

Cleavage in *Ixodes ricinus* (L.) (Acari: Ixodidae)

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ABSTRACT. The author analyzed the first 5 days of embryonic development of *Ixodes ricinus*. The cleavage takes 4 days, being terminated on the 5th day of embryogenesis, when the cells start to invaginate and differentiate. The karyomeres play a role in the initial mitotic divisions. Cellularization occurs on the 2nd and 3rd day of embryogenesis. The blastoderm cells maintain their potential for division, and as from the 5th day cellular differentiation starts.

Key words: cellularization, cleavage, *Ixodes ricinus*, karyomere.

Introduction

In ticks the penetration of the oocyte sheath by sperm cells takes place very early, already in the reproductive system of the female [1], nevertheless karyogamy proceeds only after egg-laying [2]. This point is noted as the initiation of embryogenesis. It is assumed that embryonic development is similar to that of other *Acarina* [3]. The embryonic development was previously studied for certain tick species: *Boophilus annulatus* [4], *Ixodes ricinus* [5], *Ornithodoros moubata* [6], *Hyalomma dromedarii* [7]. Nevertheless there is limited data concerning the mechanisms of the individual stages of this process in ticks.

Ixodes ricinus is a vector for many pathogens, such as *Borrelia burgdorferi*, *Babesia microti*, the tick-borne encephalitis virus, and a number of others. Reportedly these pathogens can be transovarially and transspermally transmitted between generations [8].

It seems that analysis of ticks embryogenesis is important for examination of mechanisms of pathogens transmissions.

Materials and methods

The adult specimens of the *I. ricinus* were obtained from nature, in Katowice (Poland) area, using the flagging method. In the laboratory the females were allowed to feed on rabbits. They were kept in rearing chambers at 25˚C and 90–100% r. h, after detachment from rabbits. Eggs were laid in the rearing chambers. Successive deposits were collected daily, at a fixed time, and stored separately in identical rearing conditions. The fixing of a portion of each daily deposit was followed by the proposal of a series of developmental stages. The embryos of *I. ricinus* were examined between the 1st and 5th day of development.

Tick embryos were double-fixed in a 4% glutaraldehyde in cacodylate buffer (2h at 4˚C) solution and postfixed with cacodylate buffered 1% osmium tetroxide (2h at a room temperature) following the puncturing of the chorion. This was followed by dehydratation in a graded concentration of ethyl alcohol (50, 70, 80 90, 96 and absolute for 15' each) and propylenoxide (15'), and then the embryos were embedded in an epon mixture and sectioned on the ultramicrotome. Semithin sections were stained with methylene blue and examined under a light microscope. Ultrathin sections were stained with lead citrate and uranyl acetate and examined with a JAM 100c transmission electron microscope at 80 kV.

Results

The embryogenesis of *Ixodes ricinus* in the given rearing conditions lasts 30 days. The cleavage process takes place during the first 4 days. The first day after egg-laying is a time of marked changes

occurring within the embryo.

In the period just 2-3 hours after egg-laying, inside the embryo appear numerous minute structures consisting of karyoplasm surrounded by nuclear lamina (Fig. 1). These nuclear-like structures, called karyomeres, are located within narrow strands of cytoplasm amongst the yolk spheres, which at this developmental stage preserve a regular shape.

The fusion of karyomeres was observed during first 6 hours. Four pericentric groups of karyomeres joined with bridges were visible at the end of this stage (Fig. 2). Further processes of karyokinesis occur very rapidly. The number of nuclei increases as early as the first day; this is accompanied by their translocation towards the surface. The nuclei together with surrounding cytoplasm form the blastoderm, this occurs already in the second day of embryogenesis (Fig. 4).

In addition to the proliferation of nuclei and gradual formation of the periblast, further marked changes occur in the cytoplasm on the first day of development. During the first 6 hours there is a rapid increase in the number of: endoplasmic reticulum tubules, dictiosomes of Golgi complex and

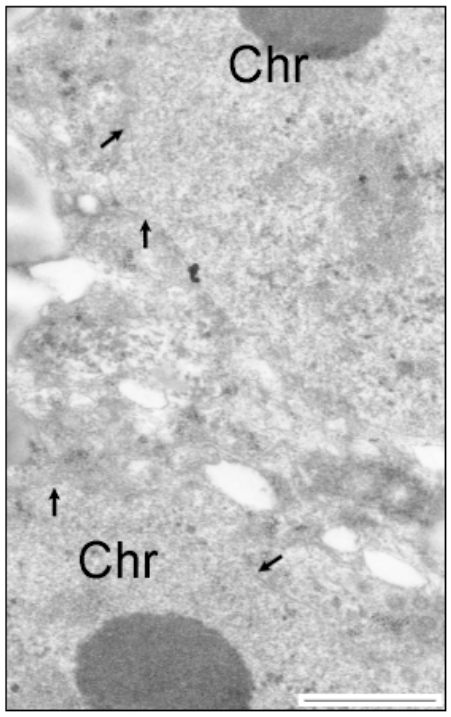

Fig.1 Transmission electron micrograph of section through 3rd-hr old embryo. Fragments of two karyomeres. Chromatin (Chr) covered by nuclear envelope (arrows). Bar: 1 μm

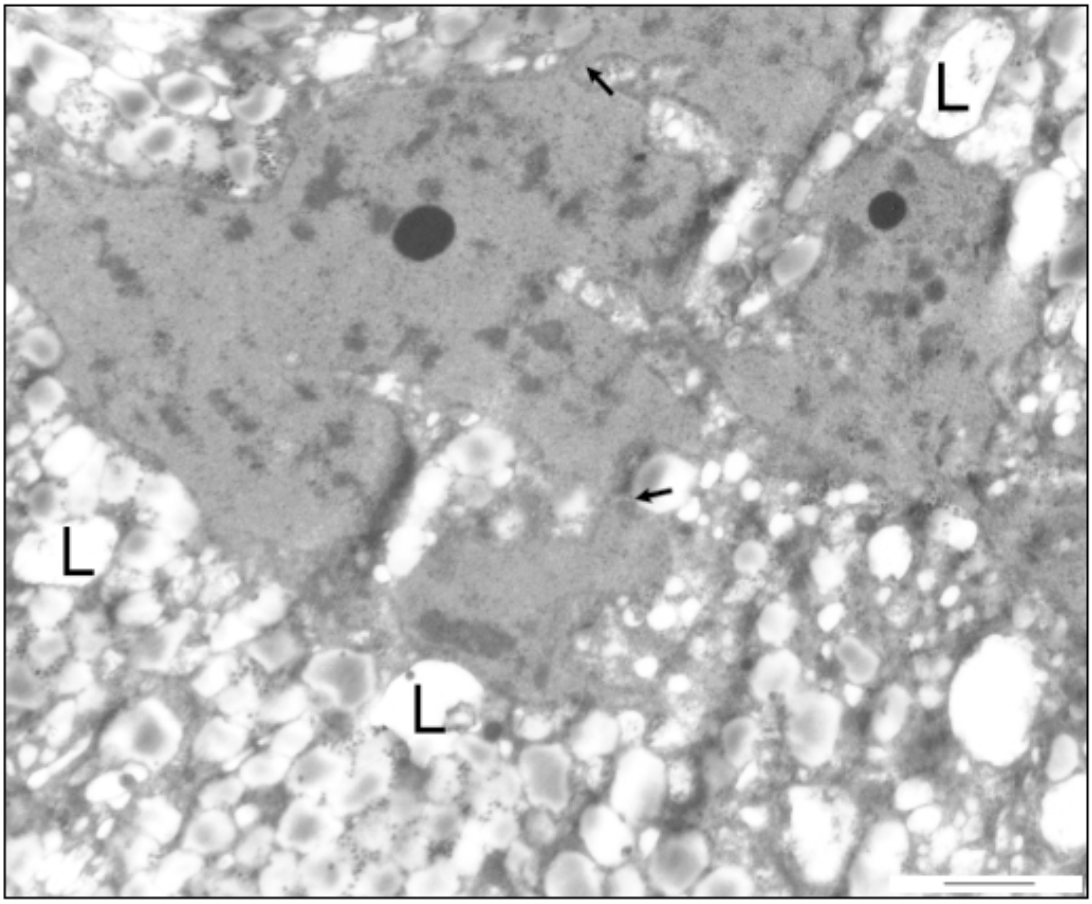

Fig.2 Transmission electron micrograph of section through 6th-hr old embryo. A group of karyomeres joined with bridges (arrows). (L) lipid droplets. Bar: 2 μm

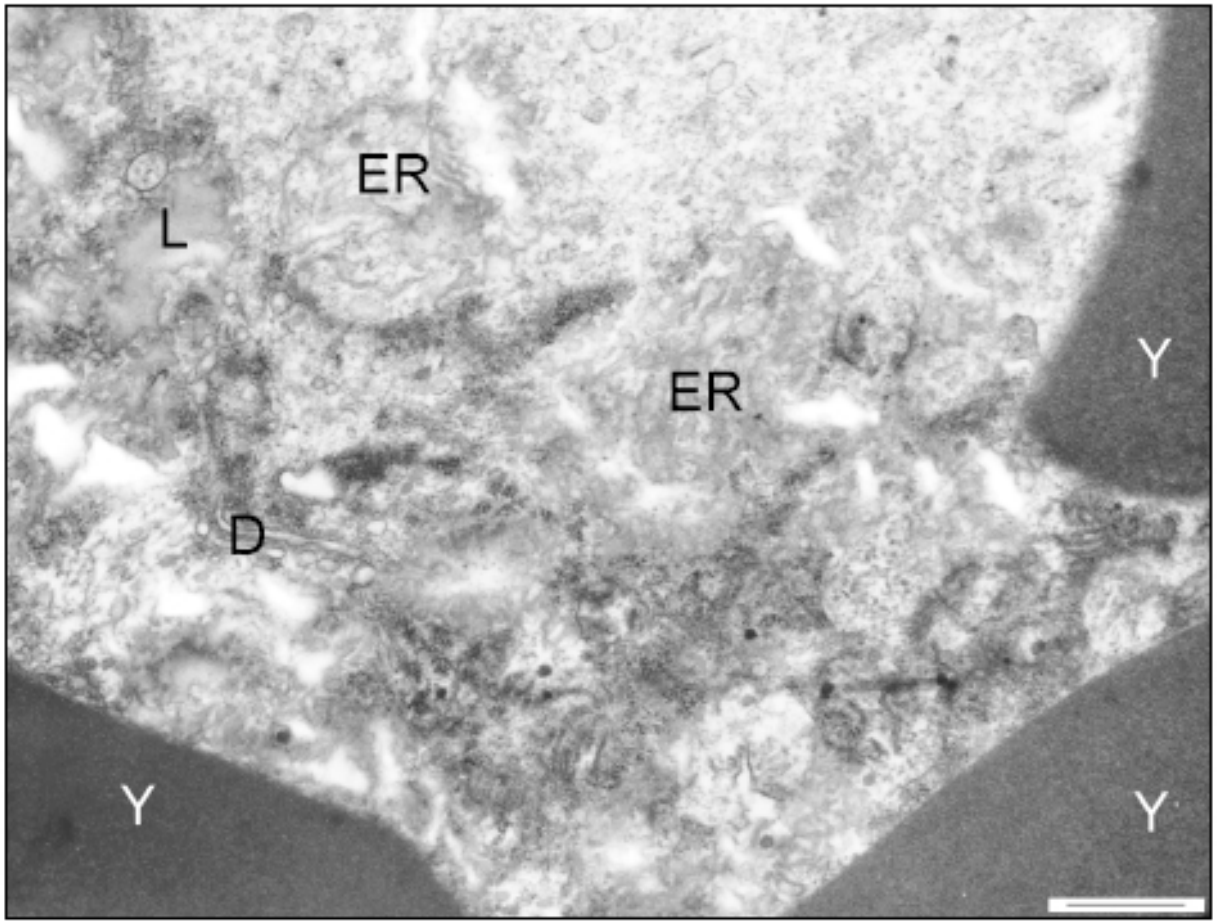

Fig.3 Transmission electron micrograph of 4th-hr old embryo. (D) dictiosome of Golgi complex, (ER) endoplasmic reticulum tubule, (L) lipid droplet, (N) nucleus, (Y) yolk. Bar: 1 μm

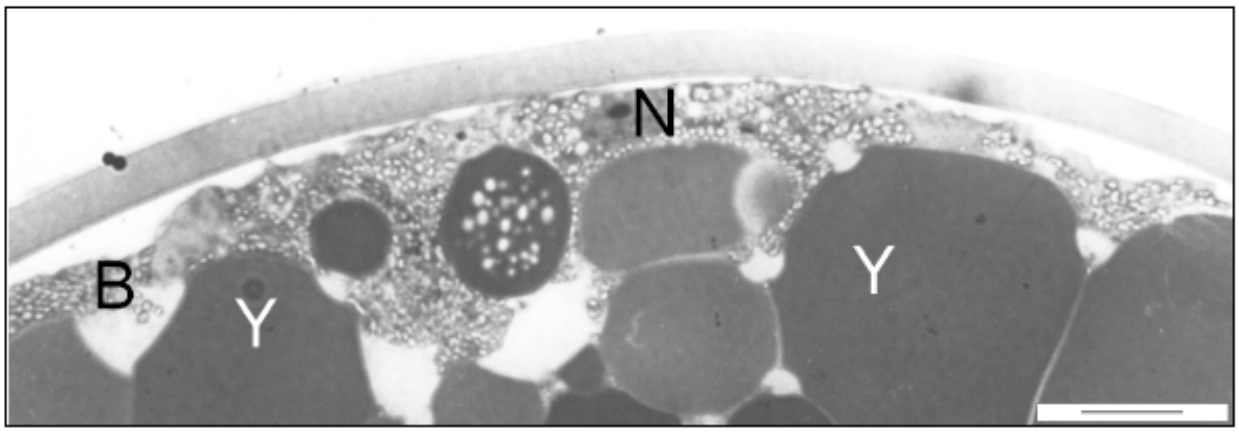

Fig.4 Photomicrograph of section through 2nd-day old embryo. (B) blastoderm, (Y) yolk. Bar: 10 μm

minute vesicles of varying electron density (Fig. 3). Characteristic of this stage is that tubules of endoplasmic reticulum are elongated, twisted and contain much electron-dense substance. Concurrently during first 6 hours of embryogenesis in the cytoplasm there is a gradual increase in storage materials, namely: lipid droplets and glycogen granules. Significant amounts of the mentioned deposits are located in the cytoplasm surrounding the joined karyomeres (Fig. 2). At this point the yolk spheres becomes irregular in shape.

The author mentioned, that on the 2nd day of the

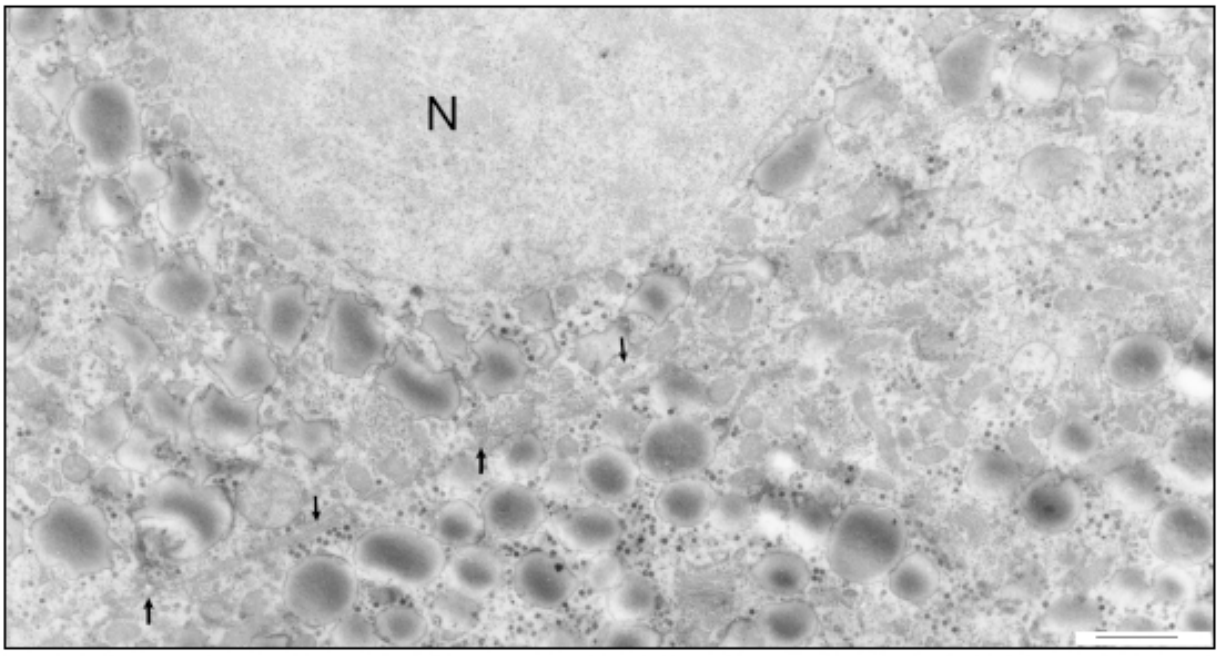

Fig.5 Transmission electron micrograph of section through 2nd-day embryo. Membranes join to form the plasmalemma (arrows), (N) nucleus. Bar: 1 μm

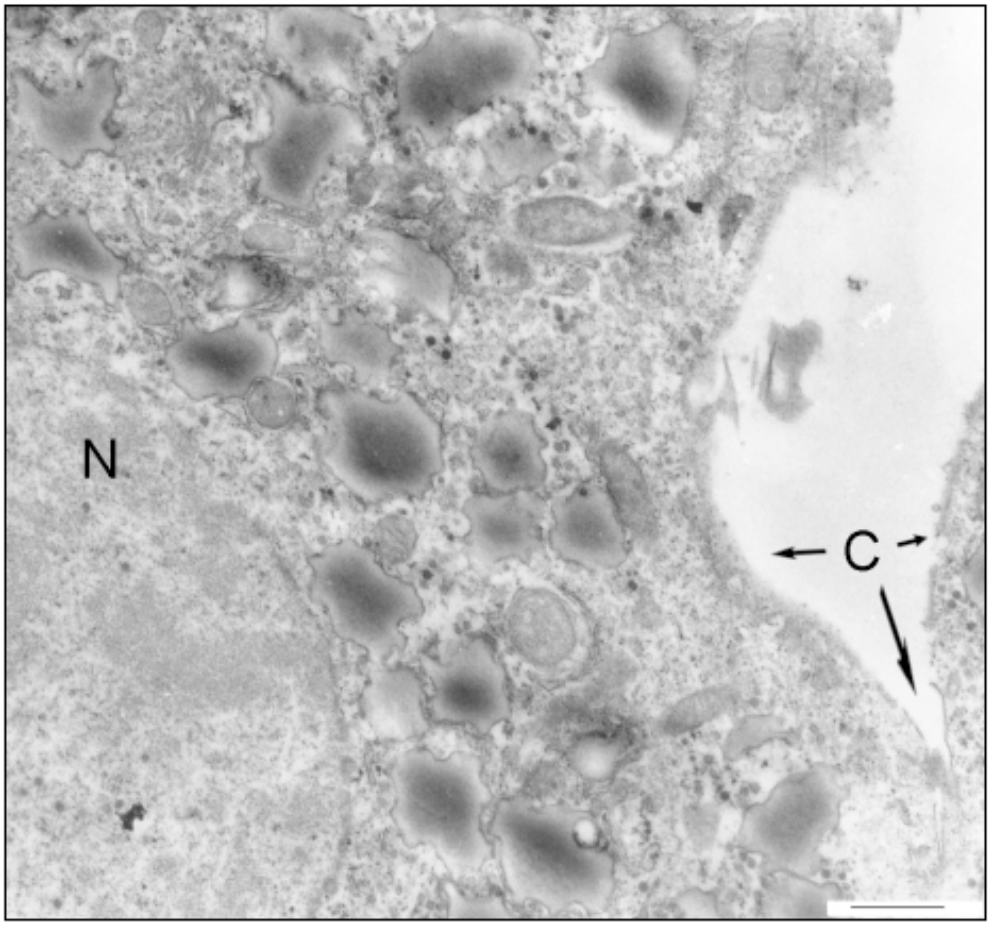

Fig.6 Transmission electron micrograph of section through 2nd-day embryo. Cleavage (C) on the external side between the forming blastomeres, (N) nucleus. Bar: 1 μm

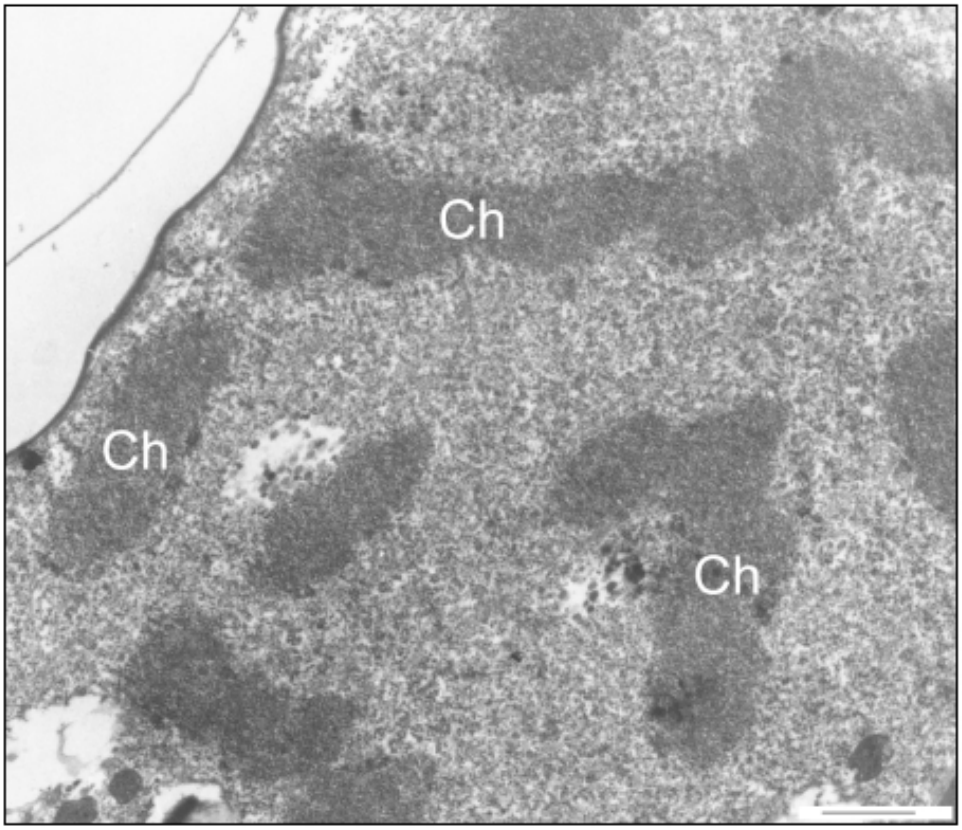

Fig.7 Transmission electron micrograph of section through 2nd-day embryo. (Ch) Condensed chromosomes. Bar: 1 μm

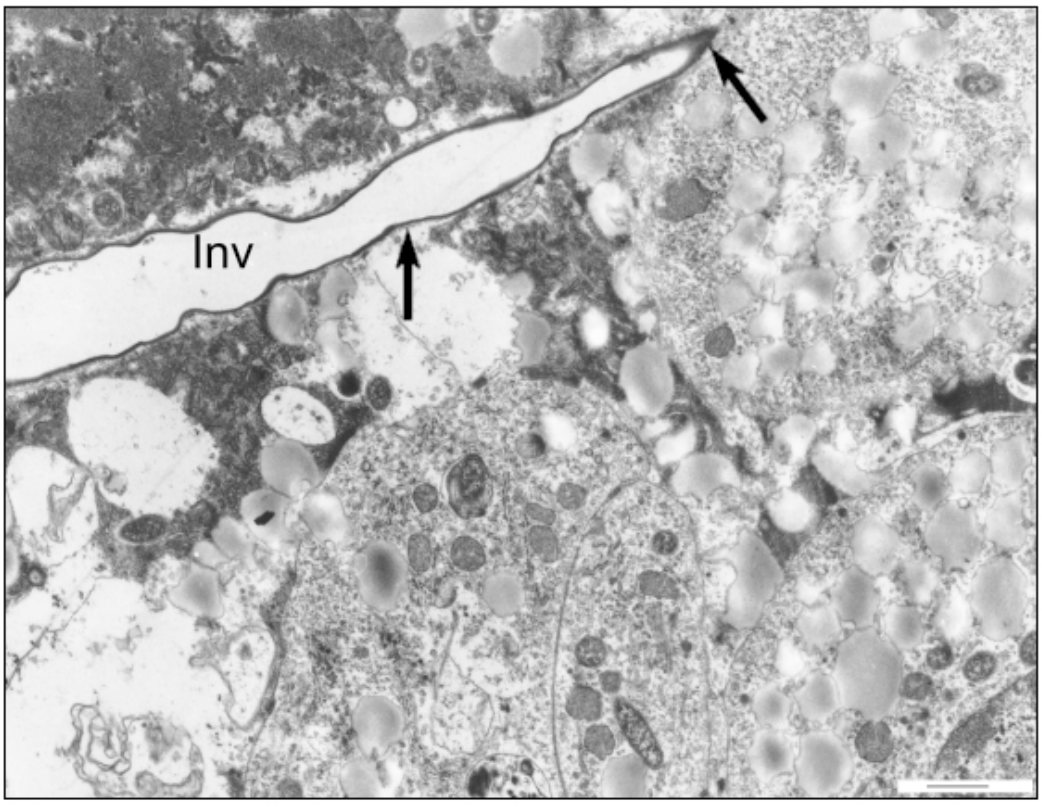

Fig.8 Transmission electron micrograph of section through 5th-day embryo. Cellular surface at the points of invagination (Inv) is thicker and strongly osmophilic (arrows). Bar: 1 μm

embryonic development — certain nuclei together with cytoplasm are located on the surface, this primal blastoderm (Fig.4) is not yet divided into individual cells. Nevertheless fragments of endoplasmic reticulum tubule membranes and minute vesicles with a linear arrangement appear in the cytoplasm, amongst the nuclei on the 2nd and 3rd day (Fig. 5). In time these membranes join to form the plasmalemma. Cytokinesis is accompanied by a simultaneous creation of cleavages on the external side between the forming blastomeres (Fig.6). Following the formation of blastoderm, the cells remain loaded with significant amounts of storage materials, however decreasing during further development. The cells maintain a potential to divide, therefore condensed chromosomes are observed within the mitotically dividing cells (Fig. 7). In certain parts of the embryo (already on the 5th day after egg-laying) the proliferation is responsible for the formation of an increased number of cell layers and their invagination. The cellular surface at the points of invagination is significantly thicker and strongly osmophilic (Fig. 8). These changes lead to a further differentiation of cells, and to the next stage of embryogenesis — gastrulation.

Discussion

Based on the published data, the process of embryogenesis in ticks follows a pattern similar to that of other arachnids [2]. The cleavage is partial and superficial, as in the majority of Acarina [3]. In *Ixodes ricinus*, as in remaining arthropods, the nuclei divide intensively and their numbers increase during the first stage of embryogenesis [9]. As described in these studies, the karyomeres take part in initial mitotic divisions. This specific and very economic method of karyokinesis during the early stages of cleavage is noted in a number of various invertebrates [10] and vertebrates [11]. It is interpreted as a method that speeds up the cell cycle by a marked shortening of the S phase, and mitosis with the elimination of G1 and G2 phases [11]. The cell cycle is again composed of all the phases, only after karyomere fusion and the restitution of a normal nucleus. Formation and fusion of karyomeres is accompanied by the growth of membranes of the endoplasmic reticulum and of the Golgi complex. This phenomenon in *I. ricinus* probably constitutes a preliminary phase for the further stages of cleavage, i.e. the formation of significant numbers of nuclear sheaths and of blastomere plasmalemma. Changes in yolk structure and accumulation of storage materials in cytoplasm are preceded by an increased activity of the endoplasmic reticulum and dictiosomes, at this stage of development it indicates the initiation of enzymatic mechanism associated with yolk digestion. The onset of this process is therefore early. A similar very early transformation of yolk into dispersed droplets has been described for *Hyalomma dromedarii* [12]. In *I. ricinus* the cellularization, and blastoderm formation starts on 1 st day of embryogenesis. The formation of cellular membranes from endoplasmic reticulum tubules and minute vesicles is a widespread phenomenon amongst other arthropods, such as *Drosophila melanogaster* [13], or *Calliphora erythrocephala* [14].

The definite moment of conclusion in cellularization in *I. ricinus* is difficult to precisely ascertain. In a 2-day-old embryo there are visible membranes separating individual cells, although in numerous locations they are not continuous. The perinuclear cytoplasm was always surrounded with a plasmalemma in 16-blastomere phase, as described for *Achaearanea japonica* [15], however for the *Achaearanea tepidariorum* this point could not be precisely stated [16]. The time of blastoderm formation varies in different tick species. For example: in the *Hyalomma dromedarii* this process takes almost half of the entire time of embryogenesis [7], whereas in the *Ornithodoros moubata* only one fifth. [8]. Therefore in *I. ricinus* this stage occurs proportionally faster. On the fifth day of embryogenesis the blastomeres are not only surrounded with a plasmalemma, but additionally start differentiating. The observed thickening of the external plasmalemma at the points of invagination is also noted during further stages of embryogenesis. As was described in other studies, during later stages of development a significant amount of an amorphous substance is formed, which amplifies the segregation of cells [17, 18]. This substance is deposited in between the observed osmophilic layer and the cellular membrane of the blastomeres. This results in a condensing of the thickened fragments of membrane, leading to the formation of characteristic seams, which delineate the shape of originating surface organs.

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