in F1, F2 and F3. 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 F2.

The number of females in each ovarian stage for each treatment group in each generation (parental, F1, F2 and F3) was not shown. In the low dose treatment group the number of females with fully mature ovarioles in F1 was 90% but declined only 60% and 50% over F2 and F3. Half of the females had fully mature ovarioles in the high dose group for F1, F2 and F3.

3.4. Morphological changes in reproductive organs of offspring when parental had been treated with high dose HC

Aberrant characteristics of offspring in experiment I (parental had been treated with 0.072 mg/mL HC in males only) were displayed in Figures 1 and 2. Analysis of F1 testes using SEM showed only the normal shape in the control (Figure 1A) but malformed shape in the treated group (Figure 1B). TEM analysis confirmed the aberrant in F1 testes. Control tissue is characterized by uniform of testes wall forming by an external layer, a peritoneal sheath and a basement membrane. Development of spermatogenesis was normal, with the image obtained showing the large patches of condensed chromatin with apparent nuclear envelope (Figure 1C) or groups of sperm having nucleus and flagellum. In contrast, observations of treated male parent exhibited evidence changes inside F1 tissue in both nucleus and cytoplasm. In nucleus, drastically alteration involved the low level of condensed chromatin (see for instance Figure 1D, arrowhead) with fade away in some cases. The double-layer nuclear envelope exhibited prominent irregularities, degenerated and sometime loss (Figure 1D, arrow). In cytoplasm, vacuoles with spherical bodies containing material of different density were observed. Analysis of F2 testes was shown in Figure 2, displaying the normal development of spermatogenesis having numerous sperm in the control group (Figure 2A). In the treated group, some of the deformity recorded during assessment included necrotic nucleus (Figure 2B, arrow), degenerated and less dense chromatin, vacuolated testes wall, mitochondrial swelling and rough endoplasmic reticulum swelling. As for the sperm *per se*, the consequence observed included under-developed (Figure 2B, arrowhead), degenerated and/or incomplete mitochondrial derivative and vacuolated flagella. Likewise phenomena were observed in F3 testes, with abundance of sperm having nucleus and flagellum appearing in the control (Figure 2C). In the treated group, deformity of testis in cellular level was still observed. The testes wall was disorganized, vacuolated and swollen. Although development of spermatogenesis existed, significant changes of this process was noted, typically involving disintegrated chromatin (Figure 2D, arrows), degeneration of axoneme, mitochondrial derivative (Figure 2D, arrowhead) as well as flagellum of sperm. The prominent of vacuolated cytoplasm was also shown.

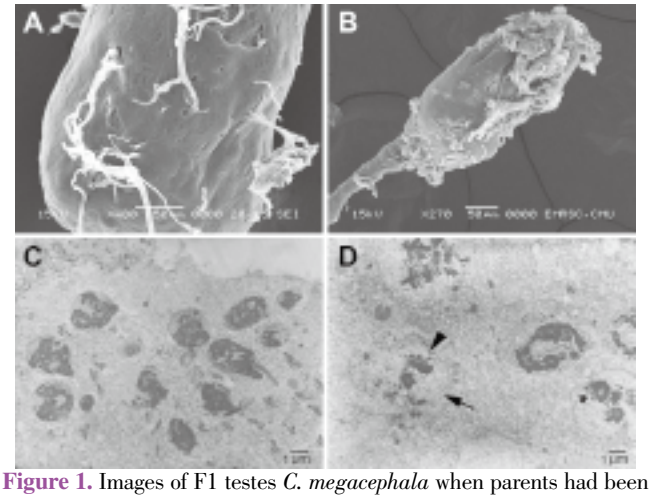

Figure 1. Images of F1 testes *C. megacephala* when parents had been treated with 0.072 mg/mL HC in males.
A: SEM image of normal shape in the control group. B: SEM image of the malformed shape in the treated group. C: TEM image of control showing the large patches of condensed chromatin with apparent nuclear envelope. D: TEM image of F1 tissue displaying low level of condensed chromatin (arrowhead) and degenerated nuclear envelope (arrow).

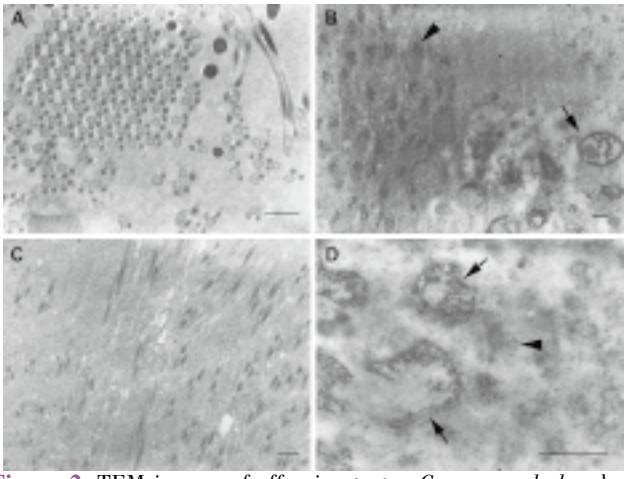

Figure 2. TEM images of offspring testes *C. megacephala* when parents had been treated with 0.072 mg/mL HC in males.
A: F2 control exhibiting numerous sperm. B: F2 treated exhibiting necrotic nucleus (arrow) and under-developed sperm (arrowhead). C: F3 control revealing abundance of sperm. D: F3 treated revealing disintegrated chromatin (arrows) and degenerated mitochondrial derivative of sperm (arrowhead)(scale=1 μ m).

Aberrant characteristics of females in experiment II (parental had been treated with 0.072 mg/mL HC in females only) were displayed in Figure 3. Unusual development of ovarioles was noticeable even in the parental generation, with the normal yolk being detected in the control (Figure 3A) while vacuolated yolk appearing as spherical bodies (Figure 3B, arrows) being observed in treated group. Malformations of treated F1 ovarioles was seen under SEM assessment, characterized by fragile enveloping peritoneal sheath, cracked ovarioles (see for instance Figures 3C; 3D, arrows), peel away chorion (Figure 3C, arrowhead), crumbled eggshell, some of ovarioles incomplete development (Figure 3D, arrowhead). Analysis of F3 ovarioles yielded similar pattern of response, with the normal yolk appearing in the control group (Figure 3E). For a closer investigation of treated group, ultrathin sections revealed numerous vacuolated vesicles (Figure 3F, arrows).

Figure 4 presented an aberrant characteristics of F1 offspring in experiment III (parental had been treated with 0.072 mg/mL HC in both males and females). Resemble consequence of experiment II, the cracked ovariole and crumbled eggshell (Figure 4A) of the treated offspring were apparent. TEM analysis exhibited the normal yolk of F1 ovarioles (Figure 4B); in contrast with much distinctive of disintegrated yolk in the treated one (Figure 4C). As for the F1 testis, normal development of spermatogenesis was evident in having numerous sperm (Figure 4D), in contrast with the undeveloped of treated group in having disintegrated chromatin (Figure 4F, arrows), malformed and disorganized mass of cells (Figure 4F, arrowheads).

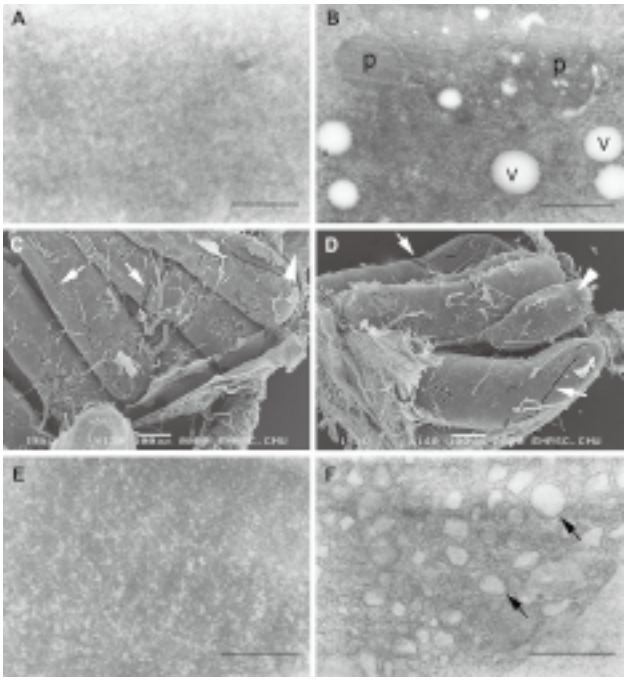

Figure 3. Images of ovarioles *C. megacephala* when parents had been treated with 0.072 mg/mL HC in females.
A: Normal yolk of parent control. B: Yolk of parent treated yielding vacuolated (v) and proteic yolk granules (p). C: SEM image of F1 treated showing cracked ovarioles (arrows), peel away chorion (arrowhead) and crumbled eggshell. D: SEM image of F1 treated showing incomplete ovarioles (arrowhead) and cracked ovarioles (arrows). E: F3 control displaying normal yolk. F: F3 treated displaying vacuolated vesicles(arrows)(A, B, E, F scale=1 μm).

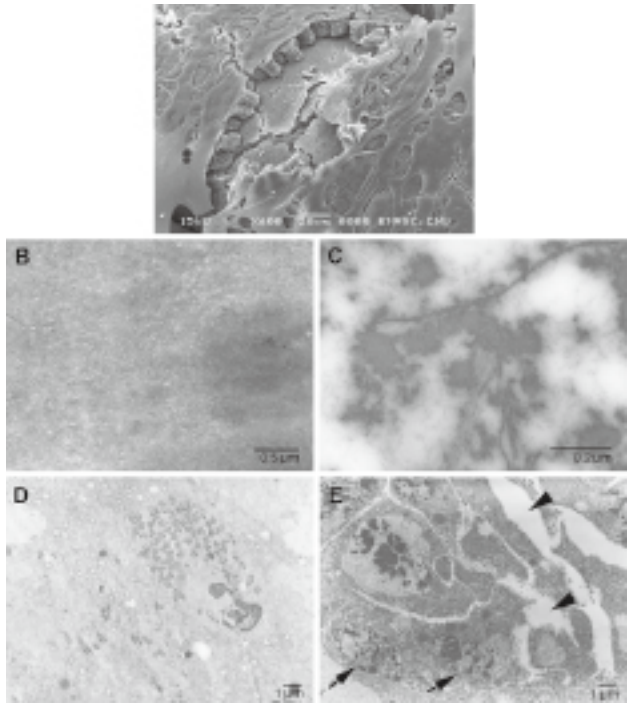

Figure 4. Images of offspring *C. megacephala* when parents had been treated with 0.072 mg/mL HC in males and females.
A: SEM image of F1 ovariole showing cracked and crumbled eggshell. B: TEM image of F1 ovariole showing normal yolk. C: TEM image of F1 ovariole showing disintegrated yolk. D: TEM image of F1 control testes showing numerous sperm. E: TEM image of F1 treated testes showing disintegrated chromatin (arrows), malformed and disorganized mass of cells (arrowheads).

4. Discussion

The effects of vertebrate steroid hormones on the reproductive system of insects have been reported by many researchers. Estradiol was found to considerably reduce the oviposition rate of adult female *Bombyx mori*[1]. The steroids 17-ethinylestradiol (EE) and bisphenol A effected the number of egg-ropes produced by *C. riparius*, as determined over 2 generations[4]; whereas the acute toxicity of estradiol-17 β as endocrine disrupter on viability of two crustaceans, mysid shrimp *Americamysis bahia* (Mysida: Mysidae) and waterflea *Daphnia magna* (Crustacea: Cladocera) was evident[5]. Three vertebrate hormones (estrogen, testosterone and thyroxine) affected the growth, development and reproduction of tomato moth, *Lacanobia oleracea* (Lepidoptera: Noctuidae)[6]. Results from our study related to such investigations in that feeding treatment of high dose HC, containing levonorgestrel and ethinylestradiol, in adult *C. megacephala* diet leads to developmental disruption up to 3 generations, according to biological activity and morphological changes in cellular level of reproductive organs. Reduction and less matured ovariole were evident in offspring treated with HC in all experiments. Such effects were greatly observed in F1, F2 and F3 but not much obvious in parents, suggesting that consequence of HC can be transferred from parents and potentially accumulate in the subsequent generations. The component of HC may have had a direct effect on the reproductive structures, as seen in the ultrastructural level. In offspring, pathological changes in testes and ovariole were apparent either parent had been

Table 2

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

Treatment[2]	Median of number of ovarioles (range)[3]			
	Parental flies	F1	F2	F3
Control	262.0(226–304)	297.3(257–335)	291.3(249–336)	329.0(305–365)
Low dose	255.9(223–288)	286.1(226–304)	272.8(251–301)	286.0(266–306)[a]
High dose	260.5(226–301)	252.0(223–282)[ab]	251.9(214–308)[ab]	287.6(272–299)[a]

[1]HC from Microgest®, [2]each beige tablet contained 0.15 mg of levonorgestrel and 0.03 mg of ethinylestradiol. [2]Each treatment involved 10 female flies; [2]Control = Male (10% sucrose solution) × Female (10% sucrose solution); [2]Low dose = Male (0.036 mg/mL) × Female (10% sucrose solution); [2]High dose = Male (0.072 mg/mL) × Female (10% sucrose solution); [3]Median followed by the different letters indicate significant differences within each column (generation) (Mann–Whitney U test; $P<0.05$), [a] significant lower comparing with control; [b] significant lower comparing with the low dose group.

Table 3

Number of ovarioles in female *C. megacephala* treated with HC after crossing with untreated male.

Treatment[2]	Median of number of ovarioles (range)[3]			
	Parental flies	F1	F2	F3
Control	262.0(226–304)	297.3(257–335)	291.3(249–336)	329.0(305–365)
Low dose	256.5(230–289)	271.0(242–296)[a]	258.9(238–275)[a]	271.0(238–293)[a]
High dose	257.3(220–306)	247.2(215–276)[a]	248.1(218–312)[a]	287.5(255–347)[a]

[a]significant lower comparing with control.

Table 4

Number of ovarioles in female *C. megacephala* treated with HC after crossing. with males treated with HC.

Treatment[2]	Median of number of ovarioles (range)[3]			
	Parental flies	F1	F2	F3
Control	205.8(180–244)	283.2(243–314)	221.6(207–236)	306.0(260–363)
Low dose	205.2(184–222)	263.2(225–302)	212.0(185–254)	208.8(189–243)
High dose	183.0(162–333)	243.8(228–2532)[a]	203.0(173–242)	220.8(186–244)[a]

[a]significant lower comparing with control.

fed in males or females. In males F1, F2 and F3, the testes distinctly showed a definite pattern of deformity, typically involving disruption of spermatogenesis. The most apparent was deformed nucleus, involving low level of condensed chromatin and degenerated nuclear envelope; whereas mitochondrial swelling and rough endoplasmic reticulum swelling were noticed in the cytoplasm. Regarding female offspring, we have found the consequence of female HC on biological and morphological deformity in ovariole of F1, F2 and F3. In the latter aspect determined at ultrastructural level, the presence of proteic yolk granules in treated female parent was resemble in the ovarioles of female tick *Rhipicephalus sanguineus* (Acari: Ixodidae) exposed to chemical agent, fibronil[7]. Comparable phenomenon in which the effect of chemicals act on the reproductive system of other insects was recorded. For example in *Rhodnius prolixus* (Heteroptera: Reduviidae) treated with 14C–pyriproxyfen (juvenile hormone mimic), it was found to be stored in the female fat body, incorporated into vitellogenin and transferred to the eggs[8].

The reproductive structures observed in this study that appeared to be effected by the application of HC are involved in vitellogenesis and oogenesis in *C. megacephala* similarly to how juvenile hormone (JH) and 20–hydroxyecdysone (20E) act in insects[9]. In previous studies, the effects of JH and 20E on viltellogenesis and oogenesis have been reported in the fruit fly *Drosophila melanogaster*[10]; the mosquito *Culex pipiens*[11]; the termite *Reticulitermes flavipes*[12]; and the tick *Amblyomma hebraeum*[13]. Ogiso and Ohishi[14] showed that estradiol is found in *B. mori* ovaries and comes from either a biosynthetic pathway in the insect or by transport from the food plant via the hemolymph. The role that estradiol plays in oocyte maturation and embryogenesis in *Bombyx mori* was examined by injecting estrogens into whole pupae but their results were inconclusive as these processes were not affected regardless of whether the application was of additional estradiol or an antagonist[14]. They found that after application of the vertebrate steroid hormones, estone, estradiol–17 α and testosterone were transported readily into the ovaries and metabolized[14]. Work published by Nieto–Fermandes *et al*[15] in the giant cockroach *Blaberus craniifer* (Dictyoptera: Blaberidae) indicated that estradiol can modulate the production of nitric oxide and release in the ovary. The observed effects of HC on less matured ovariole and the presence of cellular changes in *C. megacephala* ovariole in the current study suggested that it might transport from the digestive system to ovary as in *B. mori*. However, the presence and concentration of HC in the reproductive tissues of *C. megacephala* was not explicitly examined in this study. Regarding this, more investigation to elucidate mechanism pathway of levonorgestrel and/or ethinylestradiol in adult *C. megacephala* was warranted.

The structural characterization of the estradiol steroid isolated from B. mori ovaries[1] is similar to the structure of human estradiol[16]. Furthermore, estrogen and steroid hormone receptors have been identified in *D. melanogaster*[17]. It is possible that these types of receptors may present in *C. megacephala*. In this regard, the effects of HC on *C. megacephala* observed in this study suggest that the molecular genetics should be studied to determine the

mechanism and function of human steroid hormone in this fly.

Our results indicated that treatment only in females seems to cause more severe consequence than only males, based on the less number of ovariole in offspring. However, the number of matured ovariole in treated females and treated males was relatively similar. As for morphological investigation, alteration in cellular level determined by SEM and TEM provided similar ultrastructural changes in all experiments in detail. In view of the phenotype assessment, alteration in larva or adult offspring was not evaluated in this study. However, mouthpart deformities have been observed in *C. riparius* larvae exposed to low concentration of 17 α -ethinylestradiol[18].

Conversely, comparatively little is known about the endocrine regulation of male reproduction with the well known process being that of the mitotic division rate of spermatogonia, in the formation of spermatocytes. However, TEM images of the testes in all experiments conducted in the present study demonstrated cellular alterations during spermatogenesis, involving degenerated chromatin during developmental process, and as it reached the last stage of being sperm, deformities of nucleus was noticed. Such observations suggested that administration of HC induced aberrant spermatogenesis in *C. megacephala* in parent and subsequent generations. Sperm production might be a meaningful indicator for determining the effect of HC on male *C. megacephala*, and a sperm count could be useful in future studies.

In conclusion, administer of female HC to adult *C. megacephala* caused ovariole reduction, less matured ovariole and affected cellular changes in testes and ovariole of offspring up to F3. In this regard, HC may be included as another component used as part of an integrated pest control program to reduce reproduction of this blow fly, and additional investigation is merit.

Conflict of interest statement

We declare that we have no conflict of interest.

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