2. REVIEW

2.1 MOSQUITO-REPRODUCTIVE PHYSIOLOGY

2.1a Integral feature of egg development

The haematophagous insect group of mosquitoes are having meroistic-polytrophic ovarioles and they are said to be maturing only after taking a blood meal. However, there are certain genetically distinct populations like Culex pipiens var molestus which can lay their first batch of eggs without blood meal, but require blood meal before they can develop subsequent batches of eggs and these forms are referred as 'autogenous'. On the other hand, most of the mosquito forms like Culex pipiens fatigans (Cx. quinquefasciatus) and Aedes aegypti, they need blood meal even for the first batch of egg production and these forms are referred as 'anautogenous'.

Formerly, the reason for such difference between autogenous and anautogenous was attributed to the amount of reserves available to the adults from larval stages. Later on it was found out that the difference between the two was not in reserves but lay in the hormonal control of ovary growth as evidenced in follicle development by transplantation of active corpora allata (and X cells) from autogenous females into unfed (sugar fed) anautogenous females. Thereafter it has been postulated that a gonadotropic hormone is secreted automatically after
emergence in autogenous mosquitoes but not secreted in anautogenous mosquitoes without the stimulus of a blood meal (Clements, 1963).

Such a view has been given based on the assumption that corpora allata (CA) is not active but has been activated only after blood meal. However, Lea (1969) has demonstrated that implantation of extra CA from Culex pipiens molestus (autogenous) to Culex pipiens pipiens (anautogenous) fails to mature eggs during continued sugar feeding. According to him the CA are active in anautogenous form but some other mechanism prevents development of the ovary prior to the first blood meal. In the subsequent year the above author (Lea, 1970) has established in anautogenous Aedes taeniorhynchus that both Medial Neurosecretory Cells (MNC) and CA are producing their hormones after emergence during sugar feeding. But hormone from CA only is allowed to be in circulation whereas the hormone secreted by MNC is accumulated in corpus cardiacum (CC) and is not released until after a blood meal. Thereby it is evident that CA alone do not regulate egg development. Moreover, he has demonstrated that the implantation of extra MNC stimulate egg maturation in the absence of a blood meal thereby pointed out that anautogenous female emerges with adequate nutritional reserves for maturation of some eggs.

Maturation of egg after a blood meal is referred to the combination of vitellogenesis (the synthesis and uptake of
protein) and resumption of oogenesis (egg development) and both are triggered by the intake of blood (Banks et al., 1994). The uptake of protein is a selective process wherein the vitellogenin, a unique group of proteins that are synthesized extra ovarially by the fat bodies, released to haemolymph and they are selectively taken up by the oocyte. Once inside the oocyte, then proteins are known as vitellins, because in some cases the molecules are changed during uptake (Hagedorn and Kunkel 1979 and Raikhel, 1984).

From the foregoing account it is clear that a few studies pertaining to egg maturation in mosquito group have been dealt with regard to structure and function of different organs like ovarioles, corpora allata, medial neurosecretory cells and fat body. Moreover it is understood that all these organs act as an integrated machinery after taking a blood meal by the mosquito.

2.1b Hormonal regulation of reproduction

A survey of literature pertinent to the principle behind egg maturation reveals that egg development in ovarian follicles occur at two stages i.e., before and after blood feeding. After emergence (eclosion), growth and differentiation of primary follicles from rest of the germarium part of the follicles occur within 2-3 days and further development is ceased. At this arrested condition the ovary is said to be 'previtellogenic phase' (stage I). The maturation of the oocytes in the primary follicles
proceeds only after blood engorgement whereupon yolk synthesis and accumulation are carried out which is referred to 'vitellogenic phase' (stage II). Hormonal regulation is attributed for the above said two phases of the ovary.

The role of juvenile hormone (JH)

The view of Wigglesworth (1936) that corpus allatum as a source of gonadotropic hormone in insects particularly in *Rhodnius prolixus* and the view of Thomsen (1952) that neurosecretory hormone is required for egg development in *Calliphora erythrocephala*, were later on established in mosquito group. By ablation and implantation of corpora allata (CA) and of medial neurosecretory cells (MNC), Lea (1969 and 1970) has reported that autogenous and anautogenous *Aedes taeniorhynchus* require hormones from both tissues (CA and MNC) for egg development.

Although Spielman et al. (1971) have stated that mosquitoes may not depend upon juvenile hormone (JH) as the sole initiator of oogenesis as proposed by Lea (1969), they are of the opinion that a potential oogenic function for ecdysone can not be fulfilled until two days after adult ecdysis. However, these authors have pointed out that only arrested follicles of *Aedes aegypti* respond to ecdysone - treatment. Thereby, it may be understood that the first stage of ovarian growth (priming the ovary) may depend on the juvenile hormone.

Subsequently Gwadz and Spielman (1973) have established in the same mosquito, *Aedes aegypti* that ecdysterone does
not stimulate yolk deposition either in decapitated or allata ablated within one hour after emergence; but the steroid hormone induces yolk deposition in decapitated or allata ablated forms within one hour with topical synthetic juvenile hormone (SJH) at one day. This experimental evidence not only reveals the importance of the JH for priming the oocyte after adult emergence but also indicates that the brain's influence on the ovaries is mediated by the corpora allata.

When such priming activity has been attributed for the developing oocyte after adult emergence, the storage of neurosecretory hormone in the corpus cardiacum make blood-feeding obligatory. In the mosquito, *Aedes aegypti*, egg development is triggered by a blood meal which effects the release from the corpus cardiacum of an egg development neurosecretory hormone (EDNH - which is otherwise called ovarian ecdysteroidogenic hormone - OEH by Hagedorn et al., 1990) produced in the brain. The EDNH/OEH either directly or indirectly causes the ovary to produce vitellogenin stimulating hormone (VSH) which activates and maintains vitellogenin synthesis in the fat body (Fallon et al., 1974).

Later, the vitellogenic stimulating hormone (VSH) equivalent to ecdysone has been demonstrated its release from ovary in the form of α-ecdysone (Hagedorn et al., 1975). However, experimental evidence was given by Hagedorn et al. (1977) that presence of ecdysone before the
completion of previtellogenic phase disturbs JH dependent step in oogenesis (priming the ovarian follicles). So, it is clear that ecdysone is secreted only after the function of JH is completed.

In addition to the role of juvenile hormone in priming the oocyte, other functions have also been attributed for the JH.

According to Borovsky (1980), JH may stimulate the release of ecdysone from the ovaries which is contradictory to EDNH/OEH hypothesis. Fuchs and Fong (1981) have shown that OEH which is secreted by MNC after blood feeding will be active only in the presence of JH. Hence, juvenile hormone is assumed to be secreted even after blood feeding which in turn stimulates ecdysone secretion from ovaries. Perhaps such an observation might have made Shapiro and Hagedorn (1982) to suggest that JH not only promotes the ovarian growth but also high ecdysteroid content in Aedes atropalpus.

However, Recioppi et al. (1984) are of the opinion that JH level declines rapidly after a blood meal and it is felt by them that absence of juvenile hormone is important for normal vitellogenin synthesis due to the ecdysteroid activity. Almost the same view have been given by Shapiro et al (1986) in Aedes aegypti by measuring juvenile hormone level and JH esterase activity with are inversely correlated after blood meal.
Recently, Lu and Hagedorn (1986) have stated that JH regulates the previtellogenic events such as growth of the follicle to resting stage and the development of competence of both the fat body and ovary to respond for hormones appearing after the blood meal.

The role of ecdysone

Since the classical work of Spielman et al (1971) on the application of growth hormone (ecdysterone) in Aedes aegypti, Culex pipiens quinquefasciatus and Anopheles quadrimaculatus for initiation of vitellogensis, similar studies are made till date. They have shown that injection or ingestion of ecdysterone initiates ovarian development. According to them, ecdysterone affects the ovary directly by increased pinocytosis.

After the observation in Aedes aegypti that ecdysterone does not stimulate yolk deposition in decapitated or allata ablated specimens (within one hour after emergence), Gwadz and Spielman (1973) have established that ecdystereone induces yolk deposition only in the presence or after the function of JH. Though the above authours have observed the involvement of ecdysone in egg maturation, they have not found out its source in mosquito body.

Later, Hagedorn et al. (1975) have demonstrated the release of alpha-ecdysone from the ovary and the conversion of alpha to beta-ecdysone elsewhere in the body of Aedes aegypti. Moreover, they have indicated that the fat body response may be obtained only in high titer of ecdysone.
which occurs after blood meal. However, secretion and conversion of ecdysone should occur only after attaining previtelogenic or resting stage of the ovary have been proposed by Hagedorn et al. (1977) with the observation that presence of ecdysone before the completion of previtellogenic phase disturbs JH dependent step in oogenesis (priming the ovarian follicles).

With regard to function of ecdysone on the target tissue of fat body, Bohm et al. (1978) have reported that the fat body synthesis is programmed as evidenced by the declining trend in synthesis even when exposure of the fat body is abnormally prolonged.

In connection to the source of secretion, though Hagedorn et al. (1975) have reported the release of alpha-ecdysone from the ovary, Tadkowski and Jones (1979) are of the opinion that EDNH (OEH) activated the oenocytes to produce ecdysone after the mosquito, Aedes aegypti takes a blood meal. Later, however, Shapiro (1983) has reported that ovarian ecdysone secretion in response to factor(s) from mosquito head is mediated by cAMP as evidenced by its increase in ovary prior to ecdysone secretion.

Very recently Cho et al. (1995) have pointed out that ecdysteroid receptor is produced in both ovary and fat body in preparation for the major events of vitellogensis in which 20-hydroxyecdysone is involved. The mosquito fat body in Aedes aegypti acquires competence for vitellogenesis.
synthesis and responsiveness to 20-hydroxyecdysone during the first two days of the previtellogenic period.

It is also appropriate to point out the relationship between EDNH (OEH) and ecdysone which has been established much once again in 1980's like dose response ecdysteroid activity to the crude head extracts or partially purified OEH (Masler et al., 1983); occurrence of egg maturation only after injection of crude head extract in blood-fed, decapitated Aedes aegypti (Shapiro et al., 1986); conversion of labeled cholesterol into ecdysone in vivo by non species specific OEH (Borovsky and Thomas, 1985; Borovsky et al., 1986 and Kelly et al., 1986); the steroidogenic OEH composed of 2 fractions of peptides with the molecular weight of ~ 11 KDA and ~ 24 KDA (Whisenton et al., 1987) and the peptides from the neurons or neurosecretary cells may be like cardioexcitatory neuropeptide (FMRF amide) which involve in gonadotropic / ecdysteroidogenic activity in Aedes aegypti (Matsumoto et al., 1988a and 1988b). Finally, Wheelock et al. (1991) have succeeded in an attempt to get purified EDNH (OEH) from the heads of Aedes aegypti and have shown the ecdysone production from the ovaries.

The role of nutrition

Though the difference between autogenous and anautogenous was attributed to the amount of reserves available to the adults from larval stages, it is now found out that the difference between the two not in reserves but
lay in hormonal control. A gonadotropic hormone is secreted automatically after emergence in autogenous mosquitoes but not secreted in anautogenous mosquitoes without the stimulus of a blood meal (Clements, 1963).

Even after blood feeding, corpora allata alone secretes hormone whereas corpus cardiacum does not release the stored hormone in anautogenous mosquitoes. The neurosecretory hormone from cardiacum is released only after blood feeding (Lea, 1970).

Though the nature of the stimuli for releasing the neurosecretory hormone (OEH/EDNH) has been attributed for the act of feeding and stretching of the abdomen, Hagedorn et al. (1979) are of the opinion that a humoral agent after blood feeding is involved and this may be an early product of blood meal digestion. According to Borovsky (1982), the release of OEH / EDNH is due to corpus cardiacum stimulating factor (CCSF) from the ovary probably synthesized after a blood meal.

It is felt by Recioppi et al. (1984) that ecdysterone alone is not sufficient to stimulate a full response in connection to vitellogenin mRNA, the blood meal is also necessary. According to them it is not certain whether blood meal simply a source of nutritional reserves or whether other factors that act in concert with ecdysterone are supplied.

In connection to the reproduction of mosquito, Uchida (1992) and Uchida et al. (1993) have suggested that the
amino acids resulting from blood meal digestion are not only utilized for yolk protein synthesis but also have the potential power to initiate and promote mosquito oogenesis. Moreover, the initiation of mosquito oogenesis requires an appropriate balance in increased haemolymph amino acids.

From all the foregoing account on the roles of juvenile hormone, ecdysone and nutrition in connection to mosquito egg development, it is noteworthy that normal ovarian development in mosquitoes is dependent on a series of sequential integrated steps as stated by Fuchs and Fong (1976). To summarise the events, it may be said that after emergence (eclosion) the earlier growth of the ovary up to 'previtellogenic phase' is governed by juvenile hormone from corpora allata. Apart from priming the ovarian follicles, the hormone also makes the fat body in proper condition (competence) to respond for further action of yolk protein (vitellogenin) synthesis. After getting a blood meal which contains or provides all essential materials, for yolk preparation, the second stage of 'vitellogenic phase' of the ovary is started with the release of OEH/EDNH from cardiacum thereby production of ecdysone is carried out. The ecdysone either individually or with the support of juvenile hormone (JH) then makes the fat body to synthesize yolk protein. Under proper condition of the oocyte (stage, pH, temperature, etc.) as suggested by Koller et al (1989), the yolk protein having two sub units (Raikhel and Bose, 1988) are sequestered as such into the oocytes.
2.2 OTHER INSECTS - REPRODUCTIVE PHYSIOLOGY.

In this section, other insects are referred to the references pertinent to earlier research work on reproductive aspects of all insect forms except mosquito(es). Generally, one principle what has been established in other insect forms, it would be tried to establish in mosquito group; likewise principle what has been established in mosquito group, it would be checked whether that principle is working out in other insect groups. For instance, the corpus allatum as a source of a gonadotropic hormone in insects was first mentioned by Wigglesworth in 1936 working with *Rhodnius prolixus* (Engelmann, 1967). Later, such a view had been expressed by different authours in different insect forms at different periods.

**Role of Juvenile Hormone (JH)**

Involvement of corpus allatum in regulation of reproduction has been reported by Wigglesworth (1935) by the observation that adult female *Rhodnius* deprived of the brain and corpus allatum do not develop eggs; but if the brain is removed without corpus allatum eggs are developed normally.

Later, Highnam et al (1963) have reported that protein synthesis is controlled by the neurosecretory system during oocyte development, while the corpora allata secrete a gonadotropic hormone which fascilitates protein uptake by the growing oocytes in the desert locust, *Schistocerca gregaria.*
Yolk deposition requires the presence of juvenile hormone in addition to vitellogenin, as indicated by the failure of vitellogenin injection to stimulate yolk deposition in allectomized females of Periplaneta americana (Bell, 1969). Likewise, in Leucophaea maderae JH alone controls the synthesis of a female specific protein (Engelmann et al., 1971).

It appears that hormones from the brain and CA are requisite for post emergence egg maturation and they either directly or indirectly act to shunt fat body substrates to the developing oocytes in Munduca sexta (Sroka and Gilbert, 1971).

It is pointed out by Davey and Huebner (1974) in Rhodnius that the product of the allatum, Juvenile Hormone, acts on the ovary by bringing about the appearance of large spaces between the follicle cells, which give the oocyte surface access to the yolk proteins circulating in the haemolymph.

Kelly and Devenport (1976) also have suggested that vitellogenin uptake may be directly dependent on juvenile hormone in Oncopeltus fasciatus.

With regard to the protein uptake Keeley (1978) has substantiated the view of Davey and Huebner (1974) in the same form Rhodnius prolixus and expressed the view that the juvenile hormone seems to stimulate the uptake of haemolymph proteins by the in vitro ovaries by way of causing spaces to appear between the follicular cells. Moreover, the above
author has pointed out that juvenile hormone along with ecdysone regulate the formation of specific vitellogenic protein essential for ovarian maturation. On the other hand Riddiford and Truman (1978) are of the opinion that the alpha (α) ecdysone after conversion to β- ecdysone stimulate synthesis and release. Since fat body removed from female allectomized at emergence will not respond to ecdysone, it appears that JH has a role in priming the fat body for vitellogenin synthesis. Moreover, JH seems to be enhancing yolk uptake in oocyte by increasing intercellular spaces.

Later, Riddiford (1980) has reported that JH is thought to stimulate a tissue with abdomen to secrete ecdysone which then increase vitellogenin synthesis in the fat body of Drosophila and this tissue could well be the ovary.

Since the high rate of JH biosynthesis is observed during oocyte growth in the viviparous cockroach Diploptera punctata, Dekort and Granger (1981) have assumed that there should be a relationship between ovarian development and juvenile hormone secretion.

Once again the correlation between the uptake capacity of oocytes with the opening of intercellular spaces in the follicular epithelium has been reported in Rhodnius prolixus by oliveria et al (1986).

In Drosophila melanogaster synthesis and release of yolk proteins are presumably shut off due to the absence of normal signals from the head by decapitation. These signals
could be gonadotropic and allotropic hormones that control the production of 20-hydroxyecdysone and juvenile hormone. But it is not clear whether JH directly stimulates the production of yolk protein from the fat bodies or it mediates its action by permitting 20-hydroxyecdysone to be produced and interact with the target tissue (Wu et al., 1987).

With regard to mode of action of juvenile hormone in vitellogenesis of Locusta migratoria by the topical application of JH analog Pyriproxyfen, Edwards et al. (1993) are of the opinion that the hormone initiates transcriptional activation of the vitellogenin genes.

Response of fat body and follicle cells of Rhodnius to juvenile hormone has been viewed in different way by Wang and Davey (1993). These authors have suggested that relatively low titres of JH may be sufficient to initiate and sustain vitellogenin synthesis while the follicle cells may require a higher dose of JH to maintain a fully patent condition so as to permit the vitellogenin to have access to the oocyte surface.

Recently the analogs and homologs to juvenile hormone have been reported to stimulate vitellogenin synthesis in the fire bugs, Pyrrhocoris apterus by the application of Retionic acid (Nemec et al., 1993) and in Armyworm moths, Pseudaletia unipuncta (Cusson et al., 1994).
Role of ecdysone

Though the role of ecdysone has been attributed to vitellogenin synthesis and release from the fat body after the conversion of \( \alpha \)-ecdysone to \( \beta \)-ecdysone, the biosynthesis of \( \alpha \)-ecdysone from cholesterol has not been completely elucidated. Since insects cannot synthesize the sterol ring system, cholesterol or a plant sterol precursor is an essential part of their diet for growth and development. Injection of labeled cholesterol or 7-dehydrocholesterol into intact insects leads to the isolation of labeled \( \alpha \)- and \( \beta \)-ecdysone (Riddiford and Truman, 1978). This sort of statement might have been given based on earlier workers like Monroe (1959) who has pointed out that the house fly which has been found to lack the mechanism for sterol biosynthesis from \(^{14}\text{C}-\)sodium acetate, utilizes a high percentage of administered \(^{14}\text{C}\) cholesterol in egg production. Moreover it had been said that the lack of a dietary sterol has no effect on total egg production.

Later, Robbins and Shortino (1962) have observed that the house flies reared on larval medium supplemented with cholesterol developed mature ovaries when held on an adult diet of only sucrose and water. But, it is not known whether cholesterol as such is responsible or whether it seems as precursor for an essential steroid metabolite which regulates gonadal development.
Again Monroe et al (1967) have pointed out that when the housefly larvae, *Musca domestica* were reared aseptically on a synthetic diet containing cholesterol - 4 - $^{14}$C as the only sterol source, the uptake of cholesterol by the larvae doubled between 2 and 4 days of age reaching a maximum of about 10 $\mu$g per larva. Cholesterol accumulated in the tissues at a rate that exceeded body weight increase and the carry-over of larval sterols to the adults were nearly quantitative confirming the importance of sterol storage in the larval stage. Esterification of sterols was very minor during larval growth, but increased appreciably in the pupae and adults and was greatest in the first batches of eggs. Dahyrogenation of cholesterol to 7-dehydrocholesterol was not significant in any stage except the reproducing females and the eggs.

In *Calpodes ethlius*, Collins (1969) has reported that protein uptake into granules by the fat body involves three stages; concentration of the protein between cells, pinocytosis into small vesicles and fusion of the vesicles to form multivesicular bodies or storage granules. The first two steps are intrinsic properties of the fat body independent of the hormonal milieu. The third step is influenced by ecdysone.

Robbins et al. (1971) once again have pointed out that insects require a dietary or exogenous source of sterol for normal growth, metamorphosis and reproduction. Insects lack the capacity for the *de novo* biosynthesis of the steroid...
nucleus and thus must obtain essential cholesterol per se from the diet or from a dietary sterol that can be readily converted to cholesterol. With regard to ecdysone they have also pointed out that cholesterol is a precursor of the molting hormone in insects as evidenced by the injected labeled cholesterol into $^3$H 20-hydroxyecdysone in Callyphora stygia. A metabolite of cholesterol, 7-dehydrocholesterol occurs in a number of insects and the 7-bond that is common to both 7-dehydrocholesterol and the ecdysones points to this sterol as an intermediate in molting hormone biosynthesis. The evidence for the involvement of 7-dehydrocholesterol has been produced in C. stygia wherein 1-$^3$H-7-dehydrocholesterol like 1-$^3$H-Cholesterol has been metabolized $^3$H - 20 hydroxyecdysone. Moreover, the eggs oviposited by the 4 - $^{14}$C cholesterol injected adult female house flies contained radio labeled 7-dehydrocholesterol both in free and in the esterified form.

With regard to the metabolism of ecdysone, Gilbert et al. (1980) are of the opinion that generally in insects, synthesis of ecdysteroid is the hydroxylation of ecdysone to 20-hydroxyecdysone. The enzyme involved in the reaction, ecdysone 20-monoxygenase is distributed in various tissues (fat body, malpighian tubules, midgut etc.,). During reproduction, ecdysone is synthesized in ovaries presumably by the follicle cells and then is sequestered by the developing eggs for further uses for serosal cuticle
deposition in early embryonic development initiated by ecdysteroids.

The role of 20-hydroxyecdysone has been compared to estrogens which regulates vitellogenin synthesis in oviparous vertebrates with regard to the yolk polypeptide synthesis in Sarcophaga bullata.

The ovarian follicles of Hyalophora are shown to undergo a comprehensive transformation in altered cell potentials, cytoplasmic pH and turgidily which implicate the cell membrane in an early stage of activation (Woodruff and Telfer, 1990).

Recently Grau and Lafont (1994) have detected 20-hydroxyecdysone in the ovaries and haemolymph of Drosophila melanogaster.

By considering all the views given by different research workers on different insect forms about the role of juvenile hormone and ecdysone, priming the ovary and fat body pertinent to egg development is governed by juvenile hormone (JH) and the latter part of vitellogenin synthesis is undertaken by the steroidic ecdysone.