Miscellanea : Oocyte differentiation and vitellogenesis in "Glossina morsitans" Westw.

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Objekttyp: **Article**

Zeitschrift: **Acta Tropica**

Band (Jahr): **28 (1971)**

Heft 4

PDF erstellt am: **12.07.2024**

Persistenter Link: https://doi.org/10.5169/seals-311735

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Oocyte Differentiation and Vitellogenesis in *Glossina morsitans* Westw.

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Introduction

Glossina exhibits adenotrophic viviparity and produces eggs in a cyclical fashion. Each ovary consists of two meroistic-polytrophic ovarioles. Starting with the right ovary, eggs are produced singly in a sequence which involves alternation of ovaries and ovarioles (SAUNDERS, 1960, 1962). Degeneration of follicles followed by resorption has been shown to occur in the ovaries of some flies (SAUNDERS, 1960). Differentiation of the oocytes and yolk deposition, however, have not been studied. The study in this direction is likely to yield background information for future work on the reproductive physiology of the genus. The present paper reports investigations on the differentiation of oocytes with particular emphasis on the nucleic acids and yolk protein deposition.

Materials and Methods

The puparia of *G. morsitans* were obtained from Singida, Tanzania, and the emerging flies used in the present investigation were maintained at 25°C, 80% R.H. and fed on the blood of a live calf.

The reproductive organs of the females of different age from newly emerged to eight days old were fixed either in Carnoy's fluid, Bouin-Duboscq, Zenker's fluid or Baker's formol-calcium. In some cases the reproductive organs were fixed *in situ* and the whole abdomen sectioned.

For histological examination serial paraffin sections, cut at 6 or 8 μ, were stained either with Mayer's haemalum and aqueous eosin, Heidenhain's iron haematoxylin and light green or Gomori's trichrome stain (cf. GURR, 1962). The histochemical investigations are based on following tests. Standard Feulgen method was used for DNA (cf. PEARSE, 1968). Schiff's reagent was prepared according to the procedure of DE TOMASI (1936). The methyl-green-pyronin technique (BRACHET, 1942) was employed for differential staining of nucleic acids, with and without prior RNA-ase digestion. In order to detect protein, sections were stained by BONHAG's (1955) modification of the MAZIA-ALFERT (1953) bromophenol blue technique. Ninhydrin-Schiff method (YASUMA & ITCHIKAWA, 1953) for protein was also used. For the controls, the ninhydrin deamination was omitted or the sections were subjected to trypsin digestion. To study the living material, isolated ovarioles were placed in insect Ringer (BODENSTEIN, 1946) and examined with phase-contrast optics.

Results

The ovaries are enclosed in a common epithelial sheath (Fig. 2) consisting of epithelial cells, connective tissue and muscle fibres, and are well supplied with a system of trachea and tracheoles. Each ovariole in *Glossina* comprises the germarium and vitellarium with a single developing follicle. The anterior part of the germarium contains oogonia and prefollicular cells (Fig. 2). One of these

oogonia undergoes four consecutive apparently synchronous divisions to produce a cyst of sixteen daughter nuclei contained in a syncytial cytoplasm, which soon become surrounded by prefollicular tissue (Fig. 1). The nuclei of the syncytium are all alike: future oocyte and trophocytes cannot be distinguished at this stage.

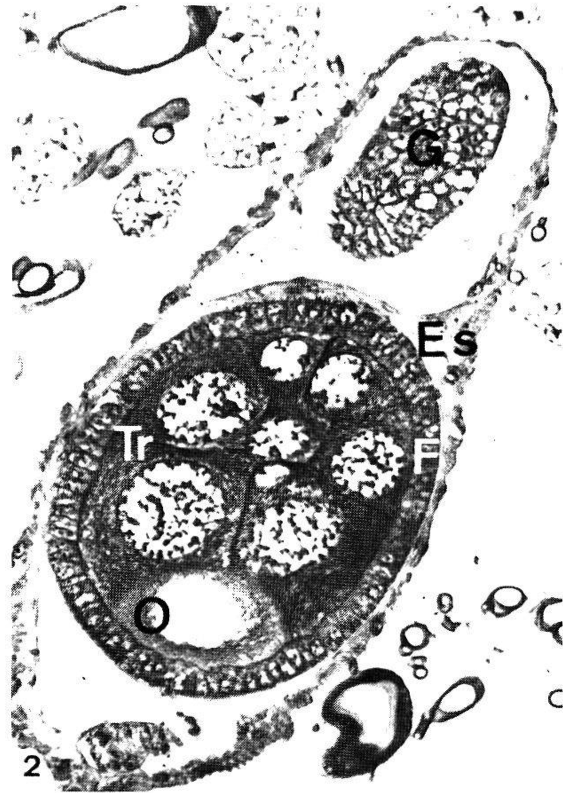
Further development of the primary follicle concerns the differentiation of the syncytium. As development proceeds, cell membranes appear separating the nuclei into distinct cells (Fig. 2). This follicle is eventually pushed into the vitellarium by the division and growth of the germarial cells. Initially the growth rate of all sixteen daughter cells appears to be identical. Soon, however, the most posterior cell, the presumptive oocyte, grows much more rapidly, its nucleus enlarges and the cytoplasm is rendered slightly more basophilic. The remaining fifteen daughter cells become differentiated into trophocytes with granular cytoplasm and large rounded nuclei each containing one to four nucleoli. The trophocytes adjacent to the oocyte are larger than the others. Each of the meroistic-polytrophic follicle is thus comprised of fifteen trophocytes and an oocyte within a follicular epithelium. Intercommunication of the cytoplasm between all members of the sixteen-cell group is mediated by fusomes, the cytoplasmic bridges with ring-shaped walls separating the adjacent cells (Fig. 6 and 7). The common origin of the oocyte and trophocytes is significant since the sixteen cells function as a physiological entity until the conclusion of vitellogenesis.

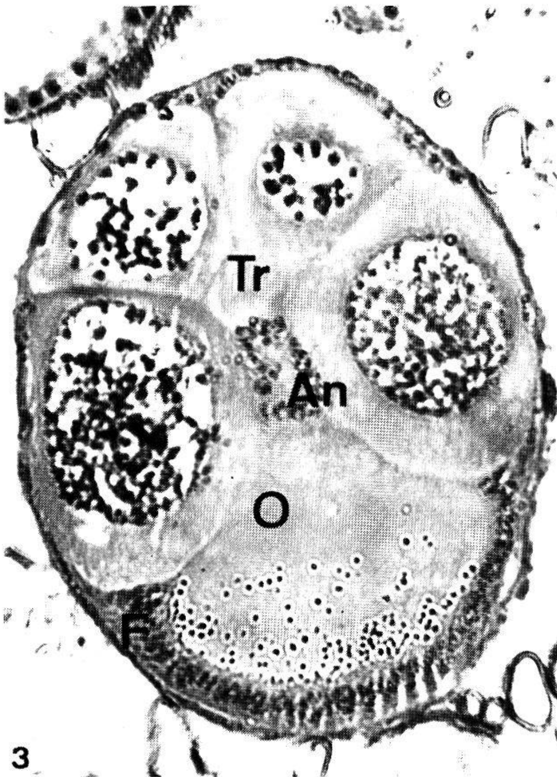
The oocyte nucleus, like the nuclei of trophocytes, is initially Feulgen-positive but unlike them, it becomes Feulgen-negative at the very early stage of vitellogenesis. During differentiation and growth of the trophocytes the chromosomes elongate, possibly by uncoiling, and concurrently undergo a series of endomitotic doublings followed by separation of homologous chromosomes, so that ultimately each nucleus becomes filled with a mass of Feulgen-positive threads.

The nucleoli of the early trophocytes are large and exhibit the phenomenon of nucleolar "emissions" (Fig. 5). A series of nucleolar protrusions gradually enlarge and eventually break off from the main body. These emission bodies become randomly dispersed in the nucleoplasm. Many of these are closely associated with the nuclear membrane and a few are seen in the trophocyte cytoplasm close to the nuclear membrane (Fig. 3). After completion of this process, the nucleolus becomes vacuolated. The constituent emission bodies give intense staining reaction with ninhydrin-Schiff, bromophenol blue and pyronin-methylgreen for RNA, while with Feulgen only slight positivity is obtained. The emission bodies apparently break down rapidly in the cytoplasm since very few are observed here and, furthermore, these bodies are relatively smaller than those in the nucleoplasm. The emission bodies are absent in the nuclei of very young follicles, appearing only when the trophocyte cytoplasmic material begin to flow into the oocyte.

Observations have revealed a number of "accessory" nuclei squeezed into the inter-trophocyte spaces as well as into the region adjacent to the growing oocyte (Fig. 3). The origin of these nuclei has not been determined. These give positive reaction with Feulgen, bromophenol blue, ninhydrin-Schiff and pyronin-methylgreen. It should be mentioned that DNA was not detected in the oocyte by the methods used.

The trophocytes adjacent to the oocyte open directly into the latter via fusomes (Fig. 6). The cytoplasm on either side of these inter-cellular bridges has similar staining properties. Methyl-green-pyronin for RNA, ninhydrin-Schiff and bromophenol-blue give intensely positive reaction in this region. Histochemical tests thus reveal a high content of ribonucleoprotein apparently streaming from trophocyte cytoplasm into the oocyte through fusomes. DNA was not detected in this stream. In the course of vitellogenesis there is an apparent gradual decrease of the RNA content of the oocyte as indicated by only slight positivity obtained for this





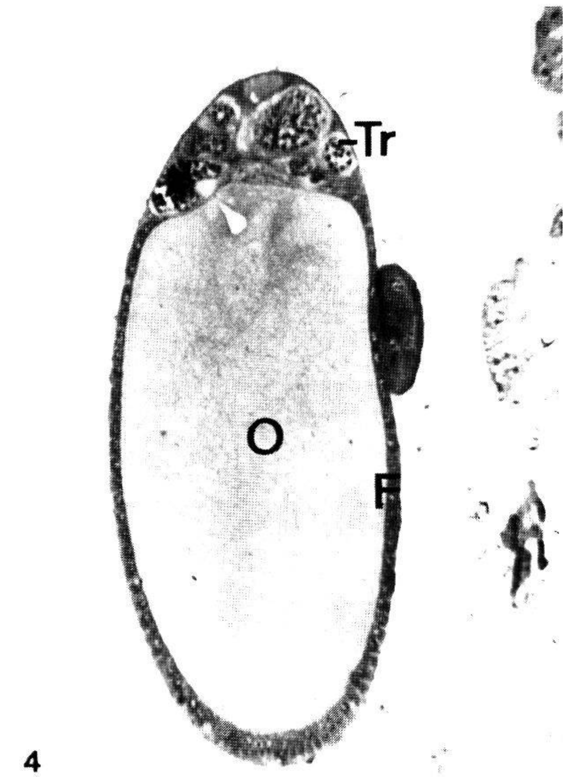


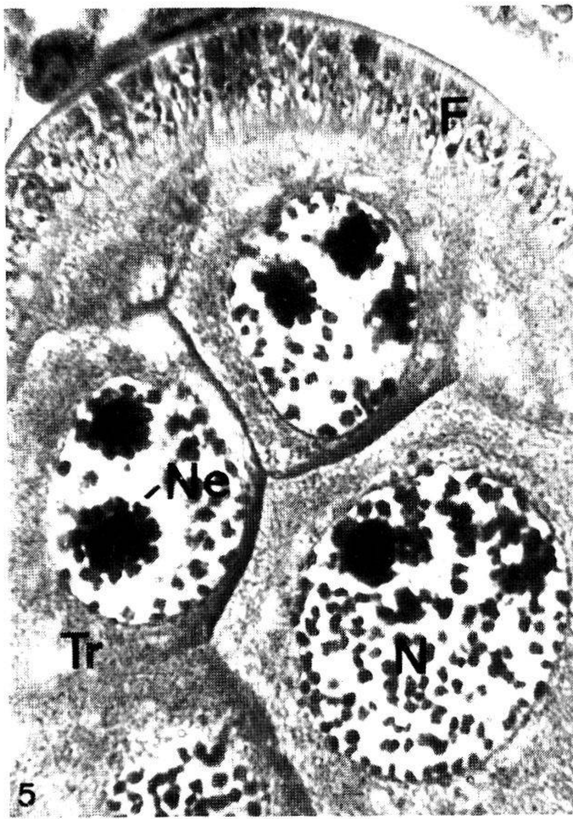
Fig. 1. Section of a follicle, showing dividing daughter nuclei within the pre-follicular tissue (Mayer's haemalum/eosin) × 860.

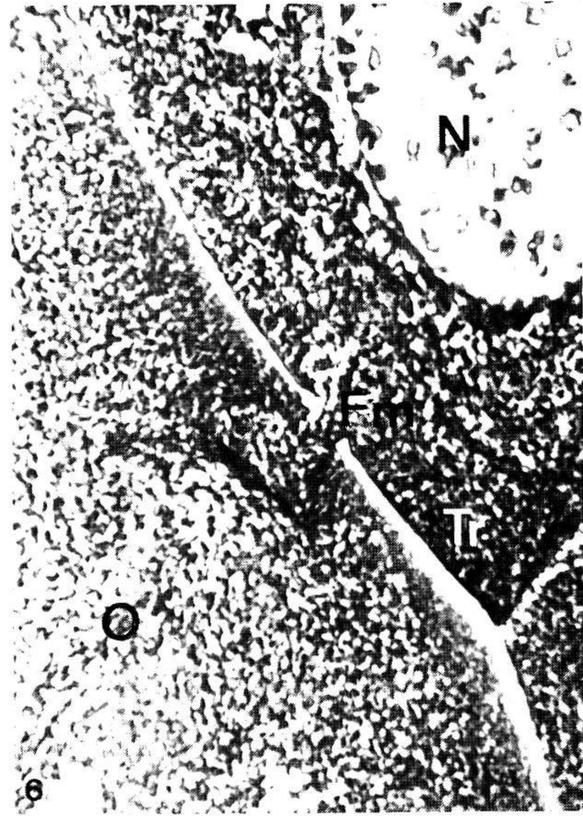
Fig. 2. Section of a young follicle, showing differentiated oocyte and trophocytes (Mayer's haemalum/eosin) × 620.

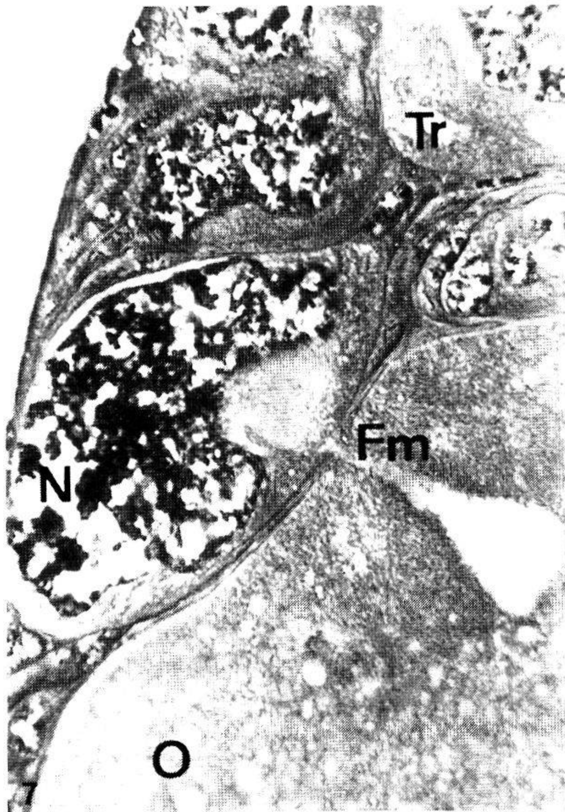
Fig. 3. Section of a follicle, showing 'accessory' nuclei (Feulgen reaction/light green) × 336.

Fig. 4. Section of an overy, showing a young and an old follicles. Note the trophocytes pushed anteriorly in the latter (Mayer's haemalum/eosin) × 130.

Fig. 5. Section of a portion of follicle, showing nucleolar 'emission' in the trophocytes (Ninhydrin-Schiff reaction) × 860.







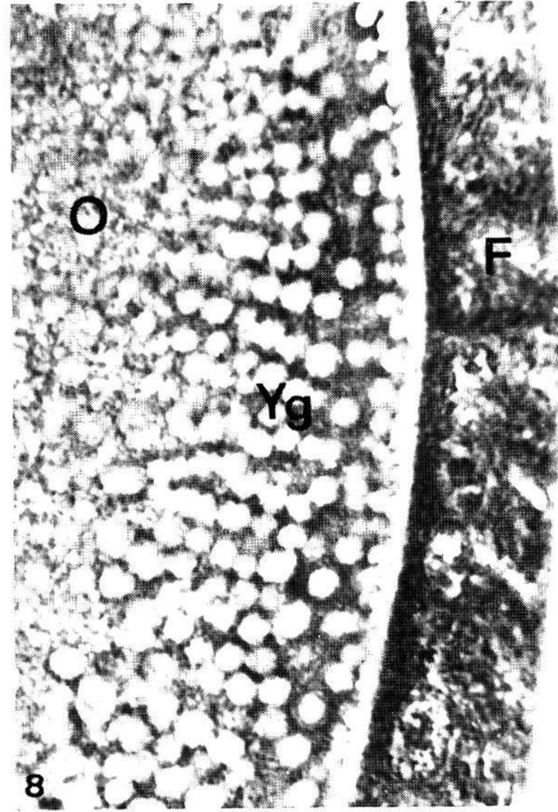


Fig. 6. Section of a portion of follicle, showing a fusome opening into the oocyte. Note the inflowing yolk (methyl-green-pyronin staining) × 1300.

Fig. 7. Section of a portion of old follicle. Note yolk deposition by the trophocyte (Ninhydrin-Schiff reaction) × 1070.

Fig. 8. Deposition of proteinaceous yolk globules by the follicular layer (Ninhydrin-Schiff reaction) × 1300.

Abbreviations: An = 'accessory' nuclei; Es = epithelial sheath; F = follicle layer; Fm = fusome; Fs = follicular syncytium; G = germarium; N = nucleus; Ne = nucleolar 'emission'; O = oocyte; Pf = prefollicular tissue; Tr = trophocyte; Yg = yolk globules.

material. This is possibly due to its dilution with the increasing volume of the oocyte.

The follicle layer stains intensely with ninhydrin-Schiff and bromophenol-blue particularly on the lateral aspects of their cells. These histochemical tests have also revealed the presence of numerous proteinaceous globules in the oocyte close to the follicle layer (Fig. 8). These globules apparently coalesce centrally to produce the proteinaceous yolk spheres. Thus, in the ovary of *Glossina* there are two entry routes of protein into the oocyte: one through fusomes from the trophocytes and the other from the follicle layer. The follicle layer and the deutoplasm adjacent to it also give positive reaction for RNA in the older follicle. It would seem that RNA is supplied by both the trophocytes as well as the follicular epithelium although the timing of its deposition apparently differs.

In the old oocyte histochemical test shows a narrow disc of RNA material at its extreme posterior end. It is conceivable that this material is concerned with the organizer activity of the future early embryo.

In the course of vitellogenesis the oocyte grows considerably and the trophocytes become gradually pushed anteriorly by the growing oocyte (Fig. 4), the nuclei of these nurse cells become ovoid or irregular in shape and the chromosomal material forms clumps (Fig. 7). Concurrently the follicle layer grows or migrates so that the oocyte gradually becomes cut off from the trophocytes by this layer. When yolk deposition is completed, the chorion is laid down by the follicle layer. Subsequently the trophocytes shrink and degenerate.

Discussion

The development of oocyte in *Glossina* may be divided into four stages: (i) An early growth period characterized by division of the germarial oogonium into a sixteen-cell group which becomes enveloped by prefollicular tissue. (ii) Cytological differentiation of the sixteen daughter cells into trophocytes and oocyte in preparation for yolk deposition. (iii) Vitellogenesis. And, (iv) degenerative changes of the trophocytes and formation of the chorion by the follicle layer.

The techniques employed in the present study can only yield qualitative information on certain aspects of vitellogenesis. It is clear that there is a considerable increase of DNA material in the trophocyte nuclei through endomitotic doublings in the course of follicle differentiation. It is interesting to note that DNA was not detected in the oocyte while in the trophocyte cytoplasm, only a few Feulgen-positive granules were observed close to the nuclear membrane. It is conceivable that DNA is depolymerized to some Feulgen-negative form before being transferred to the oocyte. Transfer of depolymerized DNA from trophocytes to the oocyte has been shown to occur in *Acanthocephala* (SCHRADER & LEUCHTENBERGER, 1952), *Oncopeltus* (BONHAG, 1955) and *Rhodnius* (VANDERBERG, 1963). In *Rhodnius prolixus* (VANDERBERG, 1963) the follicle cells also contribute DNA to the oocyte. Whether such a transfer occurs in *Glossina* could not be determined although the staining intensity for DNA increases in the course of follicle development. One can only speculate on the high turnover of DNA in the developing follicle. It seems unlikely that the genetic information is transferred to the oocyte by this process. It is possible that DNA reserve or its precursors function as a readily available source to supply the needs of the future developing embryo.

The observation that in *Glossina* both, the trophocytes and follicle epithelium function in the transfer of RNA to the growing oocyte is consistent with the results of KING & BURNETT (1959), ZALOKAR (1960) and SIRLIN & JACOB (1960) on the developing oocyte of *Drosophila*. In the present species RNA is synthesized in the trophocyte nuclei by the process of nucleolar emission. This RNA or its

precursors eventually stream into the oocyte via fusomes. The positive reaction also for protein possibly suggests that it is deposited into the oocyte in the form of ribonucleoprotein. Whether the RNA derived from the two different trophic tissues are of two functionally different classes, only further work can show.

TELFER (1961) has shown that in Saturniid moths yolk proteins are absorbed directly from the blood, reaching the oocyte by intercellular routes in the follicle layer. The presence of protein positive material in such sites suggests that a similar route for protein yolk uptake is also operative in the ovarioles of *Glossina.* There are thus two entry routes of protein into the developing oocyte; one from trophocytes via fusomes and the other through the intercellular spaces of the follicle layer. The deposition of protein yolk from two different trophic tissues has also been reported in *R. prolixus* (VANDERBERG, 1963) which differs from the present species in that the two processes occur at different stages in the life of the oocyte (PATCHIN & DAVEY, 1968).

Summary

Study of the histology and histochemistry of the developing ovariole of *G. morsitans* has been undertaken. One of the germarial oogonia undergoes four consecutive divisions to produce a primary follicle comprising sixteen daughter nuclei enveloped by follicular tissue. Further differentiation of this follicle results in an oocyte and fifteen trophocytes within a follicular epithelium. Intercommunication of cytoplasm of the sixteen-cell group is mediated by fusomes.

The trophocyte nuclei are remarkably active in DNA replication and RNA transcription. Both the trophocytes and the follicle layer function in the transfer of RNA and protein, and possibly also depolymerized DNA to the oocyte in the course of vitellogenesis. In the trophocytes, RNA and protein are synthesized by a process of nucleolar "emission" and eventually flow into the oocyte through fusomes. The transfer of protein from the follicle layer appears to occur through the intercellular routes. After the conclusion of vitellogenesis the follicular epithelium secrets the chorion and the trophocytes shrink and degenerate.

I wish to thank Mr. S. B. Kutuza for technical assistance and the Director, E.A.T.R.O. for permission to publish this work.

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