Nutritional status affects 20-hydroxyecdysone concentration and progression of oogenesis in Drosophila melanogaster

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Abstract

Drosophila egg production depends upon the nutritional available to females. When food is in short supply, oogenesis is arrested and apoptosis of the nurse cells is induced at mid-oogenesis via a mechanism that is probably controlled by ecdysteroid hormone. We have shown that expression of some ecdysone-response genes is correlated with apoptosis of egg chambers. Moreover, ecdysteroid injection and application of juvenile hormone induces and suppresses the apoptosis, respectively. In this study, we investigated which tissues show increases in the concentration of ecdysteroids under nutritional shortage to begin to link together nutrient intake, hormone regulation and the choice between egg development or apoptosis made within egg chambers. We measured ecdysteroid levels in the whole body, ovaries and haemolymph samples by RIA and found that the concentration of ecdysteroid increased in all samples. This contributes to the idea that nutritional shortage leads to a rapid high ecdysteroid concentration within the fly and that the high concentration induces apoptosis. Low concentrations of ecdysteroid are essential for normal oogenesis. We suggest there is threshold concentration in the egg chambers and that apoptosis at mid-oogenesis is induced when the ecdysteroid levels exceed the threshold. Starvation causes the ovary to retain the ecdysteroid it produces, thus enabling individual egg chambers to undergo apoptosis and thus control the number of eggs produced in relation to food intake.

Introduction

Ecdysteroids control moulting and metamorphosis in insects and are produced by the prothoracic glands in immature insects. Ecdysteroid synthesis in and secretion from prothoracic glands are activated by prothoracicotropic hormone (PTTH), which is produced in the brain and secreted from the corpora allatum (Gilbert & Goodman 1981). The prothoracic glands degenerate during pupal–adult metamorphosis (Dai & Gilbert 1991). In adult females, where the ecdysteroids are produced to regulate embryonic development, the follicle cells in the ovary are a key source of ecdysone (Riddiford 1993). 20-Hydroxyecdysone (20E) is the active hormone and Petryk et al. (2003) have established that shade, which encodes a 22-hydroxylase for converting ecdysone to 20E, is expressed in the Drosophila ovary and fat body. In addition, defective in the avoidance of repellents (dare) is also expressed in the ovary (Freeman et al. 1999). Dare encodes adrenodoxin reductase, which plays a key role in the synthesis of steroid hormones in mammals (Freeman et al. 1999). The ovaries thus have the potential for synthesis and activation of ecdysteroids.

Ecdysteroids are essential for normal oogenesis, and ecd-1 females have abnormal oogenesis, in which there are no vitellogenic stages in the ovary (Audit-Lamour & Busson 1981). During Drosophila ovarian maturation there is a developmental checkpoint at stage 8 of oogenesis (Wilson 1982), and several mutants cause a developmental arrest at this point. Yolk synthesis and accumulation are crucial for development beyond this checkpoint and to produce a mature egg. Three major yolk proteins (YPs) in Drosophila are produced in the fat body and ovarian follicle cells (Bownes 1982, Brennen et al. 1982). YP synthesis commences at stage 8 and is switched off at stage 11 when chorion synthesis begins (Bownes 1986). YP synthesis is controlled by the hormonal conditions in the fly, including juvenile hormone (JH) levels and ecdysone levels, and in addition nutritional condition and the sex of the fly also modulate YP synthesis (Terashima & Bownes 2004). Ecdysone is metabolized into its active form, 20E, and stimulates the fat body to produce YPs. The ecdysteroids are needed for normal oogenesis. On the other hand, 20E can induce abnormal oogenesis in Drosophila; thus, ecdysone levels may well be crucial in controlling oocyte progression.


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Nutritional shortage (starvation) induces apoptosis of nurse cells in stage-8 and -9 egg chambers in *Drosophila*. Similar apoptosis is observed when 20E is injected into the abdomen of flies under adequate nutrition and is suppressed by JH analogue (JHA; methoprene) application to the abdomen of females under starvation (Soller *et al.* 1999, Terashima & Bownes 2004). The apoptosis of nurse cells at stages 8 and 9 could be induced by the increased levels of 20E in the females since the 20E concentration in the females under nutritional shortage is higher than females under adequate nutrition, but the tissues responsible for producing the 20E are not known (Bownes 1989). We predict that starvation signals activate 20E synthesis in the ovary, which gives rise to the increase in the 20E concentration in the ovary and haemolymph.

To address this issue, we measured 20E concentration in whole body, haemolymph and ovaries of females flies that had been kept under various conditions, including adequate nutrition (fed), nutritional shortage (starvation) and following topical JHA application (JHA-treated). We found that the nutritional status affected 20E concentration, and that starvation induced an increase in 20E concentration in the whole body, haemolymph and ovary samples. Feeding after starvation or topical JHA application to the starved flies suppressed the high 20E concentration that was induced by starvation, again in the whole body, haemolymph and ovaries. These results indicate that nutritional shortage activates 20E synthesis in *Drosophila*. We propose a model for how these hormonal changes modulate the progress of oogenesis under various nutritional conditions.

### Materials and Methods

*Drosophila* maintenance

Figure 1 shows the scheme for maintenance of females. Flies of the *Oregon R* strain were maintained on standard yeast, maize meal, sugar and agar medium at 25 °C, and 3-day-old flies were used throughout the present study. The flies were transferred from a standard diet to a yeast diet (2 g Baker’s yeast on approximately 50 ml 1% agar medium, which contains 2:5% corn flour, 5% sucrose, 1:75% hypophilized yeast and 0:005% 10% Nipagin in 95% ethanol). After 3 days on yeast, the flies were maintained on yeast continually (FF) or transferred to a sugar diet (1% ethanolic ethanol). After 3 days on yeast followed by only sugar for 1 day, flies were transferred to yeast (FSF) or treated topically with the JHA methoprene (ZR515; Zoecon) and maintained on sugar and water (FSJH).

**Injection of 20E and application of JHA**

20E was dissolved in insect Ringer’s solution (130 mM NaCl, 4.7 mM KCl and 1.9 mM CaCl₂) at a concentration of 2 µg/ml and 50 nl was injected into individual flies (Soller *et al.* 1997). The concentration of 20E was determined according to Bownes (1989). Assuming a haemolymph volume of approximately 1 µl/female (Soller *et al.* 1997), 100 pg 20E/female corresponds to a concentration of 2 × 10⁻⁷ M in haemolymph. Methoprene in 100 nl acetone was applied topically to the ventral abdomen. Methoprene diluted 1:100 with acetone corresponds to a concentration of about 1 µg/100 nl. Controls were injected with Ringer’s alone (FR) or treated with acetone (FSA).

**Hoechst staining**

Hoechst staining was as described by Soller *et al.* (1999) with minor modifications. Ovaries were fixed in 4% paraformaldehyde in PBS. After fixation, the ovaries were stained in 1 µg/ml Hoechst (no. 33258; Sigma) and the samples were observed using fluorescein filters.

**RIA sample preparation**

RIA samples were prepared as follows. For whole-body RIA (containing ovary and haemolymph), after measuring the wet weight of 50 female flies, the flies were homogenized in 500 µl 100% methanol and centrifuged at 5000 g for 15 min at 4 °C. The supernatants were evaporated in vacuo. For ovary RIA, after measuring the wet weight of 50 pairs of ovaries, the ovaries were homogenized in 300 µl 100% methanol and centrifuged at 5000 g for 15 min at 4 °C, and the supernatants were collected and evaporated in vacuo. For RIA of haemolymph, approximately 0.1 µl haemolymph was collected from individual female flies (n=100) and 10 µl haemolymph was added to 90 µl 100% methanol. After centrifugation at 5000 g for 15 min at 4 °C, the supernatant was evaporated in vacuo.

**20E titre**

RIAs were performed according to Warren & Gilbert (1988) with minor modifications (Sakurai *et al.* 1998, Takaki & Sakurai 2003). [³H]Ecdysone (60 Ci/mmol) and standard 20E were obtained from New England Nuclear (Boston, MA, USA) and Sigma. Briefly, [³H]Ecdysone (approximately 10 000 c.p.m. in 100 µl borate buffer) and the 6000-fold-diluted 0–6 anti-ecdysone antiserum (Yokoyama *et al.* 1996) in 100 mM borate buffer (100 mM boric acid, 50 mM borax and 60 mM NaCl, pH 8·4) containing 0·02% sodium azide, 0·05% rabbit IgG (Miles, Kankakee, IL, USA) and 0·2% BSA (fraction V; Sigma) were added to aliquots of the extracts. A calibration curve was prepared for individual assays using 20E as a standard

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ecdysteroid. 20E was dissolved in ethanol and quantified by UV absorbance at 243 nm (ε_{ethanol} = 12 300), and then the aliquots were serially diluted with borate buffer to prepare the standards (800–12·5 pg/100 µl). The sample solution (10 ml) was supplemented with 100 µl antiserum solution and 100 µl [3H]ecdysone, and incubated overnight at 4 °C. Then the bound [3H]ecdysone was separated from the free form by adding an equal volume of saturated ammonium sulfate so as to give a final ammonium sulfate concentration at 50% saturation. The solution was centrifuged at 3000 g for 1 h, and the resulting pellet was washed in 50% saturated ammonium sulfate. The pellet was dissolved in 100 µl water, and then supplemented with 900 µl scintillator (Aquasol II; New England Nuclear) for counting the radioactivity. The statistical analysis was undertaken using Student’s t-test and significant differences (P<0·05) determined.

Results

The apoptosis of stage-8 and -9 egg chambers and the number of stage-10 egg chambers are affected by nutritional and hormonal conditions

We have established that starvation induces apoptosis of nurse cells in egg chambers at stages 8 and 9 in Drosophila. When 20E is injected into the abdomens of fed flies, apoptosis of nurse cells at stage-8 and -9 egg chambers is induced, whereas JHA (methoprene) treatment of starved flies suppresses the apoptosis (Soller et al. 1999, Terashima & Bownes 2004). There is a developmental checkpoint at stages 8 and 9 of oogenesis (Wilson 1982), and presumably the egg chambers undergo an apoptosis/development selection at this checkpoint. If an egg chamber is determined to be removed from the ovary, the apoptosis pathway is activated by 20E, which induces the apoptosis.

![Diagram of Drosophila maintenance under various nutritional and hormonal conditions.](https://www.endocrinology-journals.org)
of nurse cells of stage-8 and -9 egg chambers and the number of stage-10 egg chambers is therefore decreased. We have established that induction of the apoptosis requires upregulation of the Broad-Complex BR-C isoforms Z2, Z3 and E75A. These are early ecdysone-response genes which are expressed in the follicle cells of stage-8 and -9 egg chambers (Terashima & Bownes 2004, 2005a).

To establish how and when these changes in developmental progression occur, we first determined a time course of the effects of feeding and starvation on oocyte maturation by observing the number of egg chambers with nurse cells undergoing apoptosis in stage-8 and -9 egg chambers and the number of stage-10 egg chambers (King 1970).

Under adequate nutrition (FF), the percentages of stage-8 and -9 egg chambers showing nurse cell apoptosis were 1·4–2·1% at stage 8 and 4·3–5·3% at stage 9. Transfer to conditions of nutritional shortage (starvation) resulted in an increase in the percentages of stage-8 and -9 egg chambers with nurse cell apoptosis to approximately 15% at stage 8 and 35% at stage 9 and showed significant differences between FF and FS from 9 h at stage 8 \((P<0·01)\) and 12 h \((P=0·01)\) after the beginning of starvation (Fig. 2A), indicating that the nurse cell apoptosis seems to be a slow response in flies. The percentage of apoptosis increased significantly 3 h \((P<0·01)\) at stage 8; \(P=0·01\) at stage 9) after 20E injections (FE; Fig. 2B). This response was substantially faster, presumably because one of the initial responses to nutritional changes is an increased 20E level. Injection of 100 pg 20E into the abdomen of fed flies increased the percentages of stage-8 and -9 egg chambers with nurse cell nuclei showing apoptosis at approximately 75% at stage 8 and 85% at stage 9 (Fig. 2A and B). By contrast, injection of insect Ringer’s solution had no effects on apoptosis (data not shown).

The number of stage-10 egg chambers was decreased following starvation (FS) and 20E-treatment (FE; Fig. 3A and B). Under starvation, the decrease in the number of stage-10 egg chambers began 9 h after starvation started \((P<0·01)\), in spite of the fact that stage 9 egg chambers did not commence apoptosis for 12 h following starvation. Thus, in addition to apoptosis, unknown factor(s) could be involved in the loss of stage-10 egg chambers, possibility due to an arrest in the progression from stages 8 and 9 to stage 10.

When the starved flies were transferred to adequate nutritional conditions (FSF), the percentages of stage-8 and -9 egg chambers with nurse cell nuclei showing apoptosis 24 h after the feeding started was decreased to 1·5–3·1% at stage 8 and 4·7–11·9% at stage 9 (Fig. 2C) and the significant differences appeared from 15 h at stage 8 \((P<0·01)\) and 18 h at stage 9 \((P<0·01)\), after the feeding started. Moreover, the number of stage-10 egg chambers was increased from 9 h \((P<0·01)\) after the feeding started (Fig. 3C).

JHA reduced the percentage of stage-8 and -9 egg chamber showing apoptosis and increased the numbers of stage-10 egg chambers. When 1 µg JHA was applied to the abdomens of the starved flies (FSJH in Fig. 2D), the percentages showing apoptosis where decreased after 6 h at stage 8 \((P<0·01)\) and 9 h at stage 9 \((P<0·01)\;\text{Fig. }2D)\). The number of stage-10 egg chambers was increased (Fig. 3D). JHA-treated females exhibited rescue effects within 12 h of the treatment \((P<0·01)\), which was more rapid than restoring food (compare Fig. 2C with D), but the number of stage-10 egg chambers was not rescued to the same levels as with feeding.

Nutritional conditions affect ecdysteroid concentration in the whole body, the ovary and the haemolymph

Although nutritional conditions affected ecdysteroid titres in flies, the time courses of the changes in ecdysteroid levels were unknown, and also it was essential to establish whether the titre changes were in the ovary itself, in the haemolymph, or both. The change in the number of egg chambers undergoing apoptosis suggested that the ecdysteroid changes would occur prior to the induction of apoptosis. To address this issue, we measured ecdysteroid concentrations in the whole body, haemolymph and ovaries 9–12 h after the beginning of starvation (Fig. 4A, B and C). Ecdysteroid levels in the whole body remained in the range of 12·3–16·9 pg/mg body weight \((1·6–1·9 \text{ mg fresh weight/female)}\) under continuous adequate nutrition (FF), and transfer of the flies to starvation (FS) increased the titre from 12 h after the beginning of starvation \((P<0·01)\), and the titre reached high levels of 50·0–67·5 pg/mg (Fig. 4A). Similarly, ecdysteroid concentration in the haemolymph under starvation was much higher than that under adequate nutrition from 6 h after starvation started \((P<0·01)\); the ecdysteroid concentration increased to 60·1–77·9 pg/µl haemolymph \((0·7–1·0 \mu l\text{ haemolymph/female)}; \text{Fig. }4B)\). In the ovary, the ecdysteroid concentration also increased 3 h after starvation \((P<0·01)\) and reached the maximum values of 25·1–35·9 pg/mg ovary \((0·3–0·9 \text{ mg wet weight/ovary); Fig. }4C)\).

We next measured the changes in ecdysteroid levels under similar conditions to those in Fig. 2C (FSS and FSF in Fig. 1). Feeding the flies following the starvation period (FSF) decreased ecdysteroid concentration equally in whole body, haemolymph and ovaries from 3, 9 and 3 h (all \(P<0·01)\) after feeding started, respectively. Under starvation conditions (FS), ecdysteroid titres in the whole body were 51·9–67·5 pg/mg \((1·1–1·4 \text{ fresh weight/female)}\) after 24 h of starvation and dropped to 9·1–15·6 pg/mg \((1·8–2·0 \text{ mg fresh weight/female)}\) 15 h after re-feeding (FSF; Fig. 4D). Ecdysteroid concentrations in haemolymph and ovaries were also decreased by feeding (Fig. 4E and F). The concentration after 24 h of starvation was 57·8–77·9 pg/µl haemolymph \((0·7–1·0 \mu l\).
haemolymph/female) and 30·4–35·9 pg/mg ovary (0·4–0·8 mg wet weight/ovary). After re-feeding, the concentrations decreased to 13·6–19·1 pg/µl haemolymph (0·6–1·0 µl haemolymph/female) 21 h later and to 5·1–7·4 pg/mg ovary (0·8–1·2 mg/ovary) 18 h after the re-feeding. Starvation, therefore, must activate the ecdysteroidogenic pathway and feeding must maintain the low ecdysteroid levels appropriate to maintain normal oogenesis.

JHA affects 20E concentration

When 1 µg JHA is applied to the abdomens of the starved flies, apoptosis of stage-8 and -9 nurse cells is suppressed and the percentages of stage-8 and -9 egg chambers with nurse cell nuclei showing apoptosis is decreased (see Fig. 2D; also Soller et al. 1999, Terashima & Bownes 2004). Thus JH rescues, to some extent, the effects of starvation. Although the mechanisms underlying this

Figure 2 The percentage of egg chambers with nurse cell nuclei showing apoptosis at stages 8 and 9 under various conditions. The graphs show the percentage of egg chambers with nurse cell nuclei undergoing apoptosis. The percentages were calculated as follows: [(number of stage-8 or -9 egg chambers with nurse cell nuclei showing nuclear condensation or fragmentation)/(total stage-8 or -9 egg chambers)] × 100. ○ and Δ, stage-8 and -9 egg chambers showing nuclear condensation or fragmentation of the FF flies in (A) and (B), FSF flies in (C) and FSS flies in (D) respectively; ● and ▲, stage-8 and -9 egg chambers showing nuclear condensation or fragmentation of the FS flies in (A), FE flies in (B), FSS flies in (C) and FSJH flies in (D), respectively; n=12 flies. Error bars show S.D.
The percentages of stage-8 and -9 egg chambers with nurse cell nuclei showing apoptosis increased under starvation or following 20E treatment. In addition, the number of stage-10 egg chambers was decreased under starvation or following 20E treatment. To evaluate what happened under these conditions we measured the number of stage-8 and -9 egg chambers with healthy nurse cells.

Starvation arrests the development of egg chambers but not via 20E

The number of stage-8 egg chambers increased 4 h after starvation started and the increased levels were maintained until 12 h (Fig. 6A and B), whereas the number of stage-9 egg chambers did not increase after starvation started (Fig. 6C and D). These results indicate that development of egg chambers may progress from stage 7 to 8 under starvation, but not from stage 8 to 9, and hence to 10.
As a result, the number of stage-8 egg chambers increased during the period between 5 and 12 h of starvation (Fig. 6A and B). This means that stage-9 egg chambers did not develop to stage-10 egg chambers under starvation. In addition, 20E injection induced an abrupt decrease in both stage-8 and -9 egg chambers (Fig. 6). Starvation thus...
prevents stage-8 and -9 egg chambers from progressing to the next stage of development independently of the 20E concentration. Because the development of some egg chambers is arrested and others chambers undergo apoptosis, two separate events may occur in response to nutritional shortage and although apoptosis is controlled by ecdysone, the developmental arrest is not.

Discussion

Starvation increases 20E concentration

The prothoracic glands, which are the principal source of ecdysone in the immature stages, are no longer present in adults. The egg chambers produce ecdysone, which, at least in some insects, accumulates in the oocyte (Hagedorn et al. 1975, Riddiford 1993). In the fat body, ecdysone is converted to 20E, the active hormone (Chapman 1998), and shade, which encodes 20-hydroxylase for converting ecdysone to 20E, is expressed in nurse cells and follicle cells in the ovary and fat body (Petryk et al. 2003).

Ecdysteroid synthesis is affected by the nutritional status of the female, and ecdysteroids affect oogenesis in many insects. Egg production in mosquitoes is triggered by a blood meal. The digested products of the blood meal stimulate the brain to secrete egg development neurosecretory hormone (EDNH), which is also known as ovarian ecdysteroidogenic hormone (OEH; Chapman 1998). EDNH stimulates the ovary to synthesize ecdysteroids, which instruct the fat body cells to make vitellogenin for the oocytes. Vitellogenin is critical for egg production, thus without the blood meal there is no vitellogenin and no eggs, so to produce mature eggs ecdysteroids are essential.
In contrast, nutritional shortage induces an increase in ecdysteroid concentration in *Drosophila* females (Bownes 1989). As shown in Fig. 4, ecdysteroid concentration increased in *Drosophila* whole body, haemolymph and ovaries during starvation. Feeding suppressed the high ecdysteroid concentration that was induced by nutritional shortage (Fig. 4D, E and F).

Under starvation, apoptosis of nurse cells in stage-8 and -9 egg chambers is induced (Fig. 2; Terashima & Bownes 2004); 20E injection into the females under adequate nutrition also induces the apoptosis and JHA treatment of females under nutritional shortage suppresses this apoptosis (Soller *et al.* 1999, Terashima & Bownes 2004). Presumably high ecdysteroid concentrations in the haemolymph and/or the ovary, which are induced by starvation, may induce the apoptosis of nurse cells in stage-8 and -9 egg chambers. However, ecdysteroid is indispensable to produce mature eggs in *Drosophila*. Oogenesis in *ecd*-1 mutants is arrested at mid-oogenesis (Audit-Lamour & Busson 1981), and germline clones of *EcR* mutations lead to developmental arrest and egg chambers degenerate during mid-oogenesis in *Drosophila* (Buszczak *et al.* 1999). Presumably, there is an ecdysteroid threshold for inducing apoptosis of nurse cells at stages 8 and 9 and ecdysteroids induce normal development when below the threshold concentration and induce apoptosis of nurse cells at stages 8 and 9 when over the threshold. Starvation induces an increase in ecdysteroid concentration to above the threshold level in the haemolymph and the ovary through activation of the ecdysone synthesis pathway in the egg chamber. Ecdysteroid secretion from the ovary decreased following nutritional shortage. Thus, ecdysteroid secretion from the fat body or other ecdysteroid-synthesizing tissues must be stimulated to induce the high ecdysteroid concentration observed in haemolymph.

JHA suppressed the high ecdysteroid concentration that was induced by starvation (Fig. 5). JH and JHA suppress ecdysone synthesis/secretion from the prothoracic glands in larvae of *Maduca sexta* (Rountree & Bollenbacher 1986). It is likely that JHA suppression decreases the high ecdysteroid concentration in the ovary that induce s apoptosis of nurse cells in stage-8 and -9 egg chambers under starvation, and therefore JHA treatment retains minimal ecdysteroid levels needed for inducing normal oogenesis.

*Stress starvation induces oogenesis at stages 8 and 9*

There is a developmental checkpoint at stage 8 of oogenesis (Wilson 1982). YP synthesis commences at stage 8 and YP is accumulated during development into mature eggs (Bownes 1986). *Drosophila* egg chambers normally transit through stages 8 and 9 during a 6-h period (King 1970), but starvation induced an accumulation of stage-8 and -9 egg chambers in *Drosophila* oogenesis. The number of stage-8 egg chambers was increased during a 5–12-h period after starvation started (Fig. 6), but the number of stage-9 egg chambers did not increase for 0–12 h after starvation started (Fig. 6). This means that oogenesis progresses from stage 7 to 8, but does not progress from stage 8 to 9 and then to 10 under nutritional shortage. When 20E was injected into the fed flies, the accumulation of stage-8 chambers was not seen; therefore this arrest of oogenesis at stage 8 was not caused by the increasing 20E concentration in haemolymph and ovary. Perhaps starvation signals induce the arrest of oogenesis at stages 8 and 9 directly, or they could inhibit YP uptake. Some nutrient- and stress-response genes exhibit different expression patterns in the ovaries of females under adequate nutrition and starvation (Terashima & Bownes 2005b). We suggest that the genes which respond directly to stress and nutrients interact with the ecdysone-synthesis pathway, resulting in the induction of apoptosis of nurse cells in stage-8 and -9 egg chambers through activation of *BR-C Z2, Z3* and *E75A* expression in the follicle cells (Terashima & Bownes 2004, 2005a,b). Other genes could have altered their expression levels, so as to arrest oogenesis at stages 8 and 9 and to check the developmental status of the egg chamber. As a result, the decision is made to develop into a mature egg or undergo apoptosis at stages 8 and 9. The arrest in the progression of oogenesis at stages 8 and 9 is independent of increasing ecdysteroid levels.

Starvation signals are needed to activate a number of pathways to adjust the rate of egg production in *Drosophila*. These pathways could be classified into two groups: one to stimulate ecdysone synthesis in the follicle cells and/or nurse cells to activate the apoptosis pathway, including *BR-C Z2, Z3* and *E75A* expression in the follicle cells, and another one to interact with and participate in the developmental checkpoint, giving rise to an arrest in oogenesis at stage 8 under nutritional shortage. Figure 7 shows a possible scheme for the regulation of oogenesis related to nutrition in *Drosophila*. It is likely that starvation signals from the gut activate ecdysteroid synthesis in the ovary in *Drosophila* under starvation. Ecdysteroid is then accumulated in the egg chamber by decreasing 20E secretion from the ovary, and the fat body secretes 20E to haemolymph. We suggest that there are two thresholds of 20E concentration in *Drosophila* ovary – one is the concentration for normal oogenesis and the other is the concentration for inducing apoptosis – and that starvation elevates the ecdysone levels in some egg chambers over the threshold that leads to apoptosis.

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