J. Mar. biol. Ass. India, 1965, 7 (2): 284-290

ON THE REPRODUCTIVE CYCLE OF *PENAEUS INDICUS* (M. EDW.)*

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' By " reproductive cycle " is meant an entire cycle, that is the series of events from the time of activation, growth and gametogenesis in the gonads to the spawning of the gamates and recession of gonadal activity ' (Giese, 1959). One of the methods of assessing this cycle has been the microscopic examination of the gonads as adopted by Moore (1934), Loosanoff (1937, 1942 & 1953), Veevers (1949), Chipperfield (1953), Orton *et al.* (1956), Allen (1959), Carlisle (1959*a* & *b*) and Reddiah (1962) in the case of several invertebrates. By this method it has been possible to obtain cytological information defining the stages in the gonadal cycle. Crustacea received less attention in this regard, noteworthy contributions having been made by Allen (1959), Carlisle (1959 *a* & *b*) and King (1948). *Penaeus indicus* is an important species in the fishery of Madras coast and so the reproductive cycle of the species has been studied. This will also be of interest since information on tropical penaeids as regards the gonadal structure is lacking except for the study on *Parapenaeopsis stylifera* by Shaikmahmud and Tembe (1958 & 1961).

Gonads were obtained from fresh specimens and they were fixed in Buoin-Duboscq. solution Paraffin sections were cut at 3 to 6 μ thickness. Heidenhain's hematoxylin was used for staining with 5% iron alum as mordant and 3% alum as differentiator. The sections were counter stained with alcoholic eosin. Permanent slides have been made with Canada balsam.

MALE REPRODUCTIVE SYSTEM

MORPHOLOGY

The male genital system consists of a pair of testes, vasa deferentia, terminal ampoules, petasma and appendix masculina. The testis consists of four lobes (Fig. 1) located in the cardiac region dorsal to hepatopancreas. The two lobes are connected anteriorly and posteriorly each lobe is continued as tubular portion. This portion has been described by King (*loc. cit.*) and Shaikmahmud and Tembe (*loc. cit.*) as vas deferens but certain structural peculiarity warranted a rethinking and hence it is named ' *tubular portion* ' in the present study. The narrow tube which follows this portion is the vas deferens that traverses through the muscles of cephalothorax (Fig. 2) and opens at the base of the 5th pereiopod through the terminal ampoule. The petasma (Fig. 3) is formed by the fusion of the endopodites of first pair of pleopods. The endopodites of the second pair of pleopods are modified into appendix masculina (Fig. 4). Descriptions of these structures have been given by Kubo (1949).

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INTERNAL STRUCTURE

Testis (Pl. I. Fig. 4) : The lobes of the testis have a thin outer membrane. Muscle layers of any kind are absent. In a cross-section number of follicles can be seen which are formed by connective tissue septa. In between the follicles number of nutritive cells are present. In each follicle the germinal epithelium is active at the periphery. From the periphery towards the interior spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa are found in this sequence. In the lumen of each follicle free spermatozoa can be found in mature specimens. Spermatocytes and spermatogonia are larger cells, compactly packed and have large nuclei. The nuclei have nuclear ring inside which nucleolii are present. The spermatids are losely arranged and they lack in the nuclear ring. Spermatozoa are oval in shape and they are found in the lumen as stated already.

Tubular portion (Pl. I, Figs. 1, 2, 3, 5, 6, 7) : The structure shows differences with age of the prawn (*vide infra*). In young specimens two portions can be seen internally, a generative portion and a lumen. The generative portion is made of follicles similar to testis lobes. Spermatogonia, spermatocytes and spermatids are present. However this portion becomes narrower and narrower towards vas deferens and at the junction of the vas deferens it is obliterated. The lumen is covered by a thin outer membrane which is continuous with the generative portion. Below this is a layer of columnar epithelium. The nuclei of these columnar cells are large. Muscle layers are absent. In the centre is the lumen.

Vas deferens (Pl. I. Fig. 8) : This is a narrow tube which has an outer thin membrane and an inner columnar epithelium. The columnar epithelium forms an invagination into the lumen forming a typhlosole, the cells of which are glandular with large nuclei. In the middle is the lumen which is continuous from the tubular portion.

Terminal ampoule (Pl. I, Fig. 9) : This is a bulbous structure at the end of the vas deferens. Internally it has two chambers. These two are covered by an outer epithelium followed by a layer of circular and then a layer of longitudinal muscles. The inner most layer is the glandular epithelium which by an invagination divides the lumen into two. In one chamber is found the spermatophore and in the other some calcareous material that comes out on gentle squeezing. The ampoule opens at the base of the coxopodite of 5th pereiopods. The muscular layer is thicker towards the opening and this part may be pulsatory aiding in the discharge of the spermatophore. The epithelium may also be secretory in function.

Spermatophore (Pl. I, Fig. 9) : In the cross-section of the terminal ampoule the spermatophore can be seen distinctly. It has a wing like expansion of cuticular substance which stains deep with eosin. Each ampoule has one spermatophore and during copulation this is attached to the thelycum of the females together with the calcareous material. The sperms are packed in the centre of the spermatophore which stain dark.

Spermatozoa (Fig. 5) : The spermatozoan is a minute and globular body. It measures on average 9·6μ. It has a minute tail which is suggestive of motility of the sperm. This sperm is entirely different from that of *P. stylifera* (Shaikmahmud and Tembe, 1958) and similar to that of *P. setiferus* (King, 1948).

STRUCTURAL DIFFERENCES WITH AGE

As mentioned earlier the tubular portion shows variations as well as the degree of development of testis with age of prawn. Five stages have been identified in this regard.

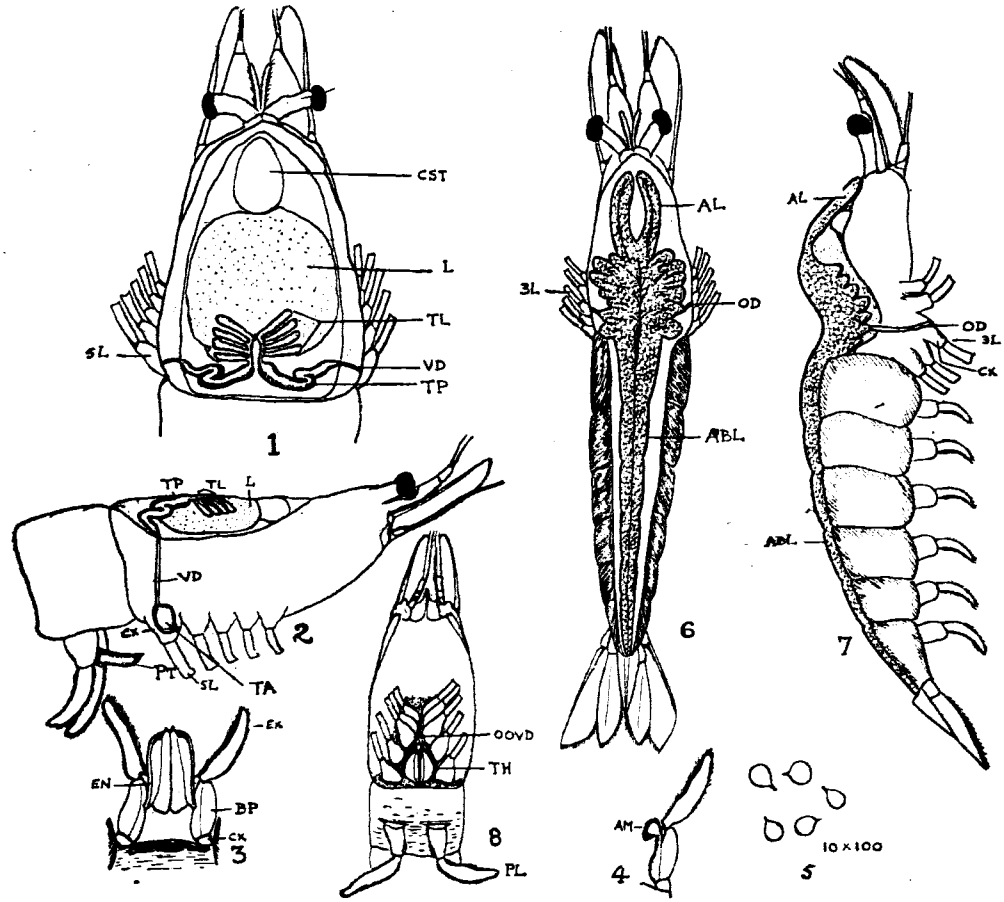

FIG. 1. Dorsal view of the male reproductive system. FIG. 2. Lateral view of the male reproductive system. FIG. 3. Petasma of male. FIG. 4. Appendix masculina of male. FIG. 5. Diagram of spermatozoa. FIG. 6. Dorsal view of the female reproductive system. FIG. 7. Lateral view of the female reproductive system. FIG. 8. Thelycum of female.

AM : appendix masculina ; AL : anterior lobe ; ABL : abdominal lobe : CST : cardiac stomach ; BP : basipodite ; CX : coxipodite ; EX : exopodite ; EN : endopodite ; OD : oviduct ; OOVD : opening of oviduct ; L : hepatopancreas ; PL : pleopod ; TA : terminal ampoule ; TL : testis lobe ; TP : tubular portion ; PT : petasma ; TH : thelycum.

110 *mm. specimen :* Testes lobes are not developed. Generative portion is present in the tubular portion up to vas deferens (Pl. I, Fig. 1). Spermatozoa are not formed.

120 *mm. specimen :* Testes lobes are fairly developed. Generative tissue is very prominent in the tubular portion (Pl. I, Fig. 2). Towards the distal region it is obliterated and only the lumen remains.

130 *mm. specimen :* Testes lobes are well developed. The two components of the tubular portion are well represented (Pl. I, Fig. 3). Vas deferens is distinctly tubular as differentiated from the earlier portion. Spermatozoa are present in the lumen of the follicles, tubular portion and in the terminal ampoules.

140 *mm. specimen :* Same as the previous stage (Pl. I, Fig. 5). Spermatophores are present in the terminal ampoules. This stage is the fully mature one and the male is ready to discharge the spermatophores.

170 *mm. specimen :* The testes lobes contain only spermatozoa but not the other stages of spermatogenesis. These may be residual sperms and the male may be incapable of producing more sperms after the discharge of the contained ones. The tubular portion lacks in the generative portion and only lumen is seen encircled by the columnar epithelium the cells of which are more rounded and have larger nuclei (Pl. I, Fig. 6). Spermatozoa are present throughout the length of the portion and spermatophores are present in the terminal ampoule. The tubular portion of this stage resembles that of *P. setiferus* described as vas deferens by King (1948). The vas deferens of *P. stylifera* is also similar in structure.

FEMALE REPRODUCTIVE SYSTEM

Morphology

The female reproductive system consists of a pair of ovaries, oviducts and thelycum. The ovary is dorsal to the alimentary canal extending from the cephalothoracic cavity to the telson inside the abdomen. It is made up of two halves and each half has 6 to 7 lobes in the cephalothoracic region located dorsal to the hepatopancreas. The anterior lobes are prominent and they encircle the oesophagus. As the prawn matures the fusion between the two halves becomes marked (Fig. 6). The oviducts are short and narrow tubes emanating from the 6th lobes of the ovary (Fig. 7). They pass through the muscles and open ventrally through the genital pores at the base of the 3rd pereiopods. The thelycum (Fig. 8) has been described by Kubo (1949). The degree of maturity of the female can be assessed by the appearance and colour of the ovary (*vide infra*).

Internal structure (Pl. II, Figs. 1-8)

The wall of the ovary consists of a thin outer epithelium, a layer of connective tissue and an inner epithelium. Muscle layers are absent. Number of follicles are formed by septa of connective tissue. The germinal epithelium is not uniformly distributed inside the ovary but distinct patches of activity are noticed. These are called the 'zones of proliferation.' Such zones are usually present on the ventrolateral side in the lobes internally. Inside the follicles, oogonia and oocytes of different sizes are seen and the internal structure varies with age as will be shown.

Oviduct (Pl. II, Fig. 9) *:* The oviduct is a slender tube. It has a thin outer epithelium, an intermediate connective tissue and an inner epithelium made up of tall cells which may be secretory in function. Muscle layers are absent.

Ripe ovum (Pl. II, Fig. 7) *:* The ripe ovum measures 360 μ in diameter. There is a distinct nuclear ring inside which are present basophilic granules. The marginal

bodies (*vide infra*), 5 to 32 are present but they stain very light. There is a tendency for them to disappear. The cytoplasm stains red and basophilic granules are absent. It is probable that just before discharge of ova they become acidophilic in nature. The ovum measures 38 times bigger than the spermatozoan.

STAGES OF MATURITY

Stage I (Pl. II, Fig. 1) : Prawns measuring up to 110 mm. belong to this stage. Ovaries are slender, colourless and measure 8 to 8.5 cm. in length. Appearance is smooth. Internally only 3 or 4 follicles are present. The zones of proliferation are located in the lateral regions. Oocytes are small without any yolk and measure 19.2 to 57.6 μ in diameter. The nuclear ring is clear with one or two nucleolii.

Stage II (Pl. II, Figs. 2, 3) : Females measuring 120 to 130 mm. belong to this stage. The ovary measures 100 mm. in length and it is slightly yellowish. This can be termed the ' yellow ovary ' stage. The external appearance is slightly granular. Internally there are a number of follicles. The septa are thick and the lateral follicles are more prolific. Follicle cells are present in between the follicles. The oocytes are mostly eliptical measuring $38.4 \times 48.0\ \mu$ to $144 \times 124\ \mu$. Nuclear ring is enlarged and 2 or 3 nucleolii are present. The ring measures 2.6 to 57.6 μ. Ooplasm shows slight yolk presence.

Stage III (Pl. II, Figs. 4, 5) : This stage females measure 130 to 140 mm. in length. The ovary measures 100 to 110 mm. in length and is greenish in colour. The appearance is also granular. This ovary can be made out through the cuticle dorsally. The two halves of the ovary show a good degree of fusion. Internally the follicles are numerous in number and oocytes of different sizes are present. They measure from $49.6 \times 57.6\ \mu$ to 192 μ. Inside the nuclear ring 3 to 4 nucleolii are seen which measure 48 μ.

Stage IV, Ripe (Pl. II, Figs. 6, 7) ; Females measuring above 140 mm. in length have fully mature ovaries. Ovary measures 120 mm. and above. The lobes show full development in the cephalothoracic region. The ovary is granular in appearance and dark green in colour. This can be easily made out through the cuticle dorsally. Internally only one type of oocytes is present. The follicles are not very distinct because the ova are fully packed inside. They measure from 240 μ to 576 μ in diameter. The salient feature of this stage is the appearance of marginal bodies at the periphery of the oocyte. 5 to 35 of them are present which stain deep. They measure 19.2 to 28 μ in diameter. These are also found in *P. setiferus* (King, 1948). The nuclear ring is very clear with 1 to 6 nucleolii. In between the ova some follicle cells can be seen. The structure of the ripe ovum is similar to this but for the marginal bodies showing a tendency to disappear.

Stage V, Spent (Pl. II, Fig. 8) : The minimum size of a spent female is 150 mm.

(a) completely spent : The ovary looks whitish and ripe ova are absent. The internal structure is similar to that of stage II ovary. Residual ova are absent.

(*b*) Rejuvenating : The internal structure is midway between stage III and stage IV. There are many follicles and large oocytes are present. They measure from

105.6×211 μ to 516.4×240 μ. The nuclear ring is distinct. Marginal bodies are not yet formed. Follicle cells are present in good number. Polar bodies are noticed in between the ova. This stage suggests that another batch of eggs is produced after spawning.

REMARKS

It has been shown that the male reproductive system does not show remarkable changes from immature to mature condition except for the different degrees of testis development and the disappearance of the generative tissue in the tubular portion. On the other hand the characteristics of each stage of maturity of the ovary are well defined. If the criterion for maturity is taken as the appearance of free spermatozoa in the vas deferens then the smallest mature male measures 120 mm. However, spermatophore is well formed in the males of above 130 mm. The ripe female measures 140 mm. and above as seen from the internal structure of the ovary. Such disparity in the sizes at which the two sexes attain maturity has been reported earlier in *P. setiferus* (King, 1948), *Metapenaeus dobsoni* (Menon, 1955) and *P. stylifera* (Menon, 1953).

The peculiarity in the male reproductive of the species studied is the presence of a generative portion in the tubular portion which has been described as vas deferens by other authors. It is significant, however, that this generative tissue is absent in males measuring above 170 mm. in length. *P. setiferus* and *P. stylifera* (*loc. cit.*) do not have this tissue, the vas deferens being a continuous duct from the testis. It is not known whether younger specimens of *P. setiferus* possess this generative tissue as King (1948) studied males measuring above 140 mm. It appears that as the males grow older this tissue is obliterated and sperms are produced only by the testes. Perhaps the capacity to produce is lost after the male reaches certain age. The structure of the vas deferens, terminal ampoule, spermatophore and sperms resemble that of *P. setiferus*. The sperms of *P. stylifera* show a different structure (Shaikmahmud and Tembe, 1958).

The different stages in the development of the ovary of the present species bear resemblance to those of the ovary of *P. setiferus* (King, 1948) and *P. stylifera* (*loc. cit.*). The marginal bodies are present only in *P. setiferus* but not in *P. stylifera*. It is possible that the development of marginal bodies in the mature ovary is characteristic of the genus *Penaeus*. That the stage IV females can be identified easily renders it possible to estimate the breeding period and extend by noting the percentage of such females in the catches. Thus this gives a correct picture of the spawning activity of the species. The spent ovary is easy to be identified by the whitish colour and smooth appearance. The presence of such females gives a positive indication of the breeding season. The spent ovary recovers after releasing one batch of eggs as seen from the internal structure of the two stages of spent condition. Therefore it is evident that a single female is capable of releasing several broods of eggs in a single season. This supports the evidence gathered that the species, *P. indicus*, has a fairly long breeding period from July to October mainly. From the anatomical study of the ovary and testes it has been possible to deduce the exact size at which the two sexes mature and also criteria to identify mature prawns in the collections. It has been possible to arrive at the exact breeding season of the species in its reproductive cycle.

The author is grateful to Dr. S. Krishnaswamy, Professor of Zoology, Madurai Centre, Madurai, for having suggested this problem and guidance.

SUMMARY

The reproductive cycle of the penaeid prawn, *Penaeus indicus* (M. Edw.) has been studied by examining the external and internal structure of the testes and ovary at different stages of maturity. The male attains maturity at 120 mm. while the ripe female measures 140 mm. in length. Although the mature males are present during the major part of the year it is the presence of ripe eggs that decides the breeding season. Five stages of maturity of ovary have been observed. It is evident that a female is capable of producing several brood of eggs. These observations agree with those on *P. setiferus* and *P. stylifera*. A peculiarity noticed is the presence of generative tissue in the so-called vas deferens which is absent in the other two species. From the study of the gonads it has been possible to arrive at the exact breeding season of the species.

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PLATE I. MALE REPRODUCTIVE SYSTEM. FIGS. 1, 2 & 3. Cross section of proximal region of tubular portion of a male specimen 110 mm., 120 mm. and 130 mm. in length, respectively (×160). FIG. 4. Cross section of testis lobe (140 mm.) (×320). FIG. 5. Cross section of proximal region of tubular portion of a male 140 mm. in length (×160). FIG. 6. Cross section of tubular portion of a male 176 mm. long (×160). FIG. 7. Cross section of distal region of tubular portion of a male 140 mm. in length (×160). FIG. 8. Cross section of vas deferens of a male 140 mm. long (×480). FIG. 9. Cross section of terminal ampoule (140 mm. male) (×160).

cep : columnar epithelium ; *ep* : outer epithelium : *iep* : inner epithelium ; *inv* : invagination ; *spm* : sperm mass ; *sph* : spermatophore ; *l* : lumen ; *tf* : follicle of testis.

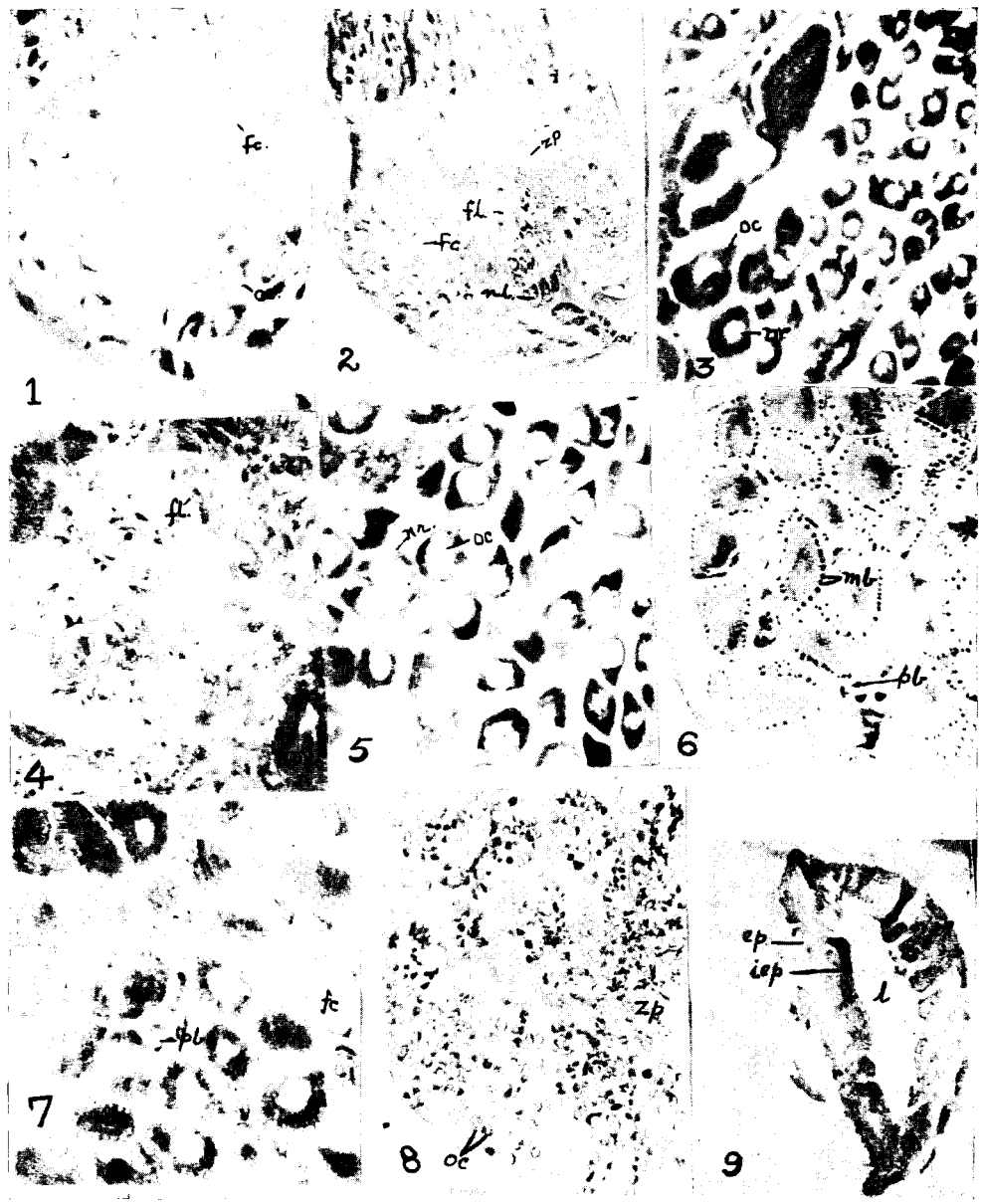

PLATE II. FEMALE REPRODUCTIVE SYSTEM. FIG. 1. Cross section of ovary in a 110 mm. female (×400). FIG. 2. 120 mm. female, Cross section of ovary (×160). FIG. 3. 120 mm. female, Magnified view of ovary (×400). FIG. 4. 130 mm. female, Cross section of ovary (×160). FIG. 5. 130 mm. female, Magnified view of ovary (×400). FIG. 6. 140 mm. female, Cross section of ovary (×160). FIG. 7. 198 mm. female, Cross section of ovary (×160). FIG. 8. 160 mm. female, Cross section of a rejuvenating ovary (× 160). FIG. 9. Cross section of oviduct (×800).

ep : outer epithelium ; *iep* : inner epithelium ; *fc* : follicle cells ; *fl* : ovarian follicle ; *mb* : marginal bodies : *nr* : nuclear ring ; *oc* : oocyte ; *l* : lumen ; *pb* : polar bodies ; *zp* : zone of proliferation.