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Nuclear vlimata and aneuploidy in embryonic cells is caused by meiosis. Behaviour and properties of meiotic cells

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Summary. This study demonstrates that human embryonic cells divide by meiosis. The use of trophoblastic tissue cells (early embryo) and amniotic cells (late embryo) exhibited the following characteristic events of meiosis: nuclear (NVs) and nucleolar (NuVs) vlimata formation; NV invasion in host cells; extrusion of chromosomes; nuclear fusion; metaphase fusion; hybrid cell formation; nuclear, nucleolar and cytoplasmic bridges, chromosomal transfer, variable-sized nuclei; nuclear fragmentation; condensed meiotic chromosomes; "0" chromosome; and aneuploidy. Two types of nuclear bridges (NBs) were identified and defined as communicative tubules through which chromosomal transfer among cells is achieved. The wall of NBs is an extension of the nuclear membrane and the lumen contained chromosomal fusion substance (CFS). Embryonic cells formed glycosaminoglycan-sacs (GSG-sacs) and rivulets, forming a cytoplasmic communicative system. The extracellular matrix (ECM), GSG-sacs and CFS were composed of glycosaminoglycan-bound protease. The protease which immuno-crossreacted with the a_1-chymotrypsin antiserum was the meiotic calcium-activated neutral proteinase (CANP). Cytogenetic analysis of early embryonic cells showed higher ratio of aneuploidy:diploidy than late embryonic cells. The results are discussed in terms of differentiation-mitosis and undifferentiation-meiosis. These observations lead to an embryonic cell life cycle identical to that of malignant cells as follows:

$$\text{Zygote} \xrightarrow{\text{meiosis}} \text{NVs and NuVs} \xrightarrow{\text{invasion}} \text{recipient host}$$

$$\text{cells} \xrightarrow[\text{metaphase fusion}]{\text{nuclear fusion}} \text{hybrid cells} \rightarrow \text{organogenesis}$$

$$\rightarrow \text{mitosis}$$

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Introduction

It has recently been documented that lymphocytic and malignant nuclear vlimata (NVs) are produced by meiosis and carry aneuploid sets of chromosomes (Logothetou-Rella, 1994a,b). Meiosis of malignant cells involves NV production, NV invasion into host cells, presence of meiotic chromosomes, extrusion of chromosomes, cell, nuclear and metaphase fusion leading to the life cycle of malignant cells as follows:

$$\text{Malignant cells} \xrightarrow{\text{meiosis}} \text{NVs} \xrightarrow{\text{invasion}} \text{recipient host}$$

$$\text{cells} \xrightarrow[\text{metaphase fusion}]{\text{nuclear fusion}} \text{hybrid cells} \xrightarrow{\text{meiosis}} \text{NV formation}$$

The malignant cell life cycle was found to be sensitive to the inhibitor (CANP-I) of calcium-activated neutral proteinase (CANP), a proteinase associated with meiosis (Logothetou-Rella, 1994c). Furthermore, the in vivo spermicidal action of CANP-I, strengthened the antimeiotic action of this inhibitor (Logothetou-Rella, 1995).

A specific extracellular matrix (ECM) of glycosaminoglycan (GSG)-CANP has been identified as common in cells dividing by meiosis (Logothetou-Rella, 1993,1995). This ECM is produced by cell to cell interaction and invasion (Logothetou-Rella, 1994d).

NVs and the GSG-CANP ECM have also been observed in embryonic cells (Logothetou-Rella et al., 1989; Logothetou-Rella, 1993) which are sensitive to CANP-I (Logothetou-Rella, 1994c). Since malignant cells resemble embryonic cells, it is necessary to examine embryonic cell division.

In this study, meiotic division is investigated in

growing human amniotic cells and trophoblastic tissue cells by cytogenetic morphology and analysis using a) the in situ cytogenetic technique, and b) the chromosomal spreading technique. Moreover the behaviour and properties of embryonic meiotic cells are examined.

Materials and methods

Cell cultures

Amniotic fluids collected for prenatal diagnosis, from 14- to 17-week pregnant women, were donated by Dr. A. Metaxotou. Amniotic cells from 10 women were cultivated in RPMI-1640 (Gibco), supplemented with 10% foetal bovine serum (Seromed), penicillin (Seromed, 100 U/ml), and streptomycin (Seromed, 100 µg/ml) and incubated at 37 ºC in CO_2-humidified incubator.

Cytogenetic analysis, morphology, cytology and immunocytochemistry

Cytogenetic analyses of 20-day-old male amniotic cell cultures, were performed by the chromosomal spreading technique, standard Giemsa, RhG banding of Dutrillaux and Lejeune (1971) and in situ by standard Giemsa. For the in situ technique, amniotic cells were cultivated in glass petri-dishes, treated with hypotonic solution (KCl 0.075 M) for 15 min, fixed in 3:1 ethanol:acetic acid, dried and stained with Giemsa (HT-Giemsa).

Trophoblastic tissues from 10 women of 9 to 11 weeks of pregnancy were collected for prenatal diagnosis. Trophoblastic tissues were treated with 1% trisodium citrate for 10 min, fixed in 3:1 ethanol:acetic acid for 24 hours, followed by 6:4 acetic acid:distilled water treatment for 5 min, smeared on glass slides and stained with Giemsa. One to two hundred consecutive metaphases were analyzed as previously evaluated (Logothetou-Rella, 1994a).

Chromosomal preparations, besides karyotyping, were also evaluated for cytogenetic morphology, such as condensed vs regular chromosomes. NV and nuclear morphology, intensity of Giemsa chromosomal staining, nuclear fragmentation, and nuclear metaphase fusion.

Amniotic cell cultures, after 10 min hypotonic treatment, were either fixed in 3:1 ethanol:acetic acid for 45 min, rinsed with distilled water and stained with Papanicolaou (HT-Papa), or fixed in 4% formaldehyde in phosphate-buffered saline (PBS) for PAS (HT-PAS), PAS-diastase staining (HT-PAS-D) and immunocytochemistry.

For immunocytochemical studies the avidin-biotin peroxidase complex method was applied (Hsu et al., 1981) using the antiserum against a_1-chymotrypsin (1:100, A022 Dako corp.) (HT-a_1-chymotrypsin). Positive controls were tested for a_1-antichymotrypsin reactivity. Negative controls were made by omitting the primary antiserum.

Amniotic cells prepared by the chromosomal spreading technique were fixed with 4% formaldehyde in PBS (instead of 3:1 alcohol:acetic acid), spread on slides, and used for PAS, PAS-diastase staining and immunocytochemistry .

Results

Cytology and cytogenetic morphology

Amniotic cell cultures consisted of mononucleated

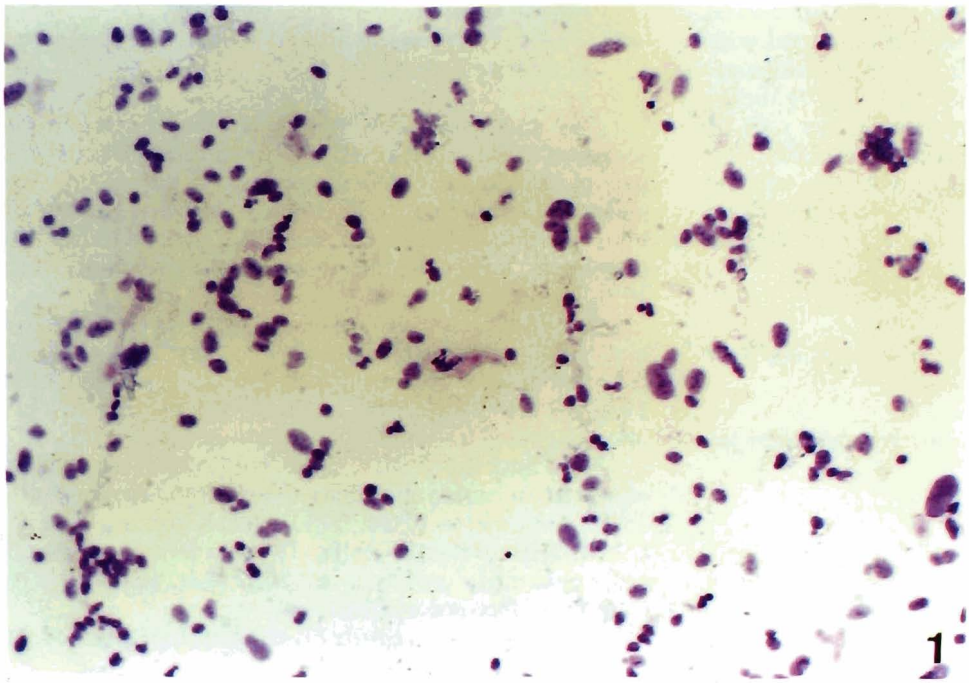


Fig. 1. Variable size and morphology of amniotic cell culture. HT-Giemsa. x 100

and multinucleated cells with abundant cytoplasm and variable-sized nuclei, ranging from very small to huge, and irregular (Fig. 1). NVs containing and protruding nucleoli (Fig. 2a,b) were observed free, or invading the cytoplasm and/or nucleus of host cells (Fig. 2c). NVs were of different head and tail size, containing eosinophilic nucleoli of a round or rod shape (Fig. 2). Nucleoli produced and extruded NVs in the form of pyknotic buds with a short tail (Fig. 3a-c). Nucleolar NVs were observed invading the cytoplasm (Fig. 3d-g), or nucleus of host cells (Fig. 3h,i), or NV nucleus (Fig. 3j). The nucleus and nucleolus of amniotic cells underwent elongation and then asymmetrical fragmentation, remaining within the same cytoplasm (Fig. 3k).

Cytogenetic morphology exhibited amniotic NVs of condensed pyknotic head (Fig. 4a-d) or extruding chromosomes (Fig. 4e,f), containing aneuploid sets of condensed chromosomes (Fig. 4g-j), or regular chromosomes in the head with condensed hyperchromatic ones along the tail (Fig. 4k) or condensed chromatin (Fig. 4l). Some NV metaphases showed ring morphology with a short tail (Fig. 4i). Trophoblastic NV chromosomes were condensed and curly (Fig. 4m). Amniotic NV metaphases were observed in host cell cytoplasm or attached onto (Fig. 5) or embracing the host cell nucleus (Fig. 5a,b). Host cell nuclei were undergoing meiosis (extrusion of chromosomes) while the host cell cytoplasm was invaded by NV metaphase (Fig. 5c,d). Nuclei extruding regular or condensed hyperchromatic, aneuploid or diploid sets of chromosomes were observed in amniotic (Fig. 6a,b) and trophoblastic tissue cells (Fig. 6c-e). Meiotic "0" chromosome was present in many trophoblastic metaphases of male sex (Fig. 6c-e) and in amniotic ones of female sex (Fig. 7a). Other groups of meiotic chromosomes, identical to those of a mature human oocyte in meiotic metaphase II (Plachot et al., 1987a) were identified in amniotic cells (Fig. 7b,c). Some amniotic cell cultures showed NVs carring pachytene chromosomes of the first meiotic division (Fig. 7e) and various sized nuclear conglomerates extruding pachytene, condensed or regular chromosomes (Fig. 7e). Chromosomal sets away from a metaphase, shown by

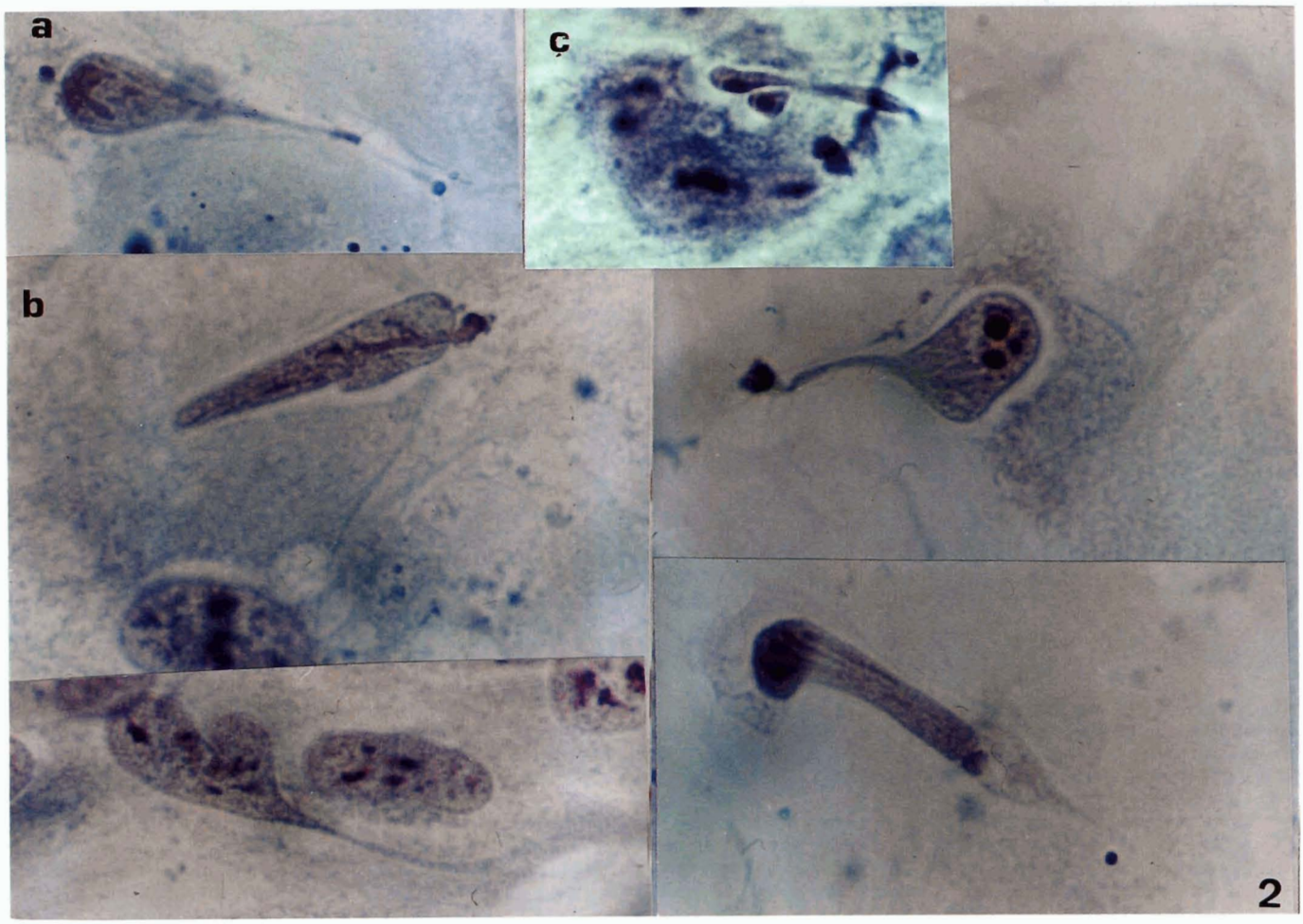


Fig. 2. Amniotic NV cytology. **Inset a,b:** NVs protruding eosinophilic nucleoli. **Inset c:** NV invading the nucleus of host cells. Papanicolaou. x 1,000

the in situ technique, belonged to an NV, since this technique does not involve chromosomal scattering and artefacts assumed to occur by the chromosomal spreading technique (Fig. 7b). Aneuploid and diploid meiotic metaphases were present in trophoblastic tissue cells (Fig. 6d,e) and amniotic cells (Fig. 6a,b, 7a,d). Symmetrical and asymmetrical meiotic division was observed within the same amniotic cell culture (Fig. 8). A cell division although of symmetrical daughter cells was characterized meiotic due the presence of NB (Fig. 8). Some cells exhibited NBs (Fig. 8a,c) containing intact or broken chromosomes at meiotic telophase (Fig. 8c). Ring metaphses formed by extrusion of chromosomes were meiotic (Fig. 8d).

Metaphase fusion occurred in both trophoblastic tissue cells (Fig. 9) and amniotic cells (Fig. 9a). Two to four nuclei extruded chromosomes simultaneously, giving rise to hybrid metaphases (Fig. 9). Metaphase fusion was abundant in trophoblastic tissue cells.

Amniotic cells showed nuclear fusion of two or more nuclei accomplished via nuclear projections (Fig. 10a,b), resulting in huge partly-vacuolated nuclei (Fig. 10c,d), which then produced NVs (Fig. 10d). During nuclear fusion one of the nuclei was simultaneously producing an NV metaphase (Fig. 10e).

Transfer and attachment of condensed hyper-

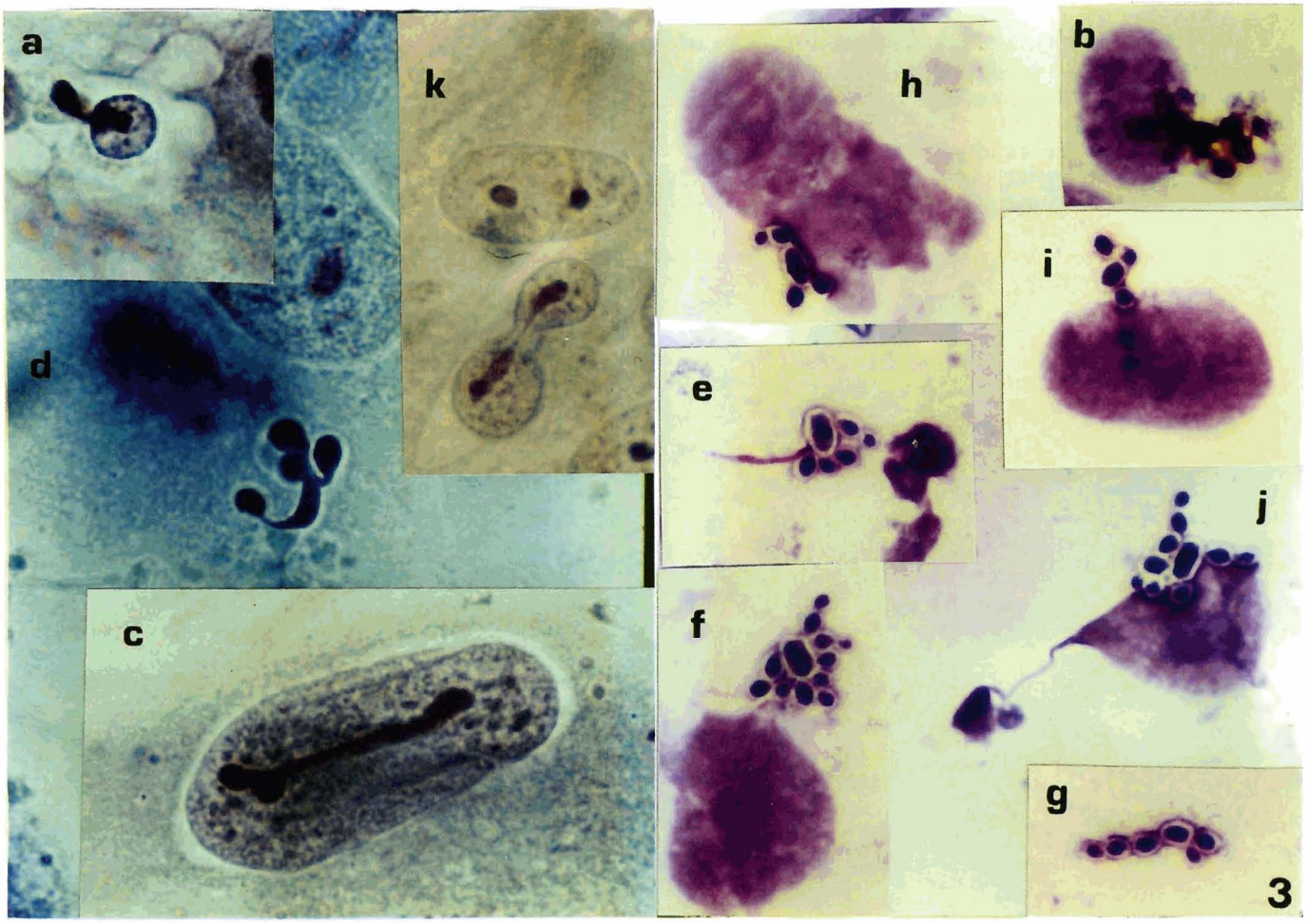
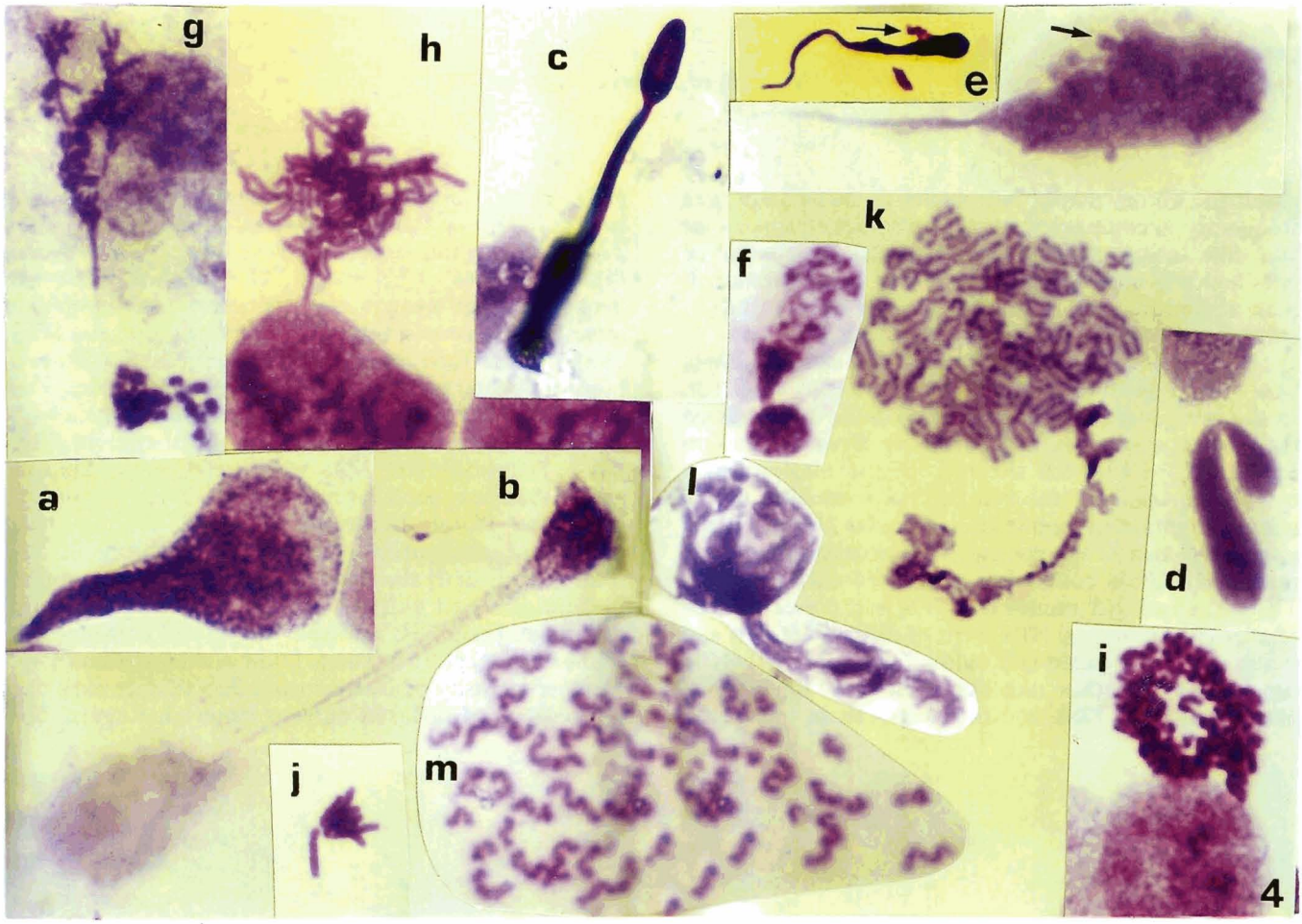


Fig. 3. Amniotic nucleolar NVs. **Inset a:** Papanicolaou. x 1,000. **Inset b:** HT-Giemsa. x 1,000. **Inset c:** Papanicolaou. x 1,000. **Inset d:** Nucleolar NVs invading the cytoplasm of host cell. Papanicolaou. x 1,000. **Insets e, f, g:** Same subject as d. HT-Giemsa. x 1,000. **Insets h, I:** Nucleolar NVs invading the nucleus of host cells. HT-Giemsa. x 1,000. **Inset j:** Nucleolar NVs invading an NV nucleus. HT-Giemsa. x 1,000. **Inset k:** Asymmetrical nucleolar and nuclear fragmentation of amniotic cell. Papanicolaou. x 1,000

Fig. 4. Cytogenetic morphology of amniotic NVs **Insets a, b, c, d:** Condensed head NVs. **Insets e, f:** NVs extruding chromosomes (arrow). **Insets g, h, I, j:** NVs containing condensed aneuploid sets of chromosomes. **Inset K:** NV with regular chromosomes in the head and condensed ones along the tail. **Inset e:** NV head of condensed chromatin. **Inset m:** Condensed, curly, meiotic chromosomes of a trophoblastic NV. HT-Giemsa. x 1,000

Fig. 5. Amniotic NV metaphases in the cytoplasm of host cells. **Insets a,b:** NV metaphases embracing host cell nucleus. **Insets c,d:** Host cell nucleus undergoes meiosis (extrudes chromosomes) while the host cell cytoplasm is invaded by an NV metaphase. HT-Giemsa. x 1,000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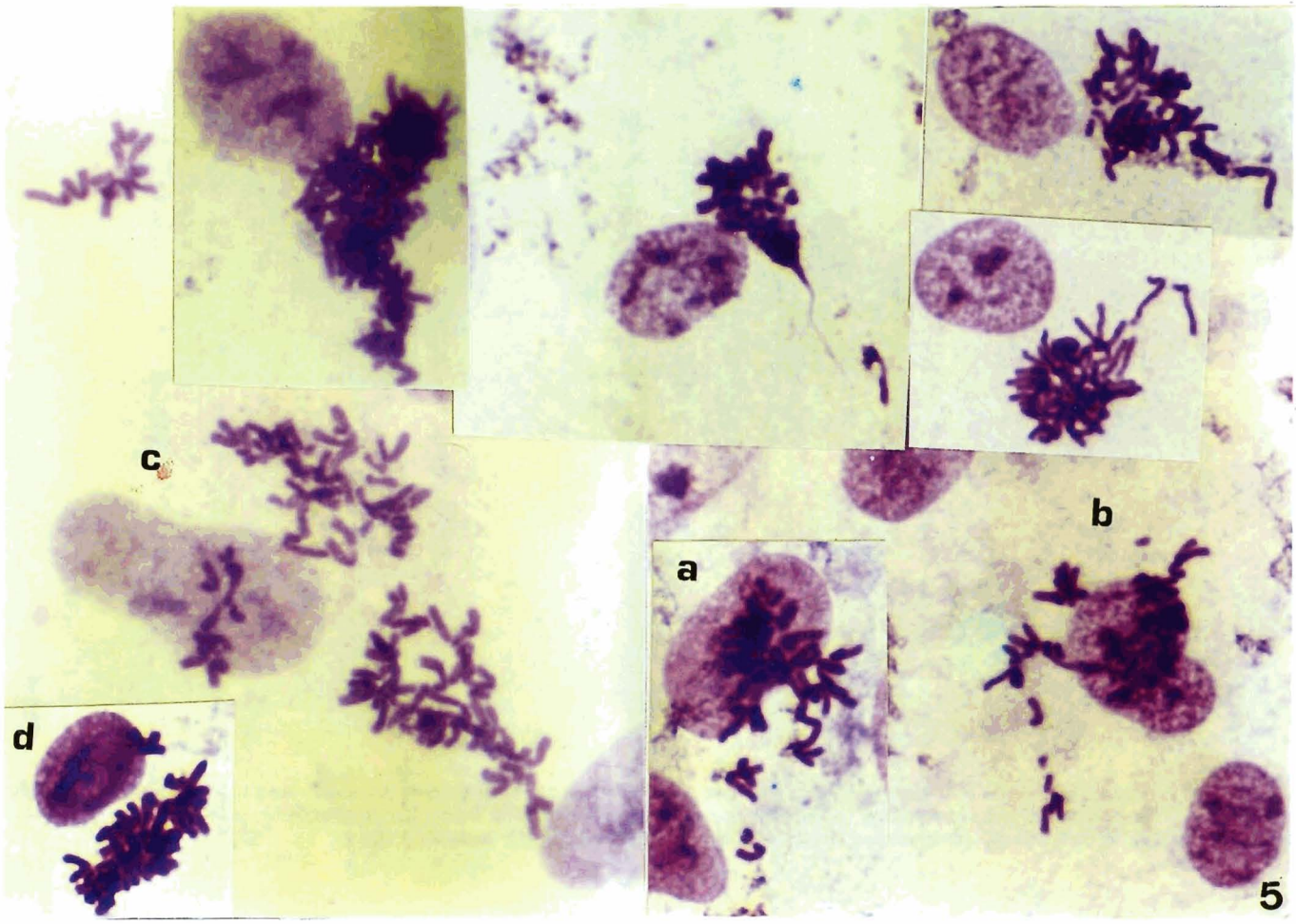
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chromatic chromosomes into nuclei or metaphases was frequently accompanied by a glassy, semitransparent, jelly-like substance. This substance was the content of NBs and was attached onto condensed chromosomes. It was abundant between two nuclei and acted as chromosomal fusion substance (CFS) (Fig. 11).

Internuclear passage of condensed chromosomes was very frequent in trophoblastic tissue cells (Fig. 12). In situ cytogenetic morphology of amniotic cells showed the presence of intact NBs running from nucleus to nucleus (Fig. 13). NB showed the morphology of a tubule. The wall of the NB was an extension of the nuclear membrane maintaining similar Giemsa stain intensity to that of the nucleus, with occasional stronger intensity at areas containing condensed chromosomes. The lumen of NB contained CFS and condensed or regular chromosomes. NBs were of different length and width within the same cell culture (Figs. 13, 14). NBs ran between mother and daughter cell, during NV production (Fig. 13a), and between cells in interphase (Fig. 13). Transfer of chromosomes from nucleus to nucleus via the NBs was obvious (Fig. 13b). Trophoblastic tissue cells showed long NBs between different types of cells (Fig. 14). NB was apparent between a small pyknotic hyperchromatic nucleus and a large hypochromatic with dispersed fine chromatin (Fig. 14a,b). More than one NB was observed between two hyperchromatic pyknotic small trophoblastic nuclei (Fig. 14b). Short NB appeared as hypochromatic vacuole with hyperchromatic condensed chromosomal content (Fig. 14e). Trophoblastic NBs were often embedded in accumulated extracellular matrix (Figs. 14c, 15). Broken NBs, after 15 min hypotonic treatment, exposed their content of condensed hyperchromatic chromosomes (Fig. 15a). Exactly the same observations were identified in amniotic cell cultures by the in situ cytogenetic technique. Long NBs ran from cell to cell embedded in ECM (Figs. 16, 17). Thick ECM accumulations kept stronger intensity of Giemsa stain than thinner ones due to presence of diffused nuclear material. Regular NV

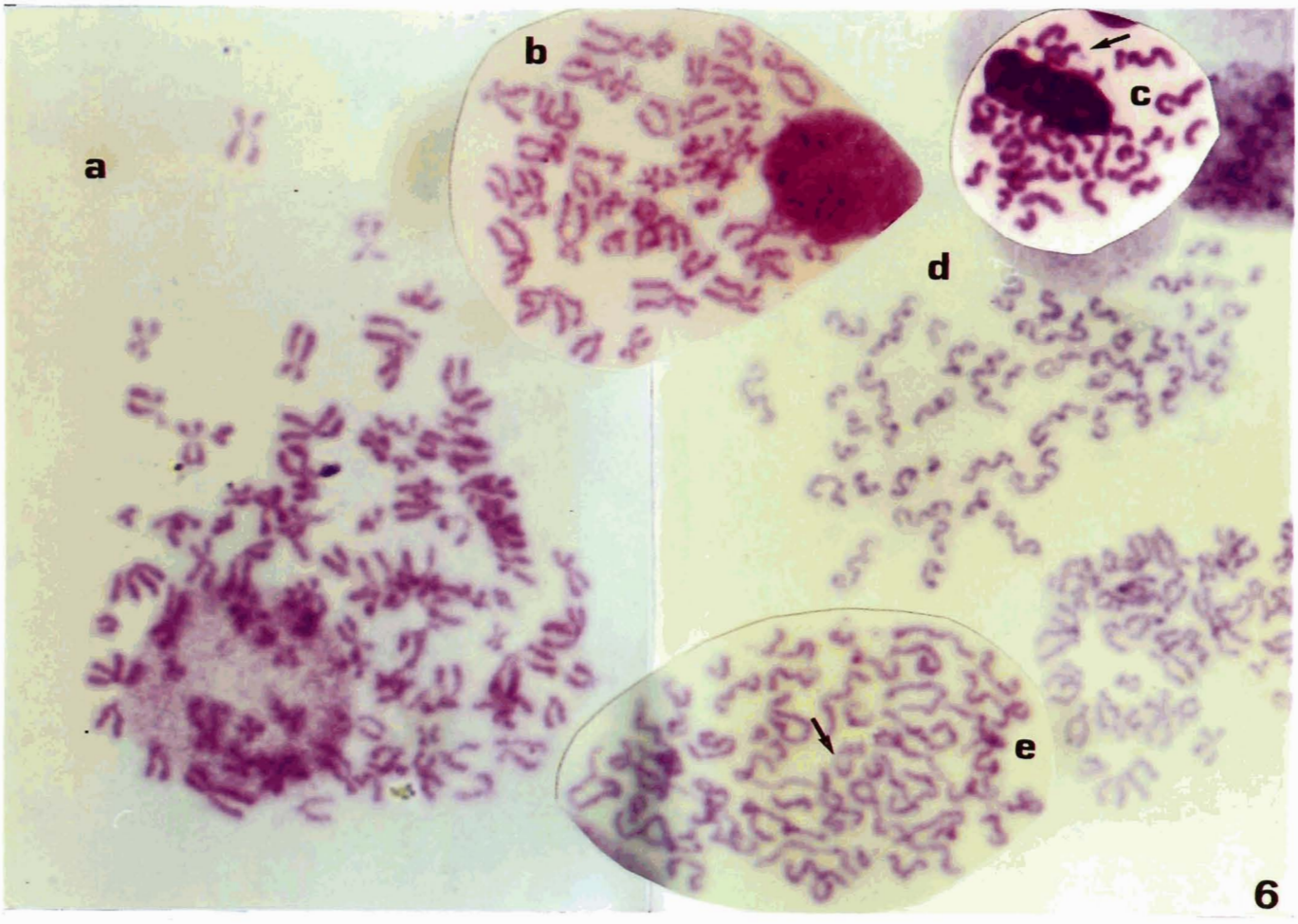
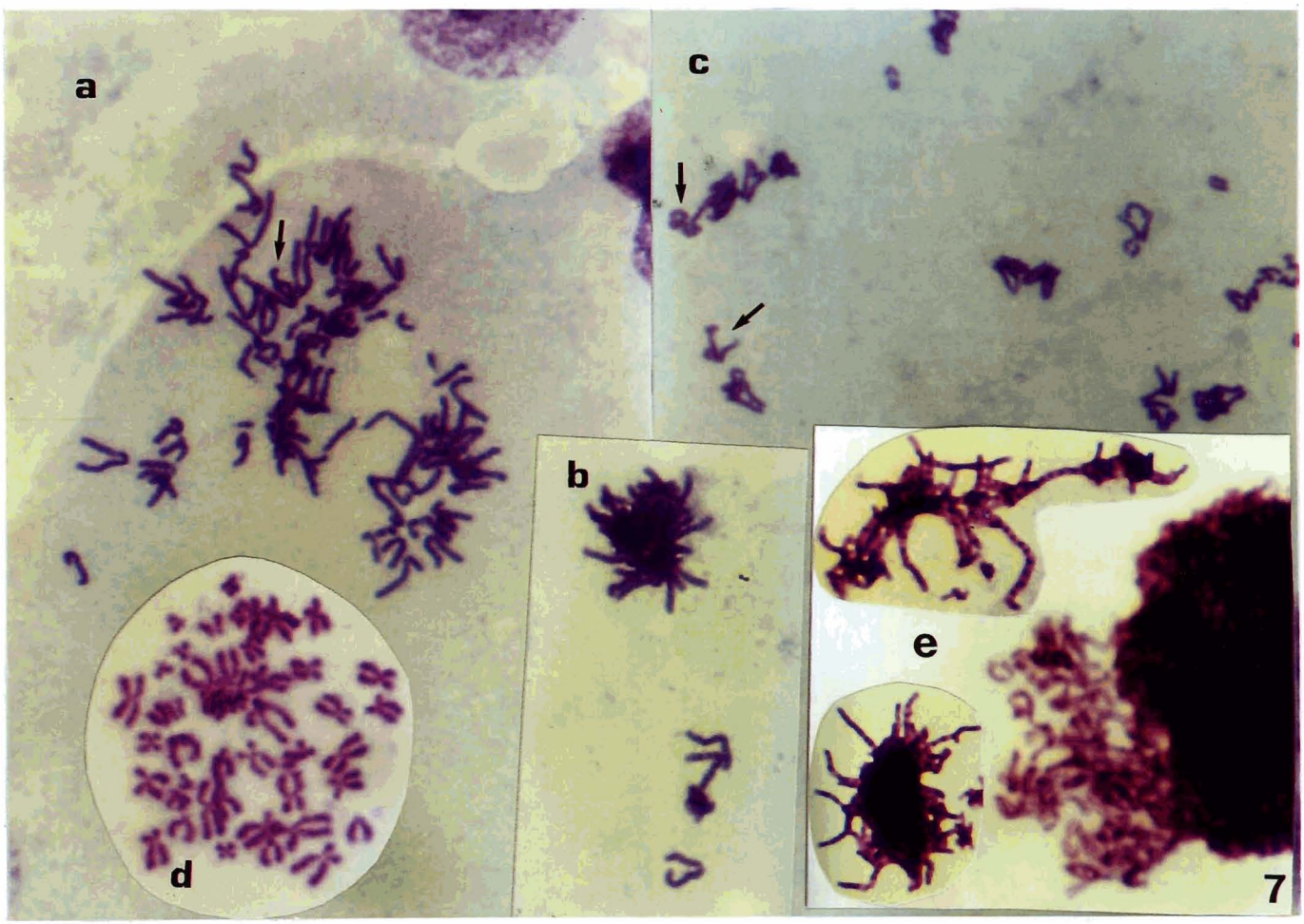


Fig. 6. Inset a: Amniotic cell nucleus extrudes regular chromosomes, a total set of 92 XY. **Inset b:** Same as inset a, but a total chromosomal set of 46 XY. **Inset c:** Trophoblastic cell nucleus extruding condensed hyperchromatic chromosomes. **Insets d,e:** Trophoblastic, meiotic diploid metaphases containing condensed, curly and «0» chromosomes. Arrows point out «0» chromosomes. HT-Giemsa. x 1,000



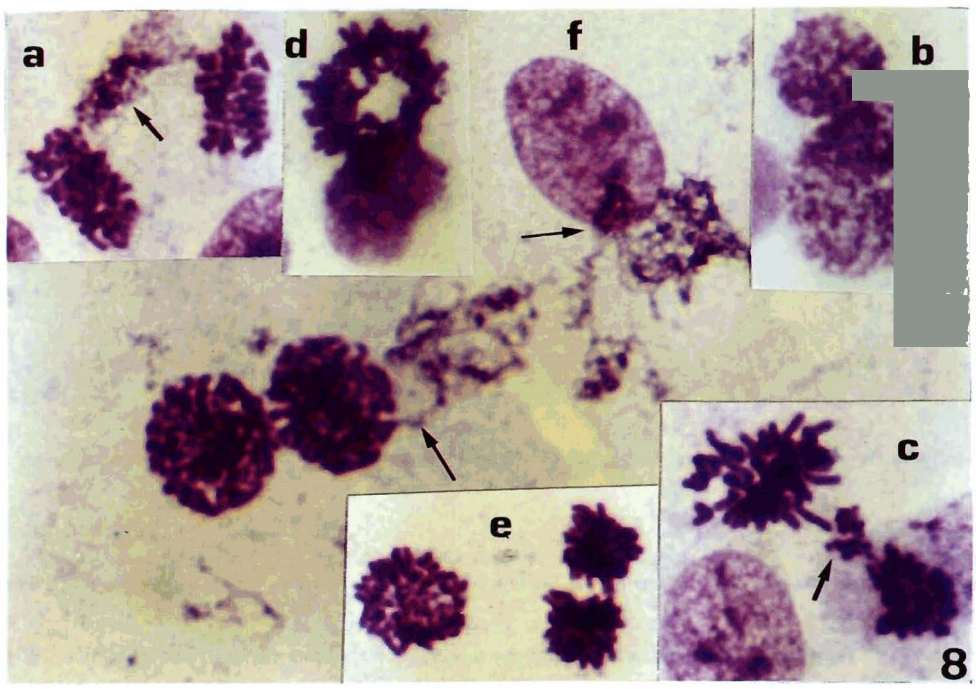


Fig. 7. Cytogenetic analysis of female amniotic cell culture by the in situ technique. **Inset a:** Presence of «0» chromosome in a female metaphase of condensed meiotic hyperchromatic chromosomes. **Insets b, c:** Scattered female meiotic chromosomes (arrows) belong to NVs and are not artefacts of spreading. **Inset d:** A diploid metaphase of regular chromosomes. **Inset e:** NV of pachytene chromosomal and nuclear conglomerates extruding pachytene or regular chromosomes. HT-Giemsa. x 1,000

Fig. 8. Symmetrical and asymmetrical meiotic division within the same amniotic cell culture with or without NBs. **Insets a, c, f:** NB containing condensed or broken chromosomes. Arrows point at NB remnants with or without chromosomes. **Insets b, e:** various sized dividing nuclei in the same cell culture. **Inset d:** Extruded ring-shaped meiotic metaphase. HT-Giemsa. x 1,000

chromosomes were also embedded in ECM (Fig. 18). The morphology of NBs was further clarified by Papanicolaou staining of amniotic cells after 10 min hypotonic treatment (HT-Papa) (Figs. 19, 20). NBs were hematoxylinophilic with strong intensity at their intranuclear position and along their wall at areas of condensed chromosomal content. The NB lumen content appeared hematoxylinophilic. NBs between interphase nuclei or from metaphase to interphase nucleus (Fig. 19a) and chromosomal transfer (Fig. 19b) were clearly demonstrated by the HT-Papa method.

Amniotic cells showed abundant cytoplasmic GSG-sacs paranuclearly and membranous rivulets running from cytoplasm to cytoplasm forming a circulatory network extracellularly, unaltered by the HT-Papa method (Fig. 21). NBs were supported and flowed into GSG-sacs (Fig. 22a) running in close parallel to the GSG-rivulets (Fig. 22b). With the HT-Papa method, NBs and their content appeared hematoxylinophilic while GSG-sacs and rivulets were basophilic (Fig. 22).

Amniotic cells showed PAS and PAS-diastase-positive perinuclear GSG, paranuclear-GSG-sacs, GSG-rivulets and NBs (Figs. 23-27). The ECM of amniotic cells consisted of PAS and PAS-diastase-positive, fibrillar to translucent GSG with unstained areas (Fig. 23a,c). Cell nodules consisted of cells embedded in GSG (Fig. 23b).

Moderate to strong PAS staining of the ECM was

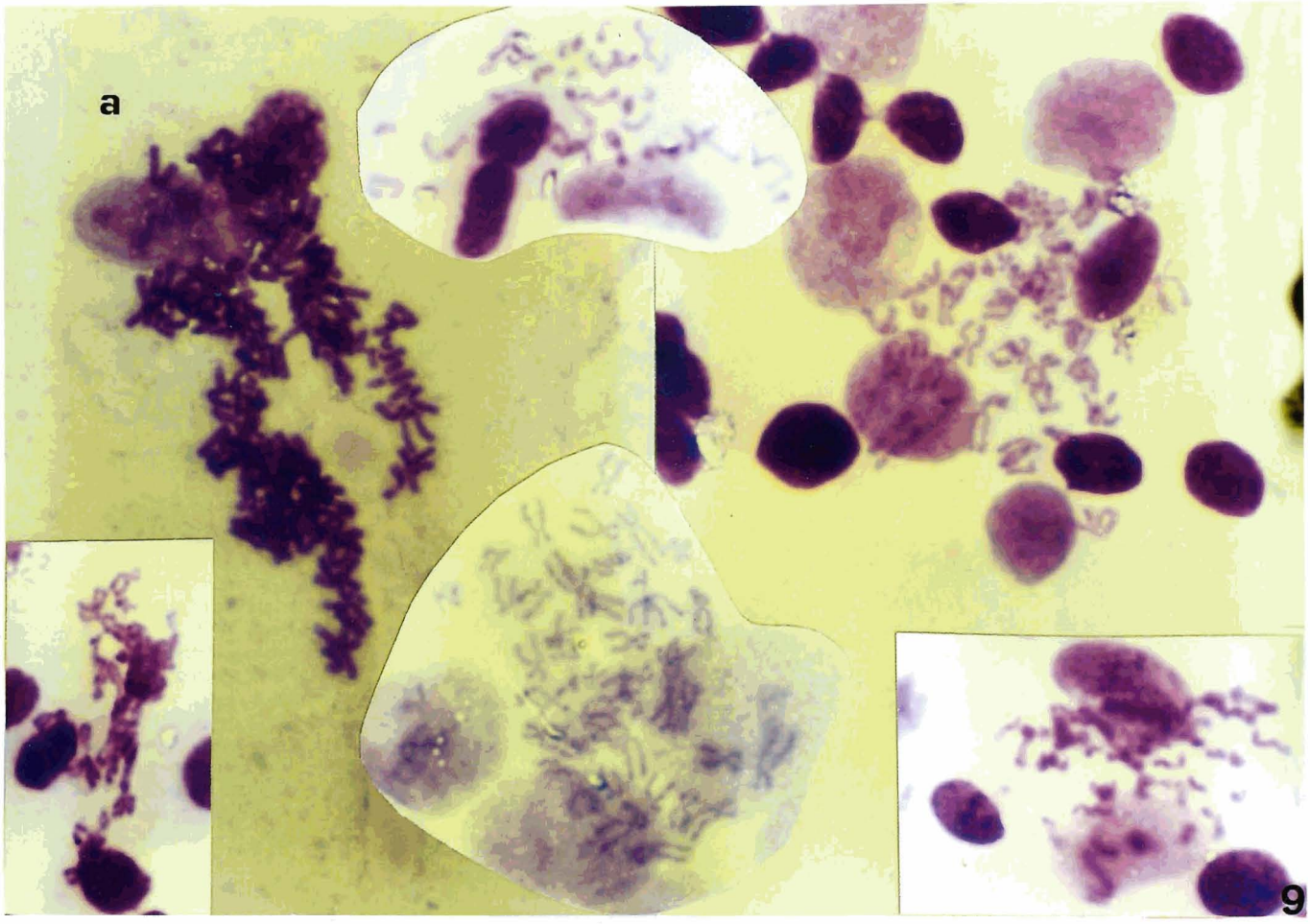
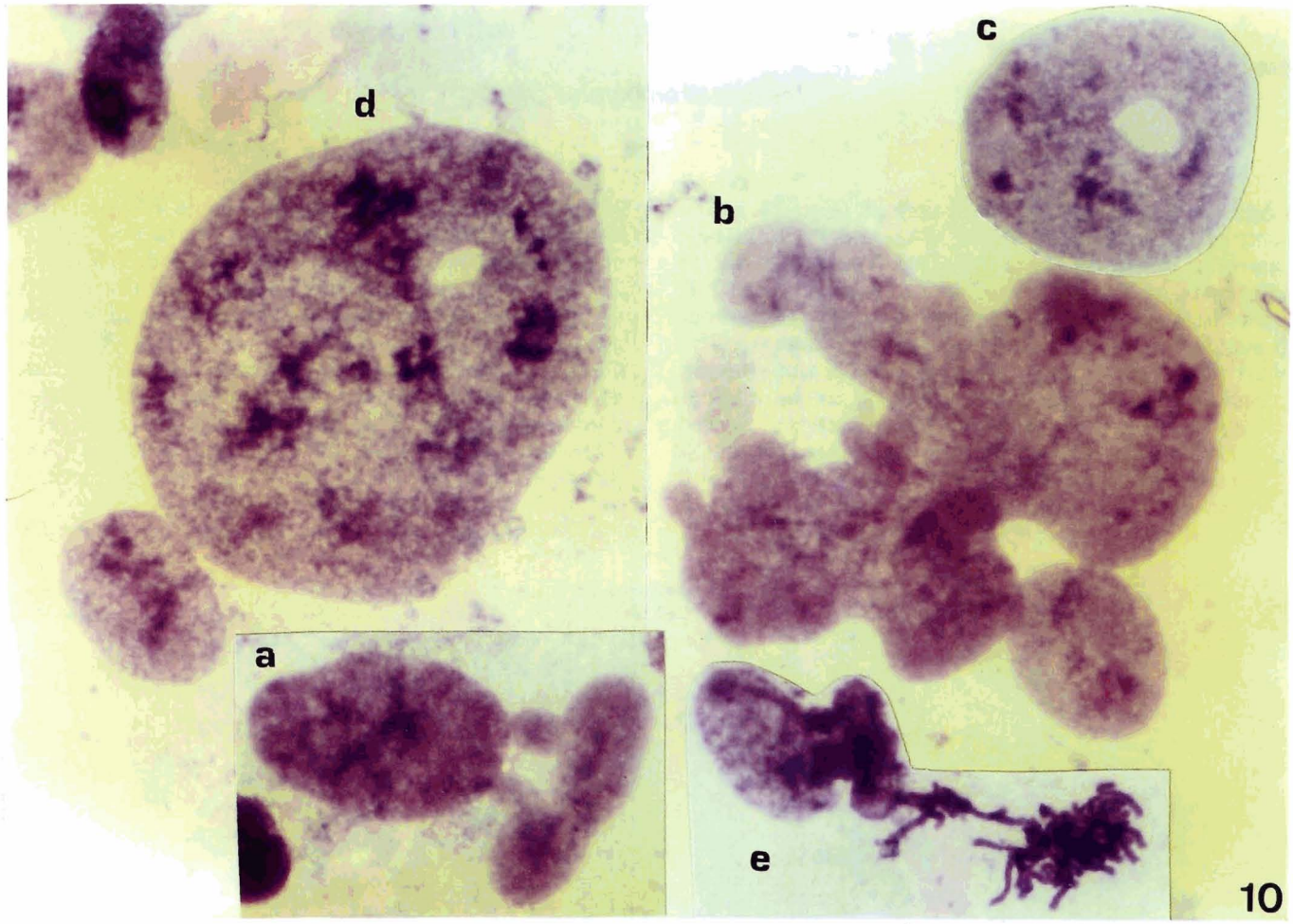


Fig. 9. Metaphase fusion of trophoblastic tissue cells of regular or condensed chromosomes. **Inset a:** Metaphase fusion of amniotic cells. HT-Giemsa. x 1,000

Fig. 10. Nuclear fusion of amniotic cells. **Inset a:** Two nuclei fuse via nuclear projections. **Inset b:** Fusion of more than two nuclei. **Inset c:** Nucleus with vacuole resulting from nuclear fusion. **Inset d:** Nucleus with vacuole producing NV. **Inset e:** Simultaneous nuclear fusion with NV metaphase production. HT-Giemsa. x 1,000

Fig. 11. Transfer and attachment of condensed, hyperchromatic chromosomes onto amniotic cell nuclei or metaphases via CFS. Arrows point at CFS. Chromosomal spreading technique-HT-Giemsa. x 1,000



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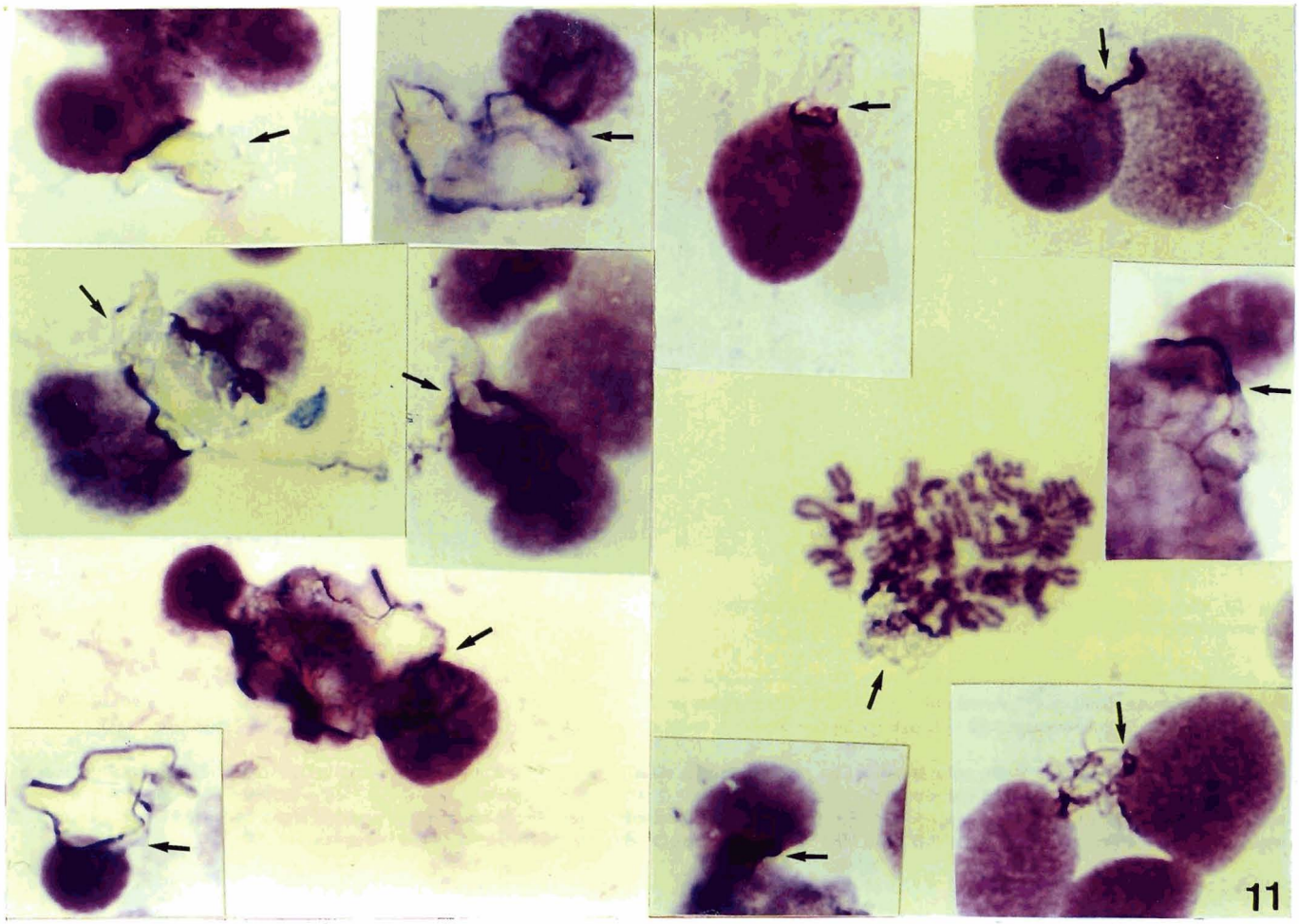

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observed within the same cell culture (Fig. 24a). The wall of NBs exhibited strong PAS and PAS-diastase staining, while its content varied from negative, to moderate or strong (Fig. 24b-d). Accumulated NB content in the cytoplasm, in the form of membrane-bound vacuoles, was PAS negative to moderate. The wall of these vacuoles was connected and showed identical hematoxylinophilic texture to the nuclear membrane (Fig. 24e). NBs were connected and flowed into PAS positive GSG-sacs and rivulets (Fig. 24f). PAS-diastase positive NBs, intracellular GSG-sacs and chromosomes are shown in Fig. 25. Cells in metaphase exhibited one or more PAS-positive NB (Fig. 26a-d). NBs of cells in interphase appeared as PAS-positive intranuclear vacuole (Fig. 26e). NBs, NV and condensed chromosomes were in close association with GSG (Fig. 26f-i). Observations of PAS stained amniotic cells prepared by the chromosomal spreading technique agreed with those of the in situ PAS staining. Chromosomes attached onto other cells (Fig. 27a,b), tails of NVs (Fig. 27c), intact NBs transfering condensed chromosomes (Fig. 27d) and meiotic cell division (Fig. 27e), were accompanied by moderate to strong PAS-positive GSG. Figure 27 shows the same subject as Figure 11 documenting that CFS involves GSG.

The chromosomal spreading technique broke the

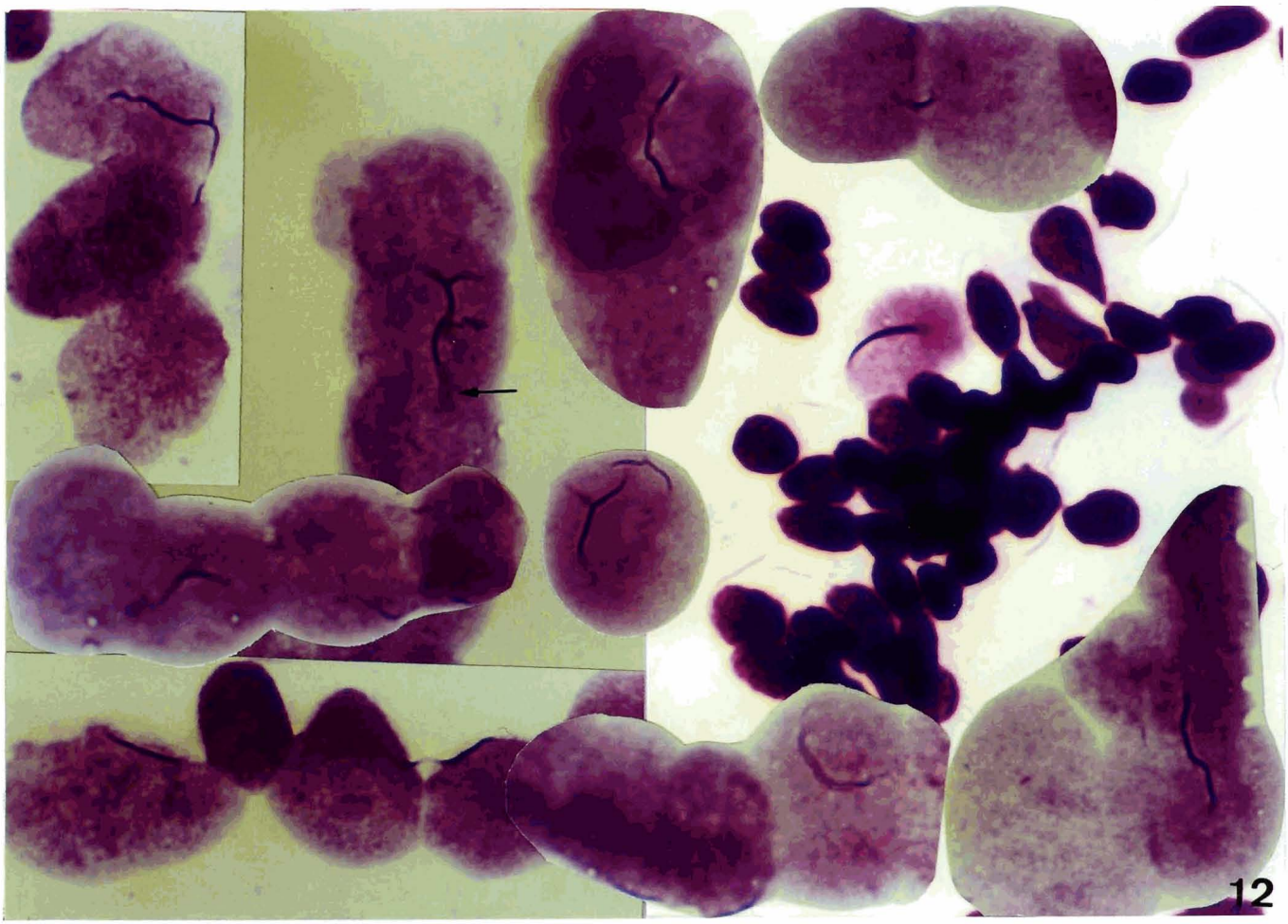
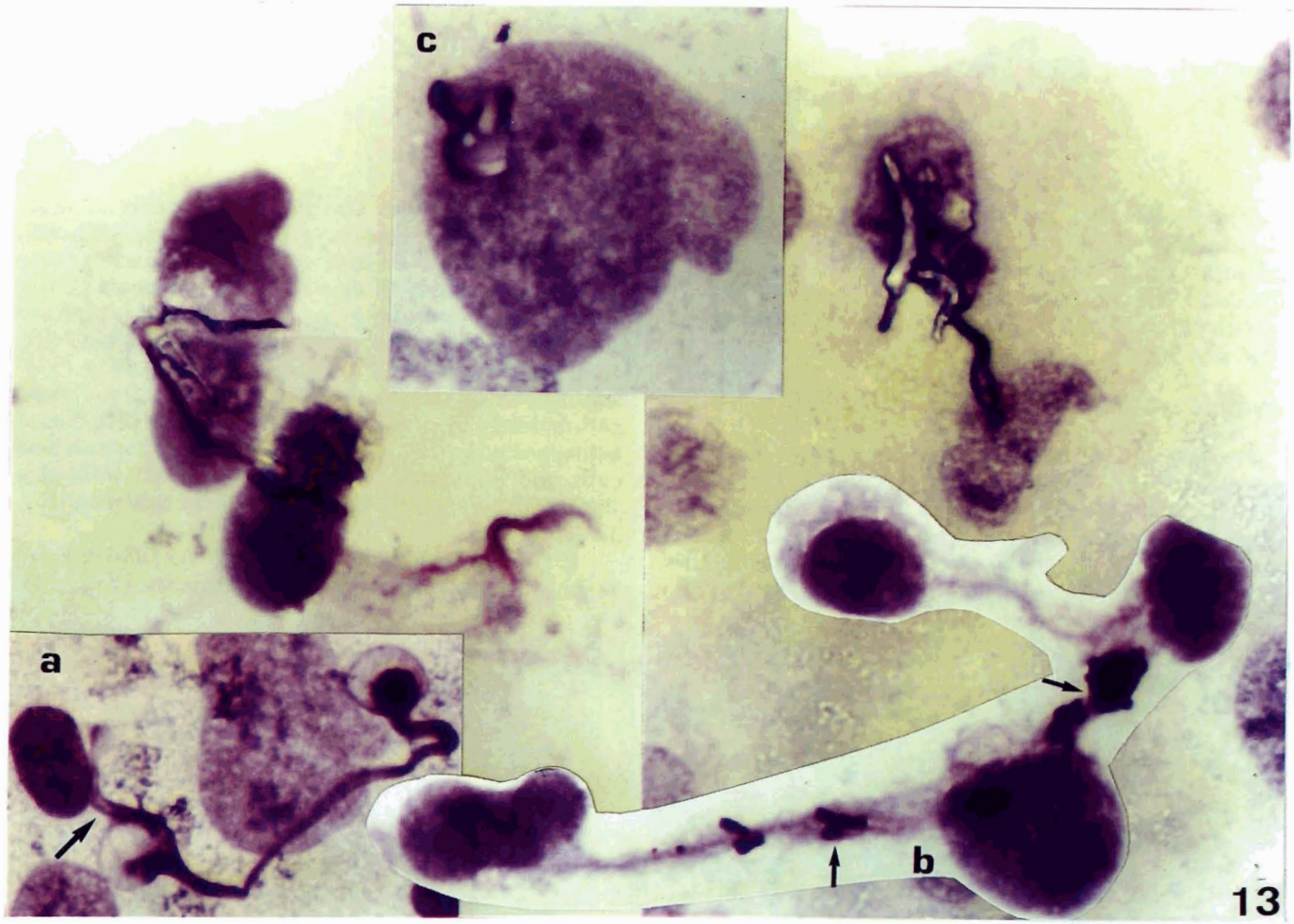


Fig. 12. Inter and intranuclear passage of condensed chromosomes in trophoblastic tissue cells. Arrow points at the two arms of a decondensing chromosome. HT-Giemsa. x 1,000

Fig. 13. Tubule morphology of amniotic NBs. **Inset a:** Arrow points at an NB from a cell producing NV to an interphase cell. **Inset b:** arrow points at chromosomes transfered through NBs between nuclei in interphase. **Inset c:** Onset of NB. In situ, cytogenetic technique-HT-Giemsa. x 1,000

Fig. 14. NBs in trophoblastic tissue cells. **Inset a:** Long NB between a small hyperchromatic, pyknotic nucleus and a larger hyperchromatic. **Inset b:** Presence of more than one long NB between two small, hyperchromatic, pyknotic nuclei. **Inset c:** NBs embedded in jelly-like ECM. **Inset d:** NB during meiotic production of NV. **Inset e:** Arrow points at a condensed hyperchromatic chromosome in a short NB resembling a vacuole. **Inset f:** Condensed hyperchromatic chromosomes along the wall of an NB attach themselves to other nuclei. CFS is visible. HT-Giemsa. x 1,000



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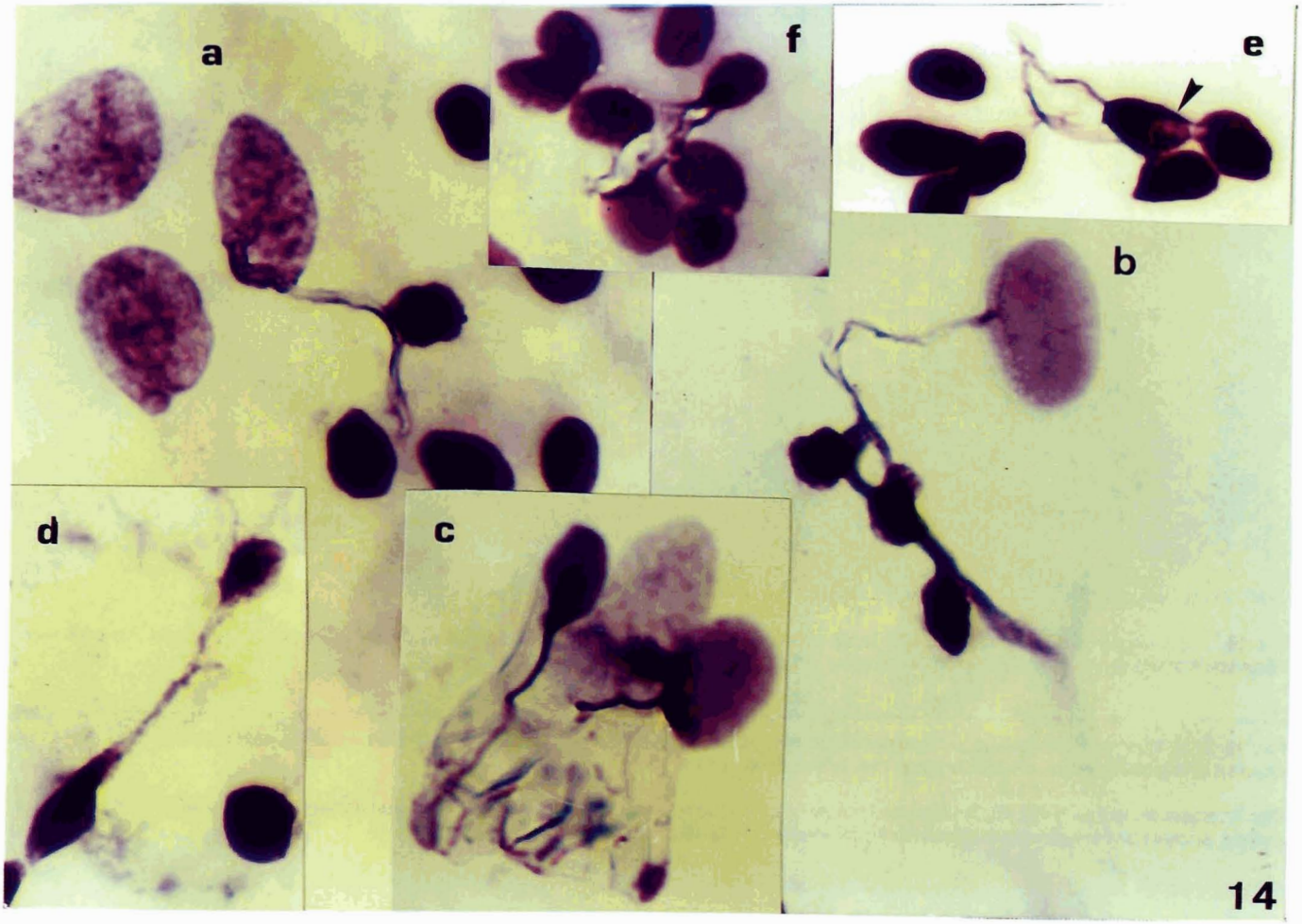

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continuity of NBs, which appeared as intranuclear vacuoles of thick nuclear wall, filled with clear, moderate to strong PAS-positive GSG (Fig. 27f). Identical NB morphology could be observed in the cytogenetic morphology of trophoblastic tissue cells (Fig. 27g,h).

Immunocytochemistry

Amniotic cell cultures showed strong immunoreactivity for a_1-antichymotrypsin perinuclearly, at GSG-sacs, GSG-ECM and NBs (Fig. 28). Immunoreactivity (Fig. 28a) was exhibited in the same cell areas as PAS-positive GSG (Fig. 26b). Many a_1-antichymotrypsin-positive NBs originated from each nucleus (Fig. 28, 29). The nucleolus also formed NBs with immunoreactivity for a_1-antichymotrypsin (Fig. 29a-c). NV heads, nuclear content NBs and condensed chromosomes during meiotic division showed strong immunoreactivity for a_1-antichymotrypsin (Fig. 30). Metaphase chromosomes and chromosomes, transfered via NBs, also showed positive immunoreactivity (Fig. 31). Immunoreactivity in the NV head was distributed perinuclearly in dense granules (Fig. 32a) or diffused homogeneously (Fig. 32c). Interaction of NVs with host cells and implanted chromosomes in host cells also showed immunoreactivity for a_1-antichymotrypsin (Fig. 32b).

Extruded condensed chromosome via NBs retained a_1-antichymotrypsin immunoreactivity (Fig. 32).

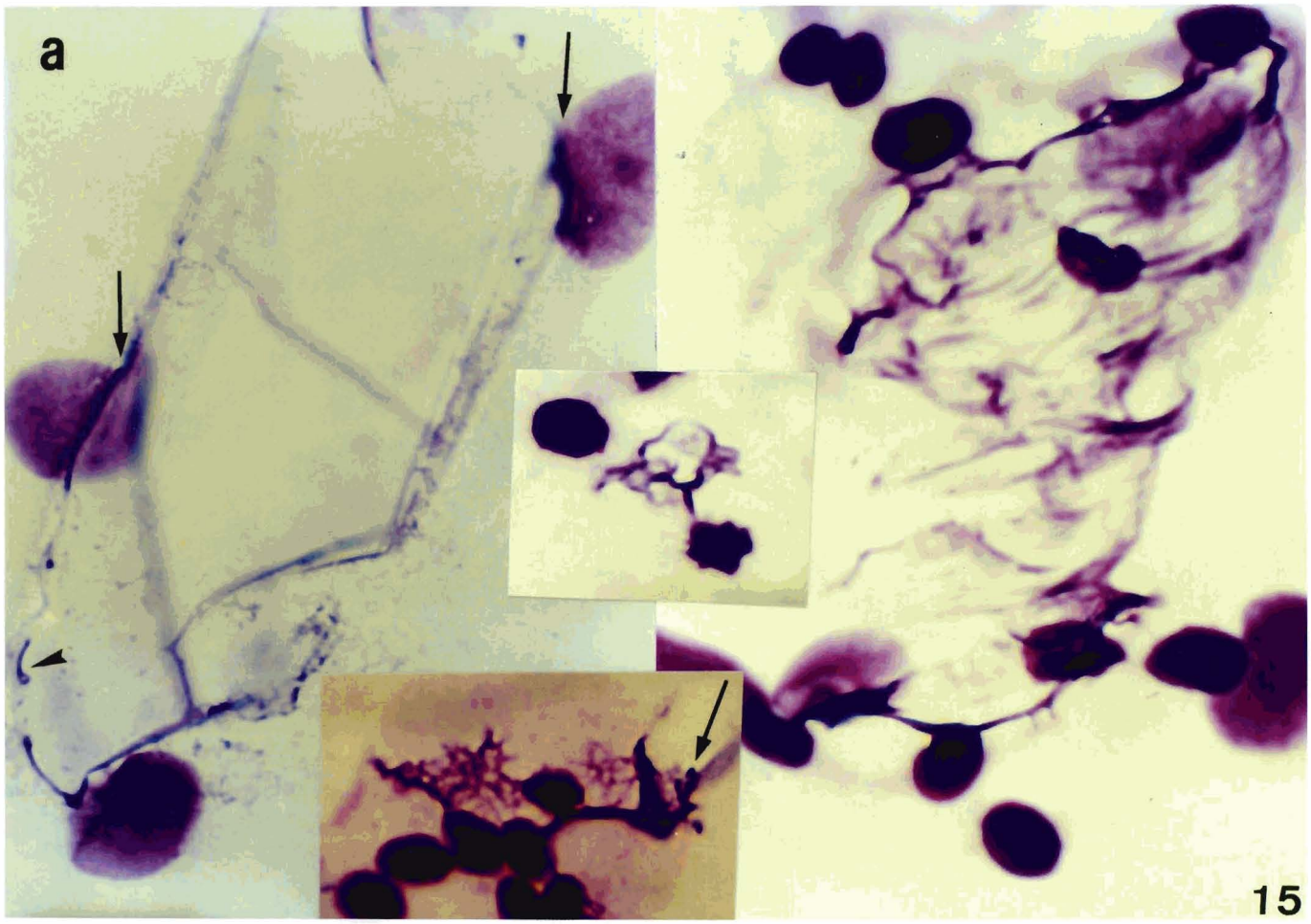
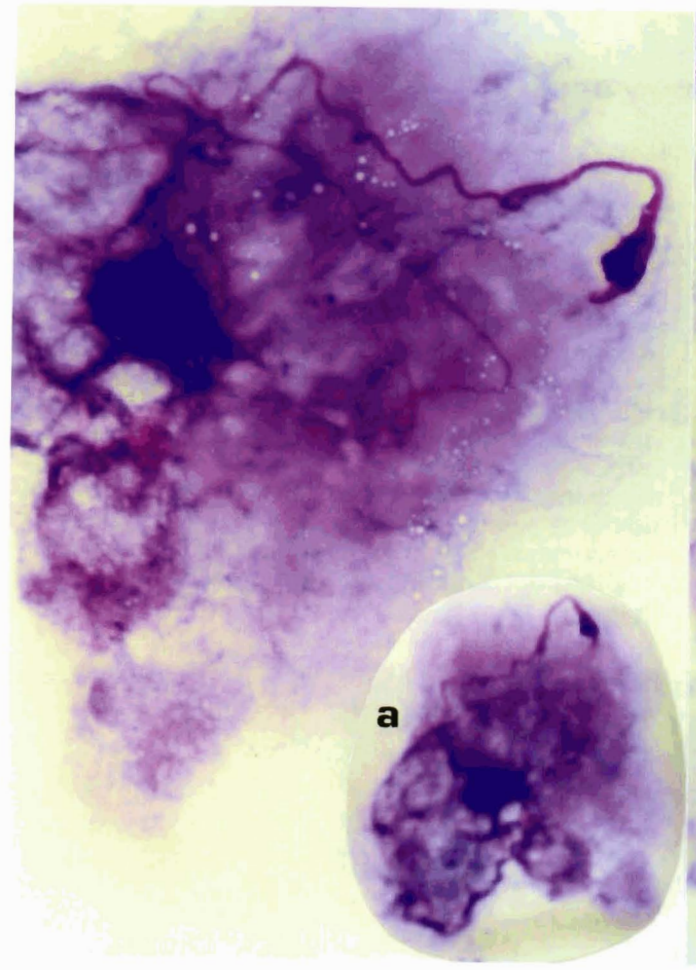


Fig. 15. Trophoblastic NBs embedded in ECM. **Inset a:** Arrows point at condensed chromosomes of broken NBs after 15 min hypotonic treatment. Short arrow points at an «0» chromosome. HT-Giemsa. x 1,000

Fig. 16. Long NBs run from nucleus to nucleus through accumulated ECM in amniotic cell cultures. Arrows show the chromosomal content and the direction of NBs. HT-Giemsa. x 1,000. **Inset a:** The same subject. x 100

Fig. 17. Same subject as in Fig. 16. Thick accumulations of ECM retain Giemsa stain while thinner ones are transparent due to various diffused nuclear content. Arrows point at condensed implanted chromosomes. HT-Giemsa. x 1,000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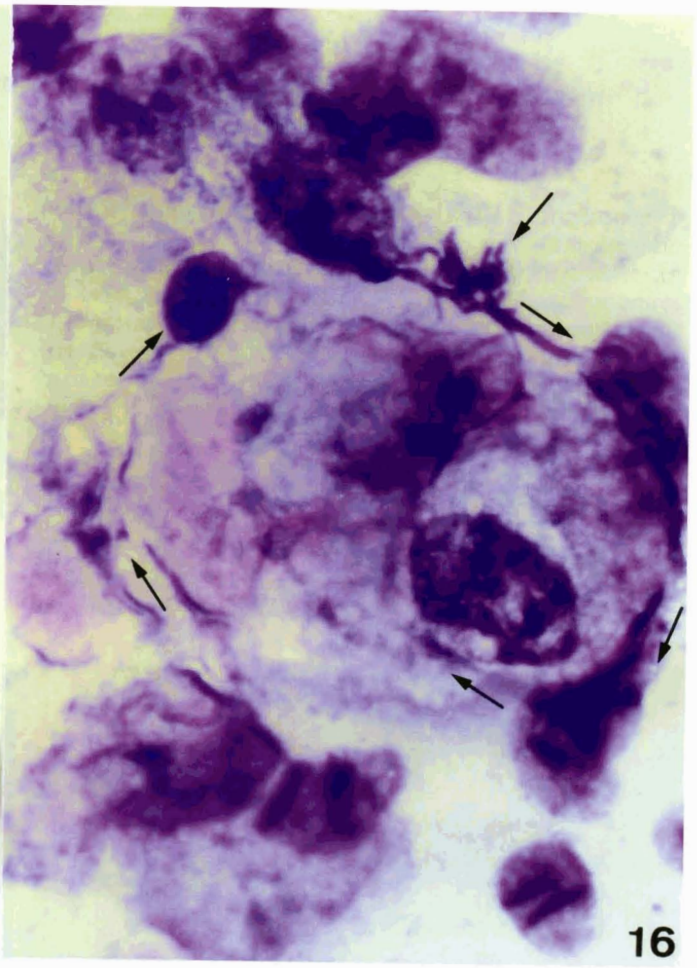
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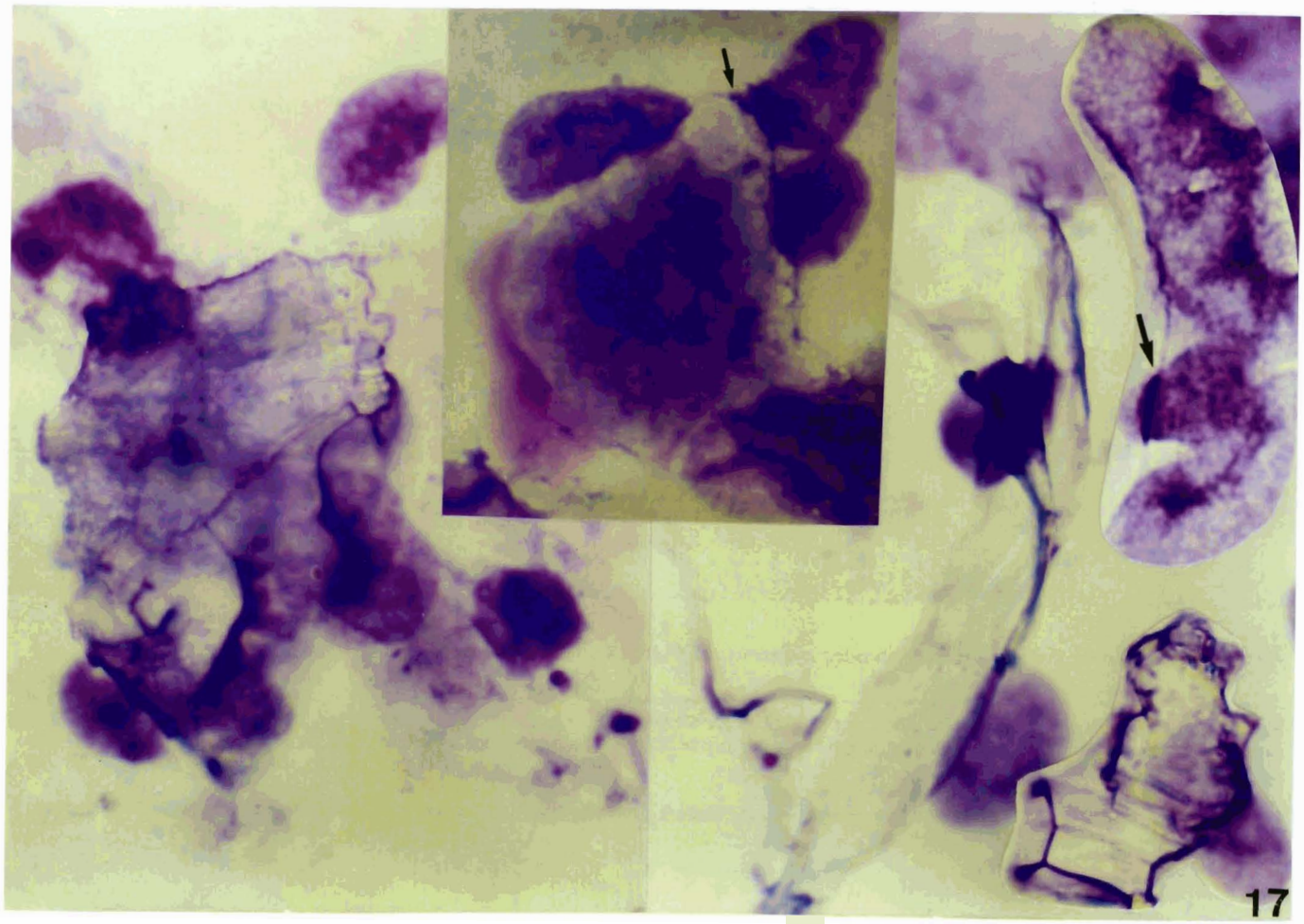
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Cytoplasmic NV tails were less immunoreactive than nuclear ones (Fig. 32c, 33). Nuclear heads of free NVs extruded condensed chromosomes with strong immunoreactivity (Fig. 33). Amniotic cells, prepared by the chromosomal spreading technique, also showed immunoreactivity for a_1-antichymotrypsin associated with NBs (Fig. 33a), condensed chromosomes (Fig. 33b), nucleolus (fig. 33c) and nuclear vacuoles (Fig. 33d).

Cytogenetic analysis

Trophoblastic tissue cells exhibited 15% to 47% diploidy with a mean of 27% and 53% to 85% aneuploidy with a mean of 73%. Aneuploidy consisted of hypodiploidy (Table 1). Amniotic cells showed 46% to 82% diploidy with a mean of 72% and 18% to 54% aneuploidy with a mean of 28%. Aneuploidy consisted of a mean 17.7% hypodiploidy and 10.3% tetraploidy (Table 2). The high ratio of aneuploidy: diploidy exhibited by trophoblastic tissue cells (early embryo) was reversed in amniotic cells (late embryo). There was insignificant poliploidy and no hyperdiploidy observed in these embryonic cells. The karyotype of aneuploid metaphases was not identical within the same cell population, and similarity was only numerical. Tetraploid metaphase chromosomes amniotic cells were extruded by a nucleus (Fig. 6a) documenting that tetraploidy is a result of meiosis.

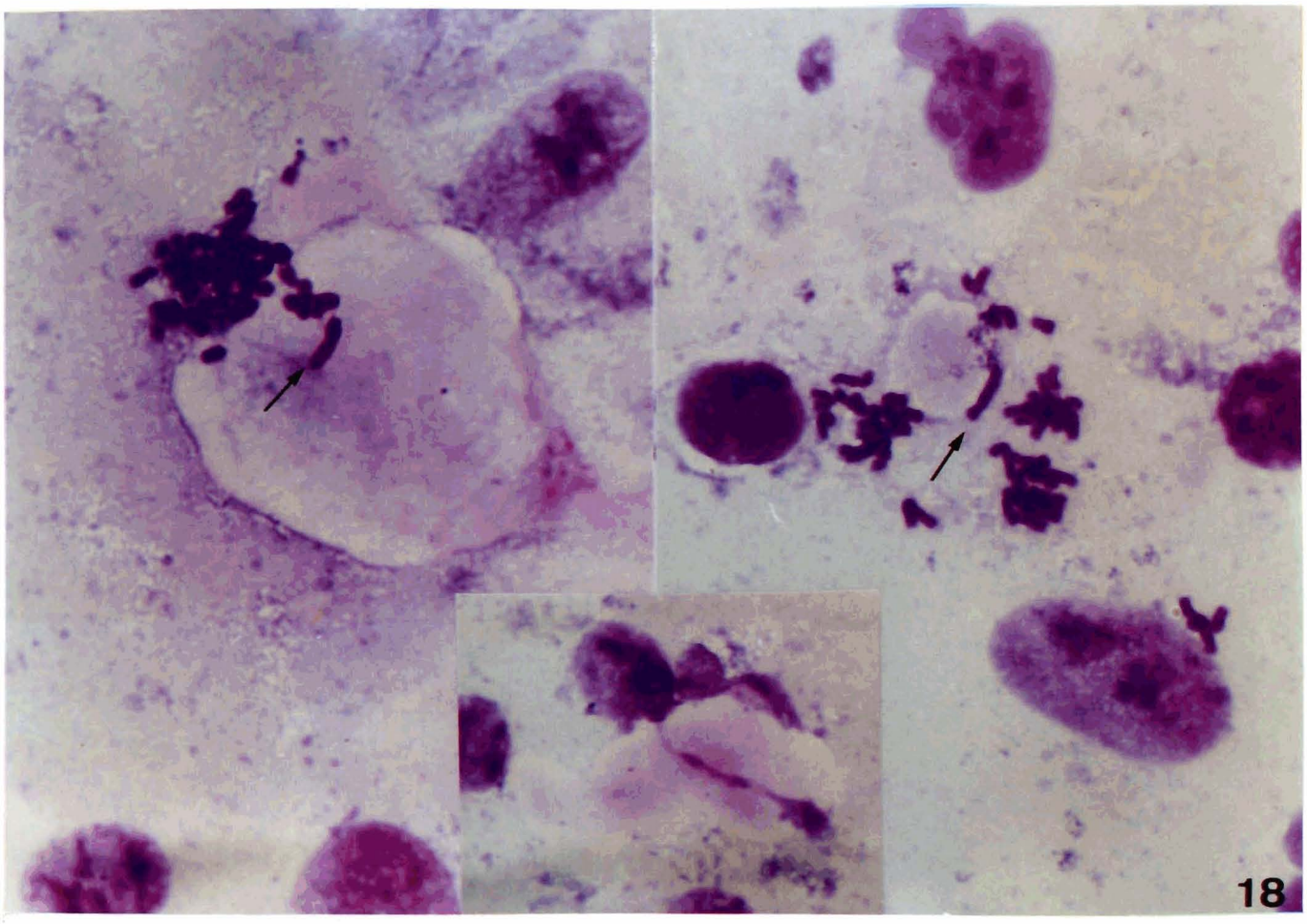
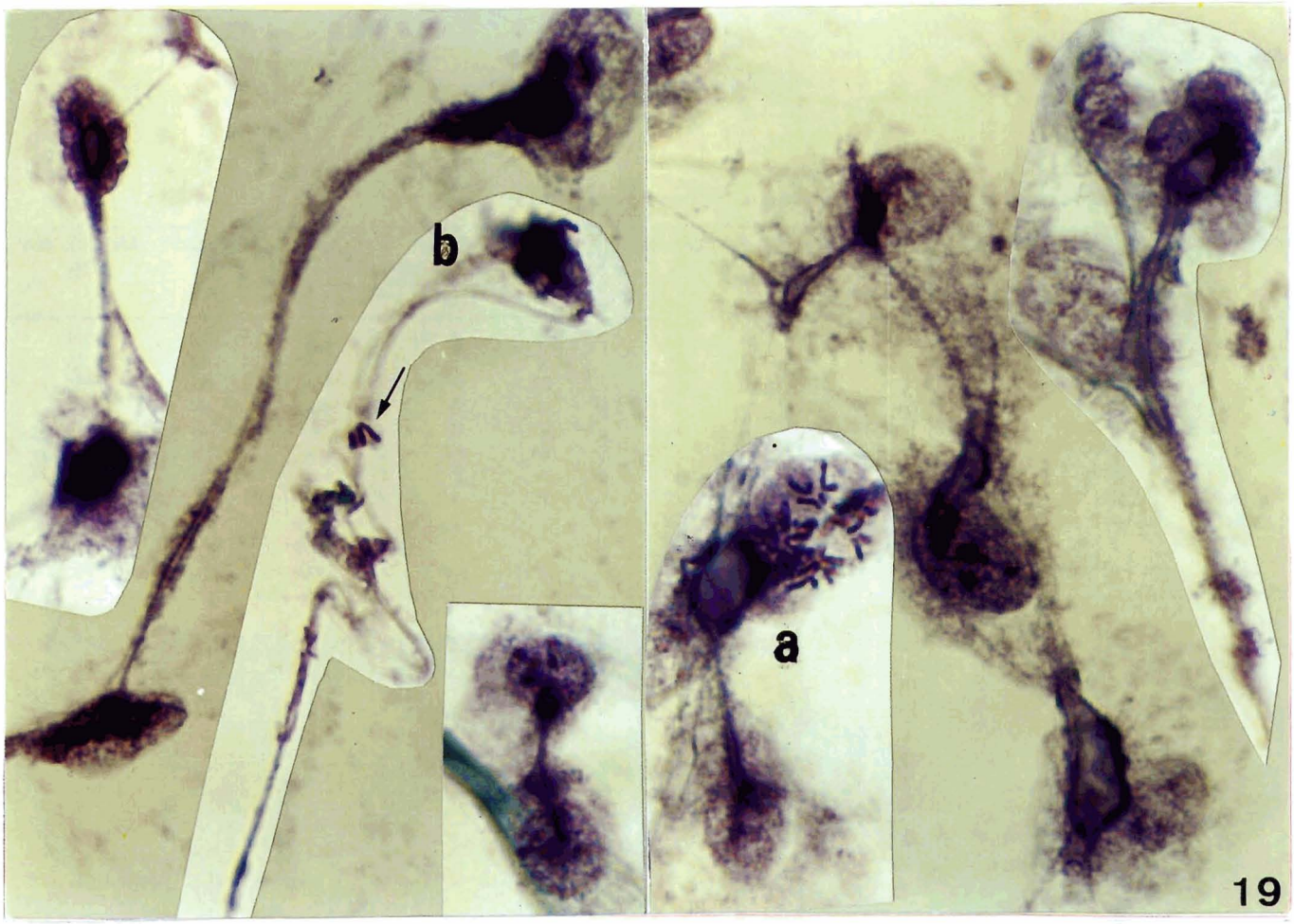


Fig. 18. Regular NV chromosomes (arrows) embedded in accumulated ECM in amniotic cell cultures in situ. HT-Giemsa. x 1,000

Fig. 19. Morphology of amniotic cell NBs after hypotonic cell treatment and Papanicolaou staining. Intranuclear points and wall areas of NBs are strongly hematoxylinophilic. **Inset a:** NB from a cell in metaphase to a cell in interphase. **Inset b:** Chromosomes (arrow) are transferred from a metaphase cell via NB. HT-Papa. x 1,000

Fig. 20. Different length and with of NBs width hematoxylinophilic content, within the same amniotic cell culture. Arrow points at transfered condensed hyperchromatic chromosomes. HT-Papa. x 1,000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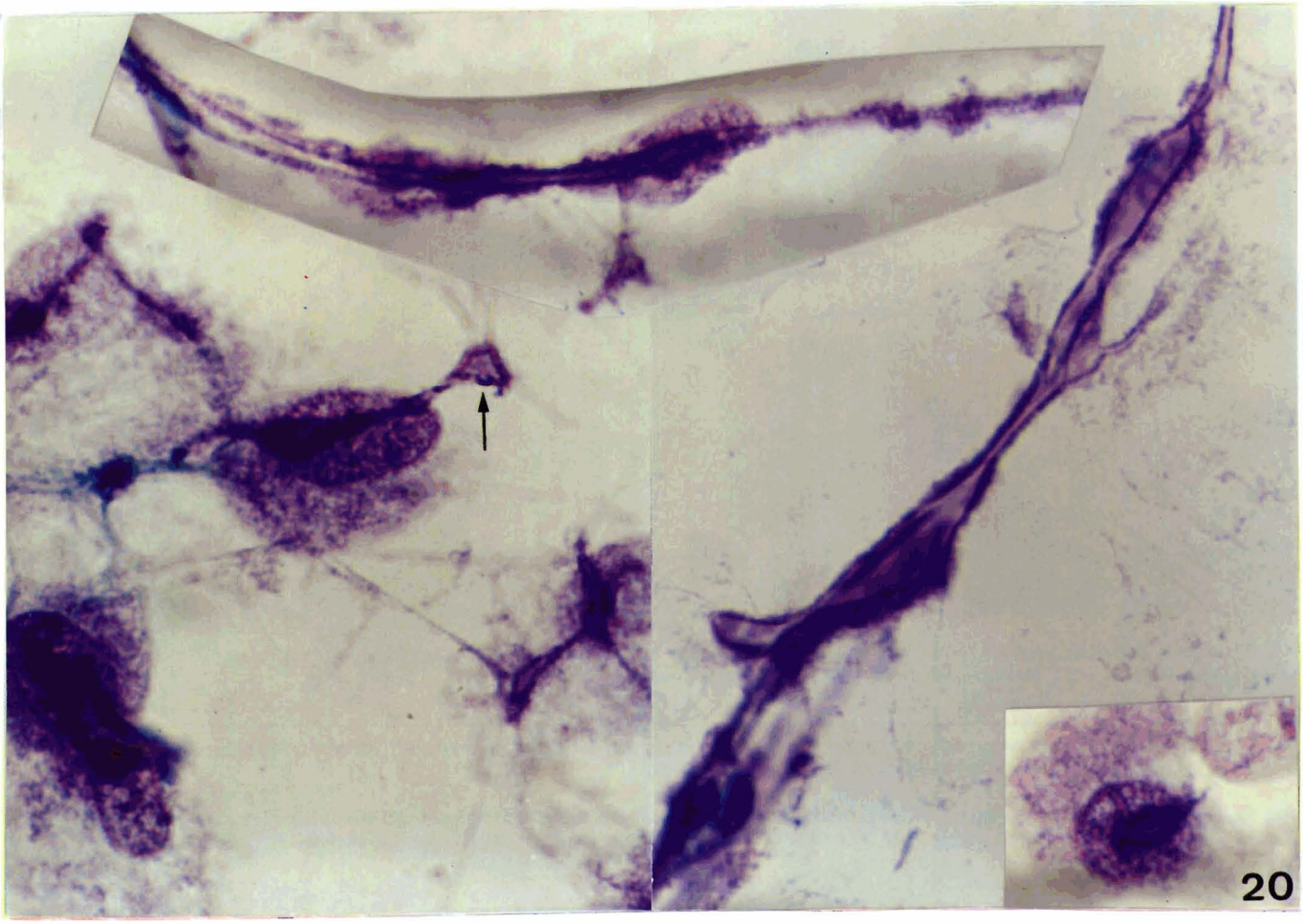
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Table 1. Numerical chromosomal analysis of trophoblastic tissue (early embryonic) cells.

CASE No.	HYPERPLOIDY %	TETRAPLOIDY %	POLYPLOIDY %	HYPOPLOIDY %	% CHROMOSOMAL HYPODIPLOID AND HYPOHAPLOID SETS OF				DIPLOIDY %	ANEUPLOIDY %
					1-10	11-20	21-30	31-45		
1	0	2	0	51	9	2	12	28	47	53
2	0	0	0	70	4	9	8	49	29	71
3	0	0	0	85	54	11	4	16	15	85
4	0	0	0	71	6	6	6	53	29	71
5	0	0	0	84	43	10	8	23	16	84
6	0	0	0	71	24	12	0	35	29	71
7	0	0	0	75	8	11	11	45	25	75
8	0	0	0	72	15	8	6	43	8	72
9	0	0	0	69	17	7	11	51	20	80
10	0	0	0	69	17	11	8	33	31	69
$\bar{X}$	0	0.2	0	72.8	19.1	8.7	7.4	37.6	27.0	73.0

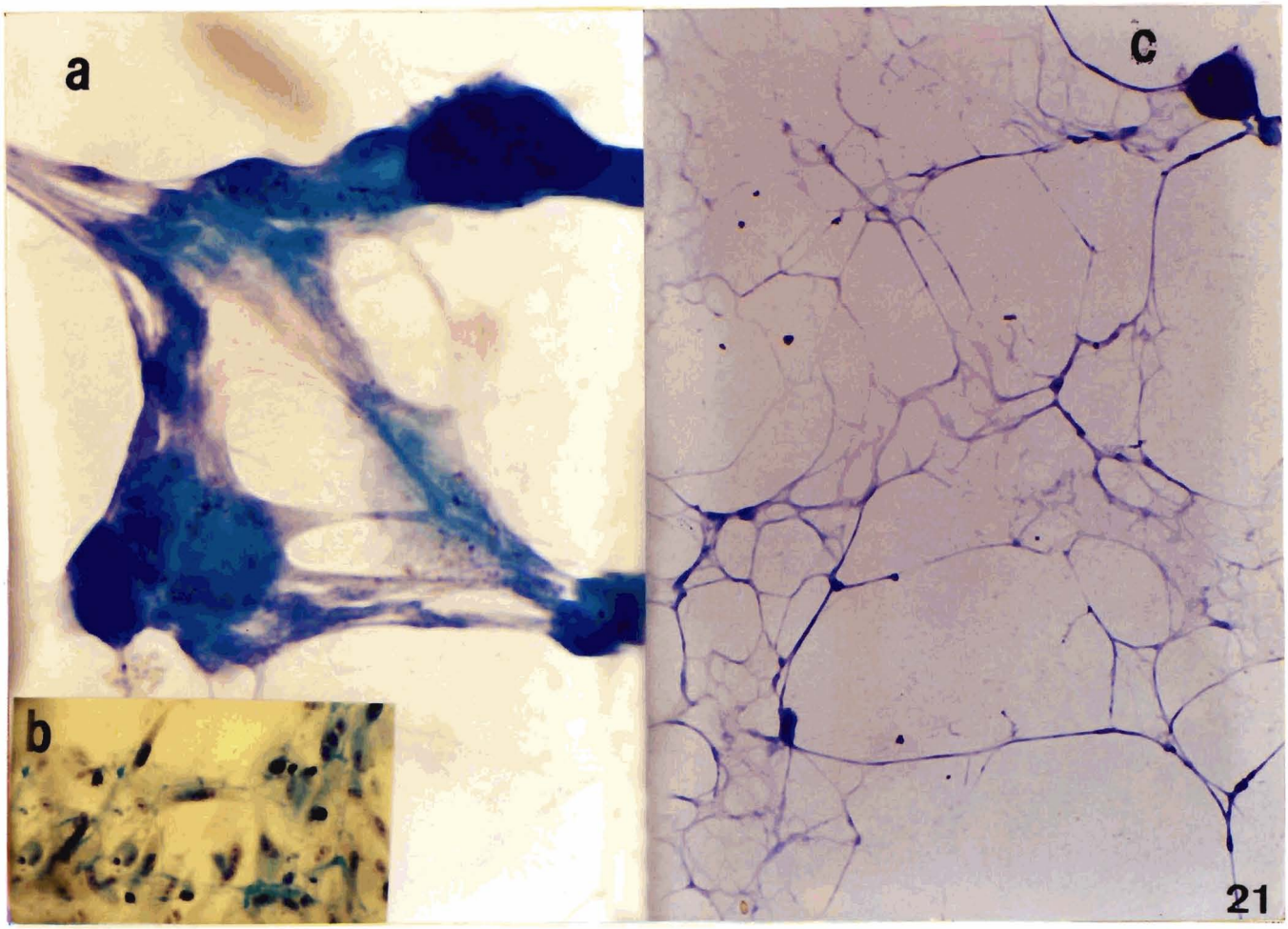
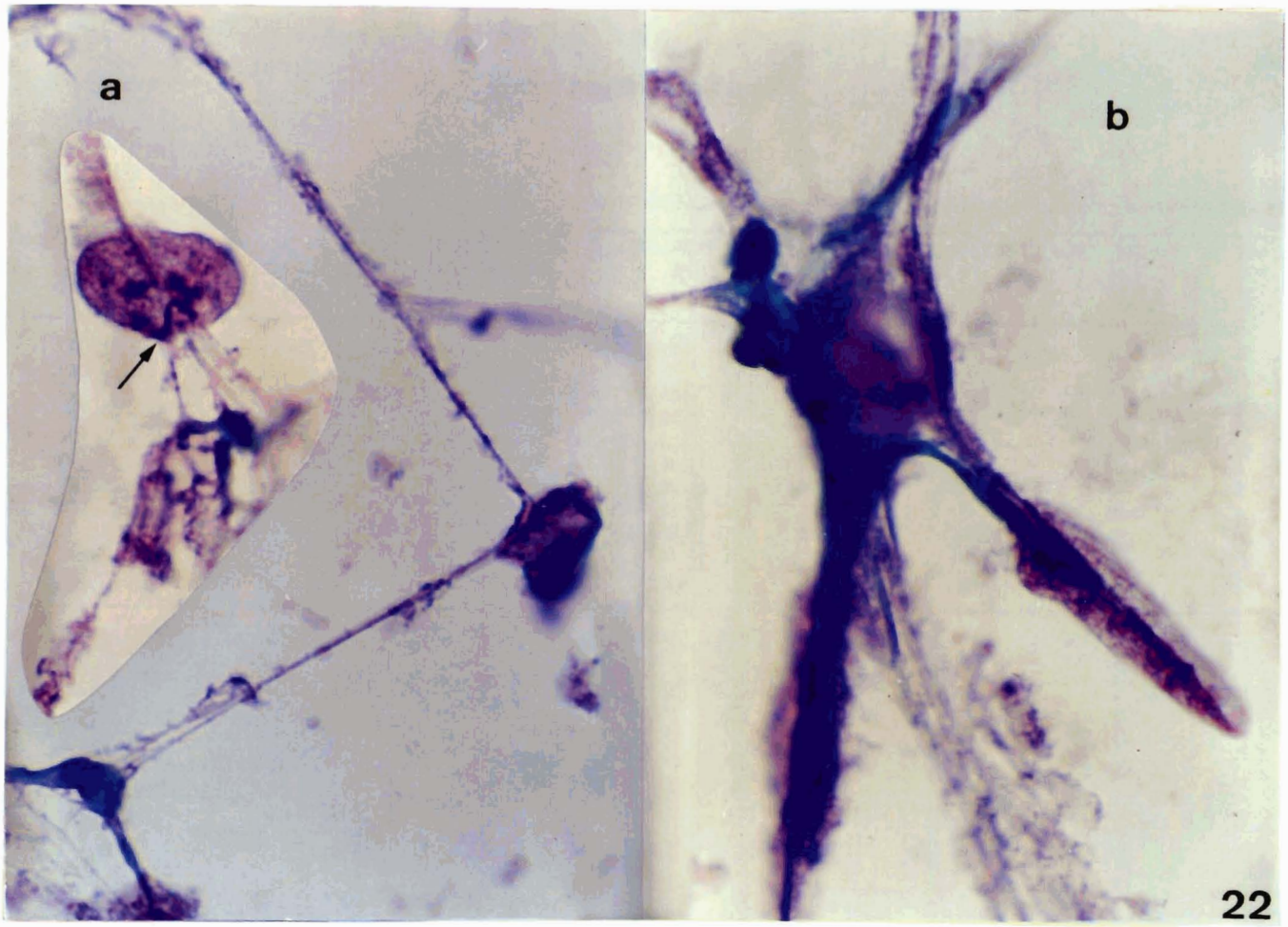


Fig. 21. Inset a: Basophilic GSG-sacs in amniotic cell cultures. HT-Papa. x 1,000. **Inset b:** GSG-sacs running from cytoplasm to cytoplasm via GSG-rivulets. Papanicolaou. x 100. **Inset c:** GSG-sac and circulatory network of rivulets. HT-Papa. x 400

Fig. 22. Amniotic cell NBs flowing into a GSG-sac. Arrow points at an NB chromosome. **Inset b:** NBs and GSG-rivulets running in parallel. Accumulation of hematoxylinophilic NB content is obvious. HT-Papa. x 1,000

Fig. 23. Inset a: ECM of amniotic cells consisting of PAS-positive and negative material. NBs are embedded in GSG-ECM. **Inset b:** PAS-positive cell nodule. **Inset c:** PAS-positive and negative ECM of amniotic cells prepared by the chromosomal spreading technique. HT-PAS. x 1,000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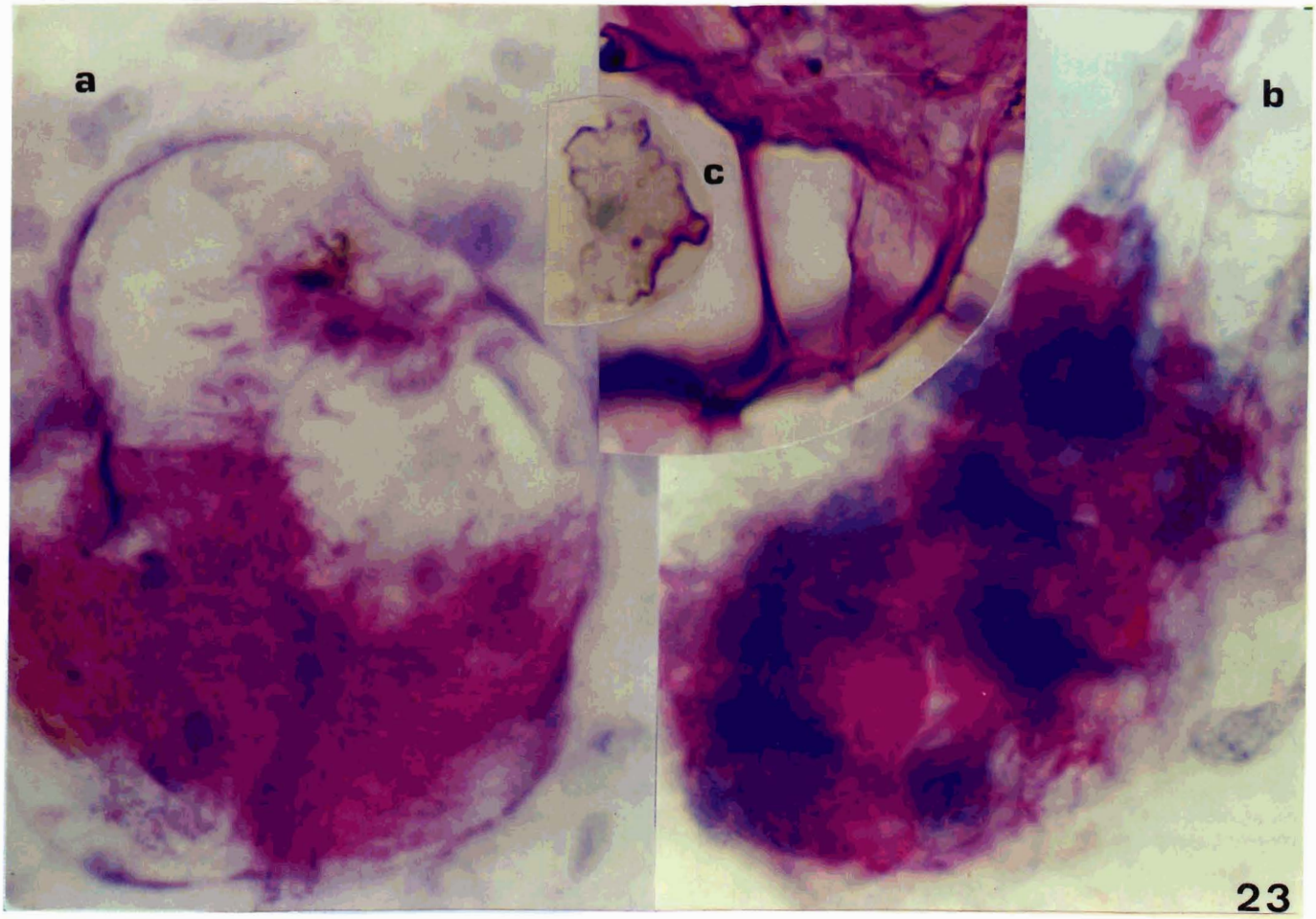
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Table 2. Numerical chromosomal analysis of amniotic (late embryonic) cells.

CASE No.	HYPERPLOIDY %	TETRAPLOIDY %	POLYPLOIDY %	HYPOPLOIDY %	% CHROMOSOMAL HYPODIPLOID AND HYPOHAPLOID SETS OF				DIPLOIDY %	ANEUPLOIDY %
					1-10	11-20	21-30	31-45		
1	0	9	0	11	4	0	2	5	80	20
2	0	13	0	7	3	0	1	3	80	20
3	0	20	0	34	11	4	3	16	46	54
4	0	4	0	32	22	1	2	7	64	36
5	0	8	1	13	2	1	3	7	79	21
6	0	12	0	6	0	3	0	3	82	18
7	0	4	1	28	9	2	2	15	67	33
8	0	7	1	12	0	3	1	8	80	20
9	0	11	0	20	0	1	3	16	69	31
10	0	15	1	14	4	5	3	2	70	30
$\bar{X}$	0	10.3	0.4	17.7	5.5	2	2	8.2	72.0	28.0

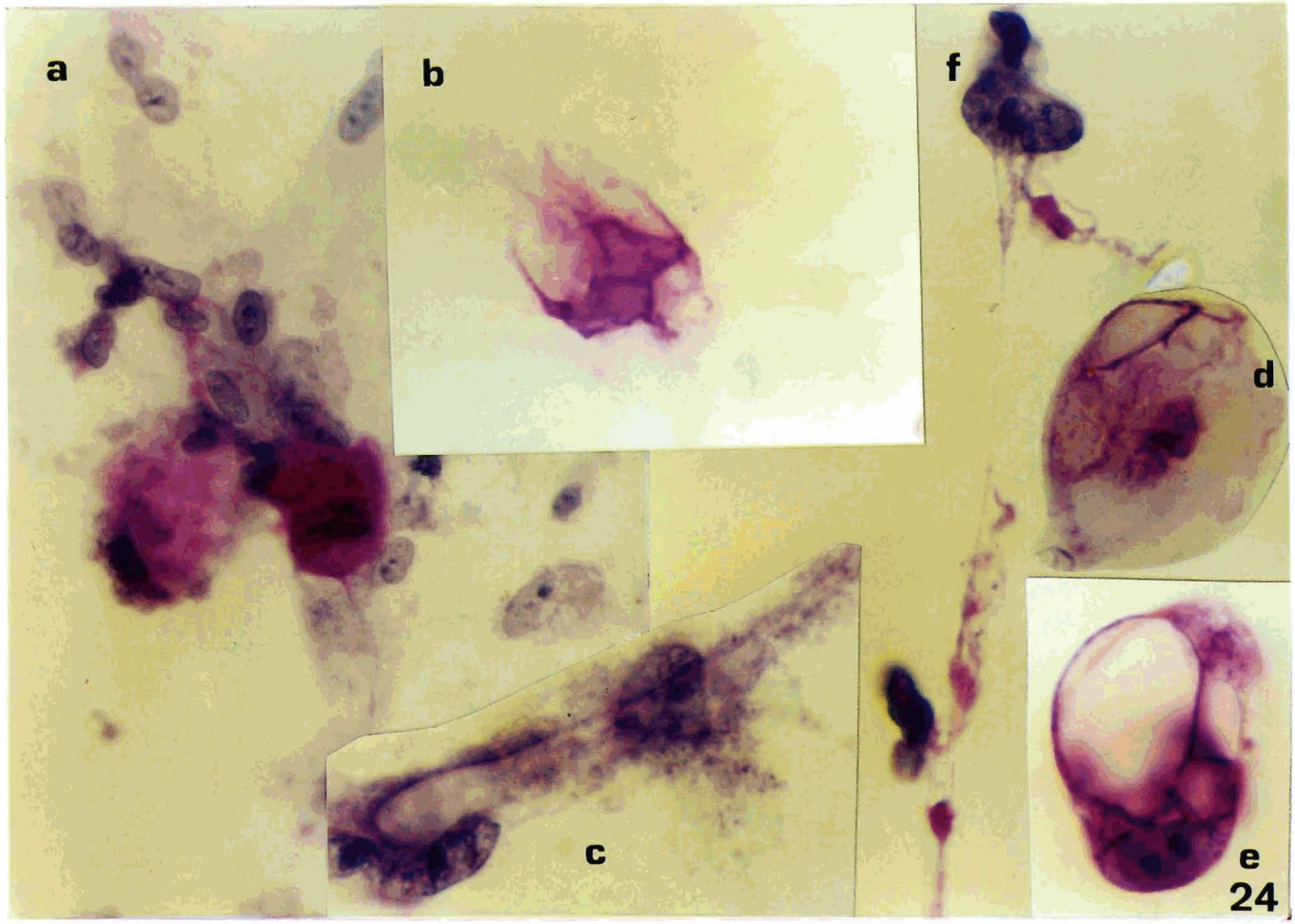
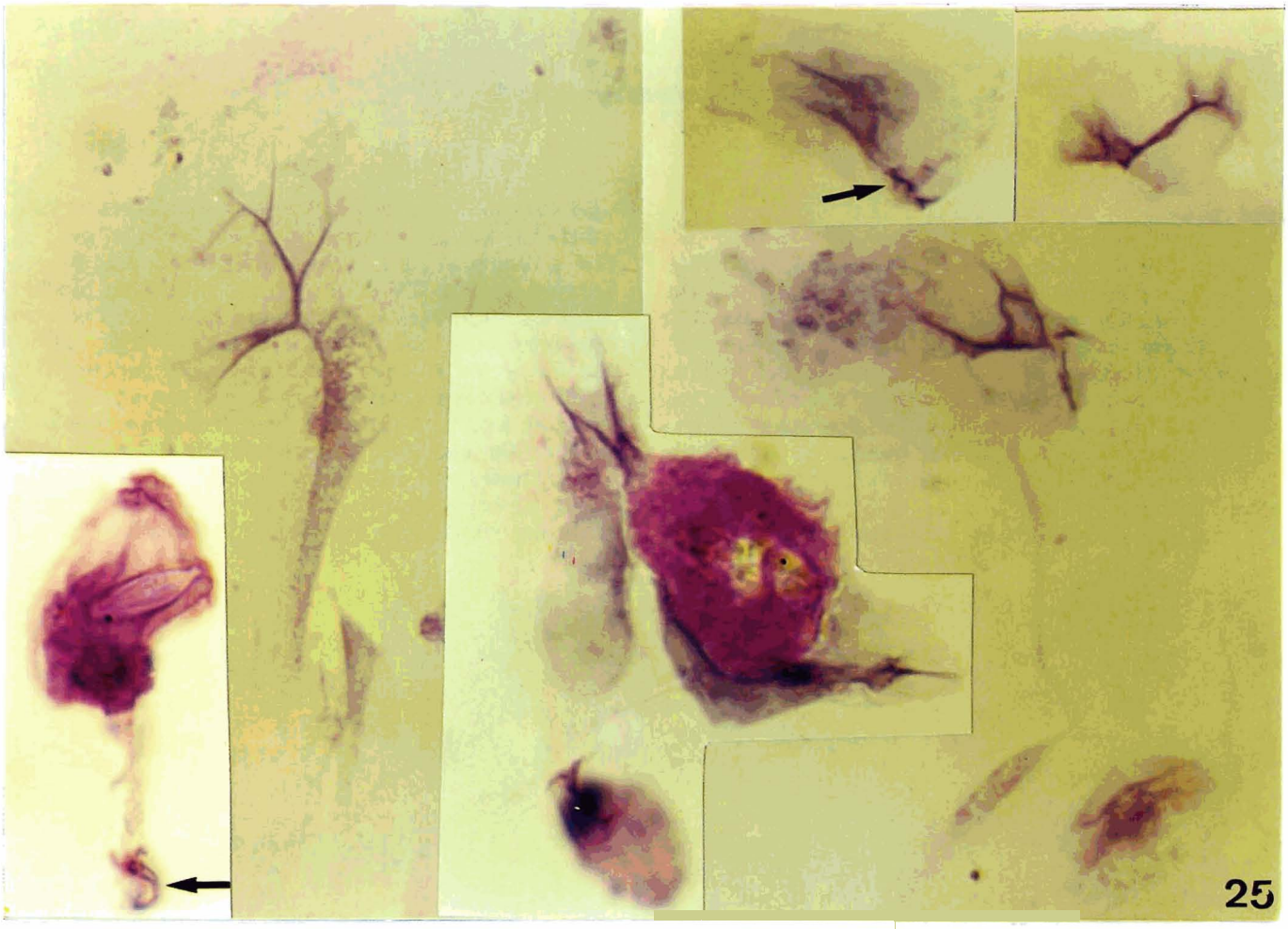


Fig. 24. Inset a: Moderate and strong PAS-positive ECM of amniotic cells. HT-PAS. x 400. **Insets b, c, d:** Negative, moderate and strong PAS-positive NBs. PAS. x 1,000. **Inset e:** NB content accumulated in the cytoplasm in the form of membrane-bound vacuole. The wall of this vacuole is connected to nuclear membrane. In situ-HT-PAS. x 1,000. **Inset f:** NB is connected and flows into GSG-sacs and rivulets. HT-PAS. x 1,000

Fig. 25. Amniotic cell NBs, GSG-sac and chromosomes are positive for GSG. Arrows point at chromosomes. In situ-HT-PAS-diastase. x 1,000

Fig. 26. Insets a, b: NBs of cells in metaphase contain GSG. **Inset c:** A metaphase embedded in GSG. **Inset d:** A metaphase NB flows into GSG-rivulet. **Inset e:** NB of a cell in interphase appears as GSG-positive intranuclear vacuole. **Inset f, g:** NBs flowing into GSG. **Inset h:** Wall of NB containing condensed chromosome attached on GSG. **Inset I:** NB embedded in GSG. In situ-HT-PAS. x 1,000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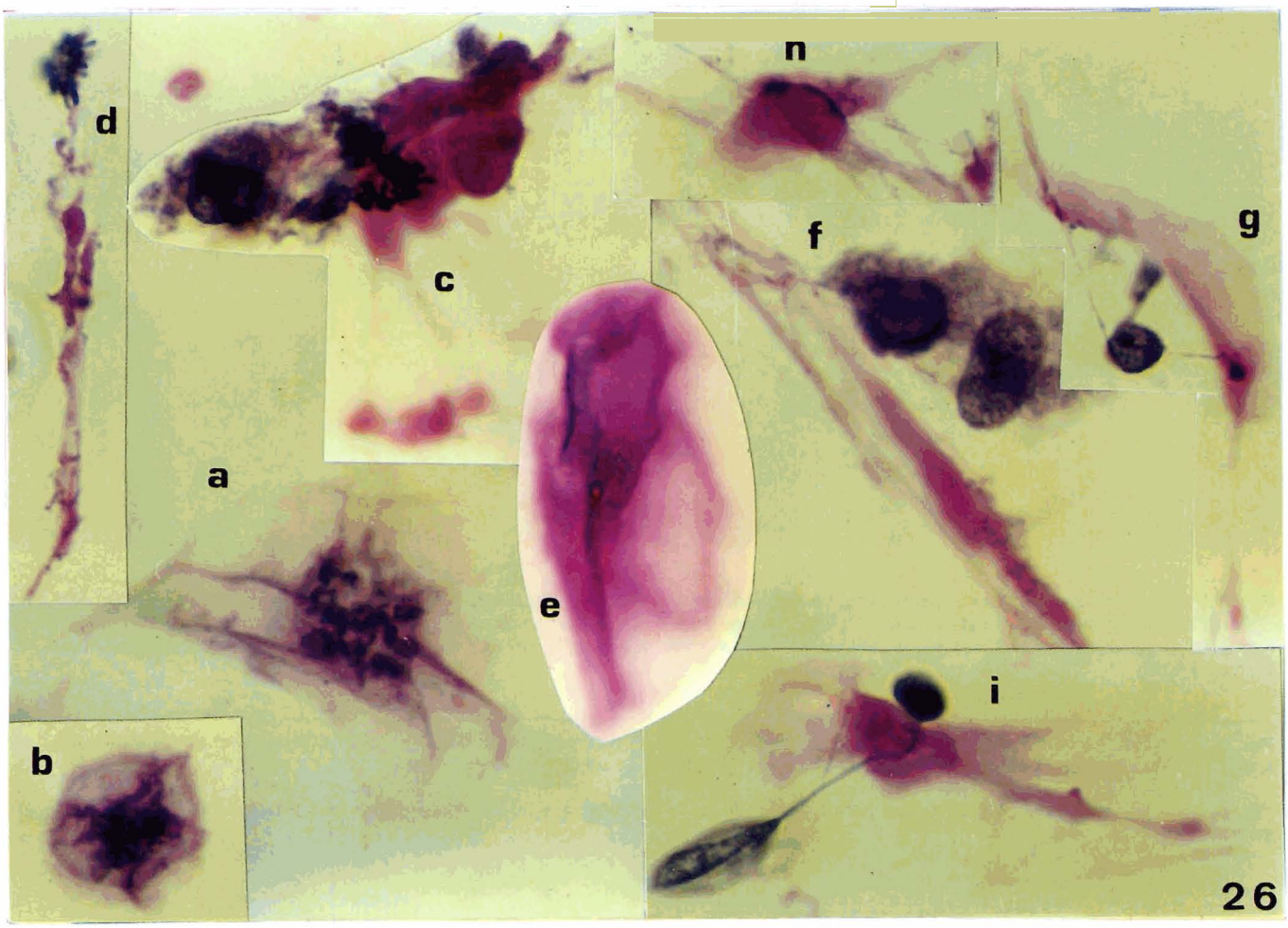
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Discussion

The observations of this study documented that embryonic cells divide by meiosis and follow the life cycle of malignant cells (Logothetou-Rella, 1994b). Meiosis was documented by the presence of NVs, NV invasion, extrusion of chromosomes, nuclear fusion, metaphase fusion, nuclear, nucleolar and cytoplasmic bridges, chromosomal transfer, variable-sized nuclei, nuclear fragmentation, condensed meiotic chromosomes, «0» chromosome, aneuploidy and nuclear conglomerates.

Embryonic NVs are identical to malignant and lymphocytic NVs (Logothetou-Rella, 1993, 1994a,b), which consisted of a nuclear head and tail. NV tail is part of a cytoplasmic or nuclear bridge formed during NV production connecting mother with daughter cells. NVs carry aneuploid sets of chromosomes mainly in the head and fewer along the tail. NVs of different size, invade and fertilize other host cells within the same cell population. NV invasion into host cells is accomplished by proteases, as NVs showed strong immunoreactivity against the a_1-chymotrypsin antiserum, detected mainly in the head. Embryonic cells, in addition to NVs, formed nucleolar vlimata (NuVs) which also invaded host cell

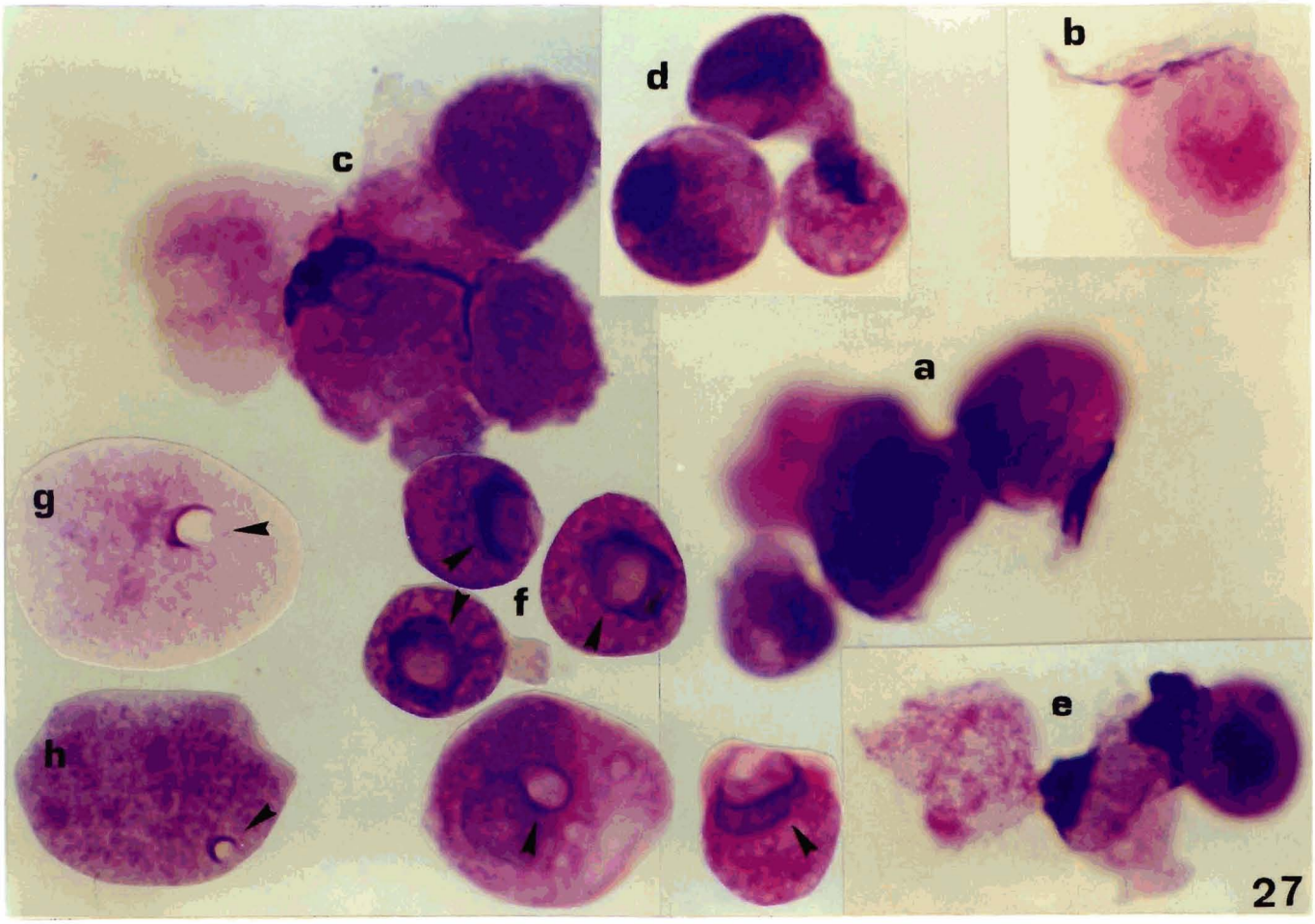
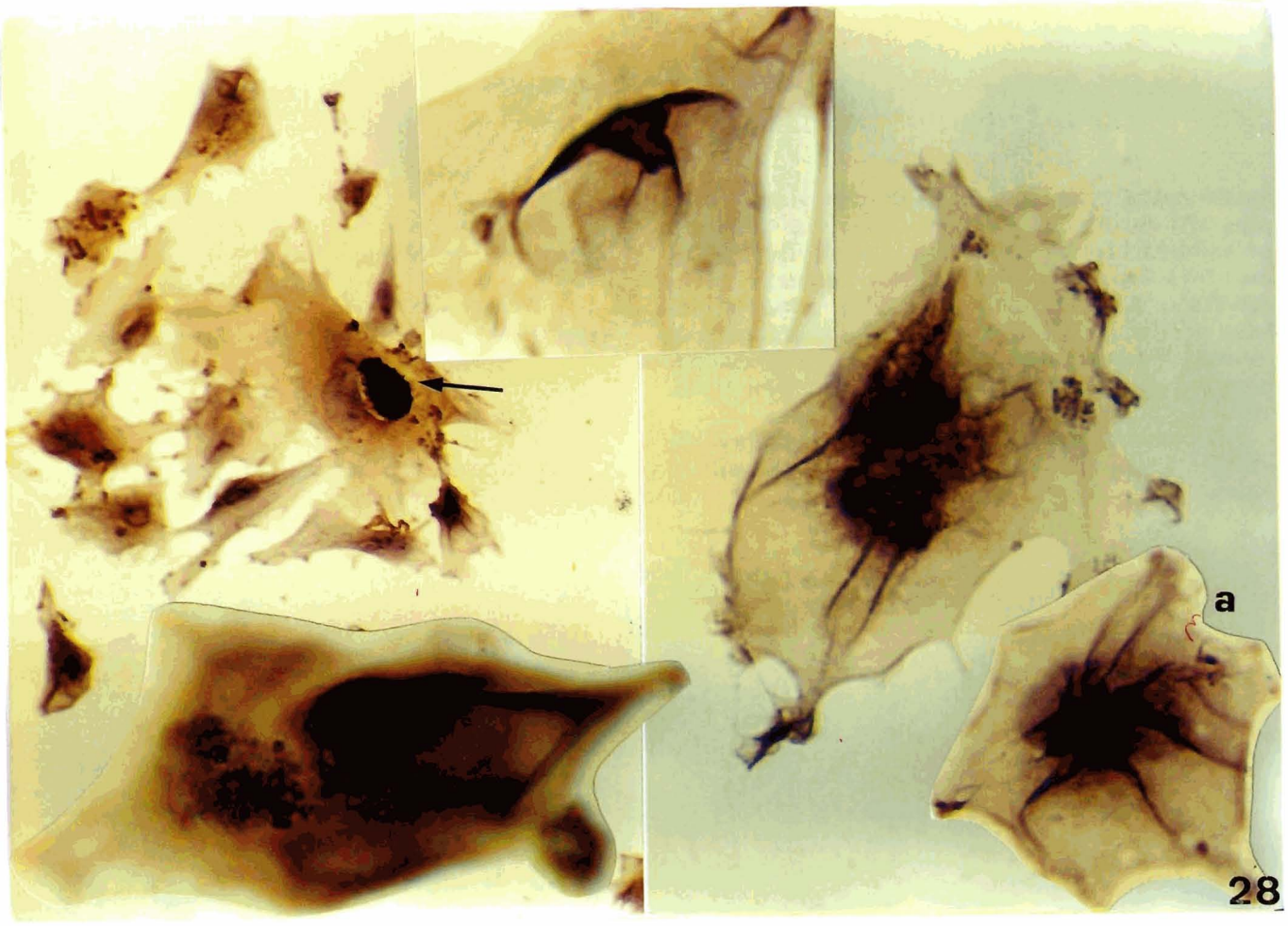


Fig. 27. PAS staining of amniotic cells prepared by the chromosomal spreading technique. **Insets a, b:** Condensed chromosomes attached onto other cells are associated with GSG. **Insets c, d, e:** NV, intact NB and meiotic cell division are accompanied by GSG. **Inset f:** Vacuole morphology of NBs and GSG by the chromosomal spreading technique. HT-PAS. x 1,000. **Insets g, h:** Vacuole morphology of NBs in trophoblastic tissue cells. HT-Giemsa. x 1,000

Fig. 28. Strong immunoreactivity of amniotic cells for a_1-antichymotrypsin, perinuclearly and at GSG-sac (arrow). HT-a_1-antichymotrypsin. x 200. **Inset a:** Strong immunoreactivity shown by NBs, originating from the nucleus. HT-a_1-antichymotrypsin. x 1,000

Fig. 29. The various morphology of immunoreactive NBs of amniotic cells. **Insets a, b, c:** Immunoreactive nucleolar bridges. HT-a_1-antichymotrypsin. x 1,000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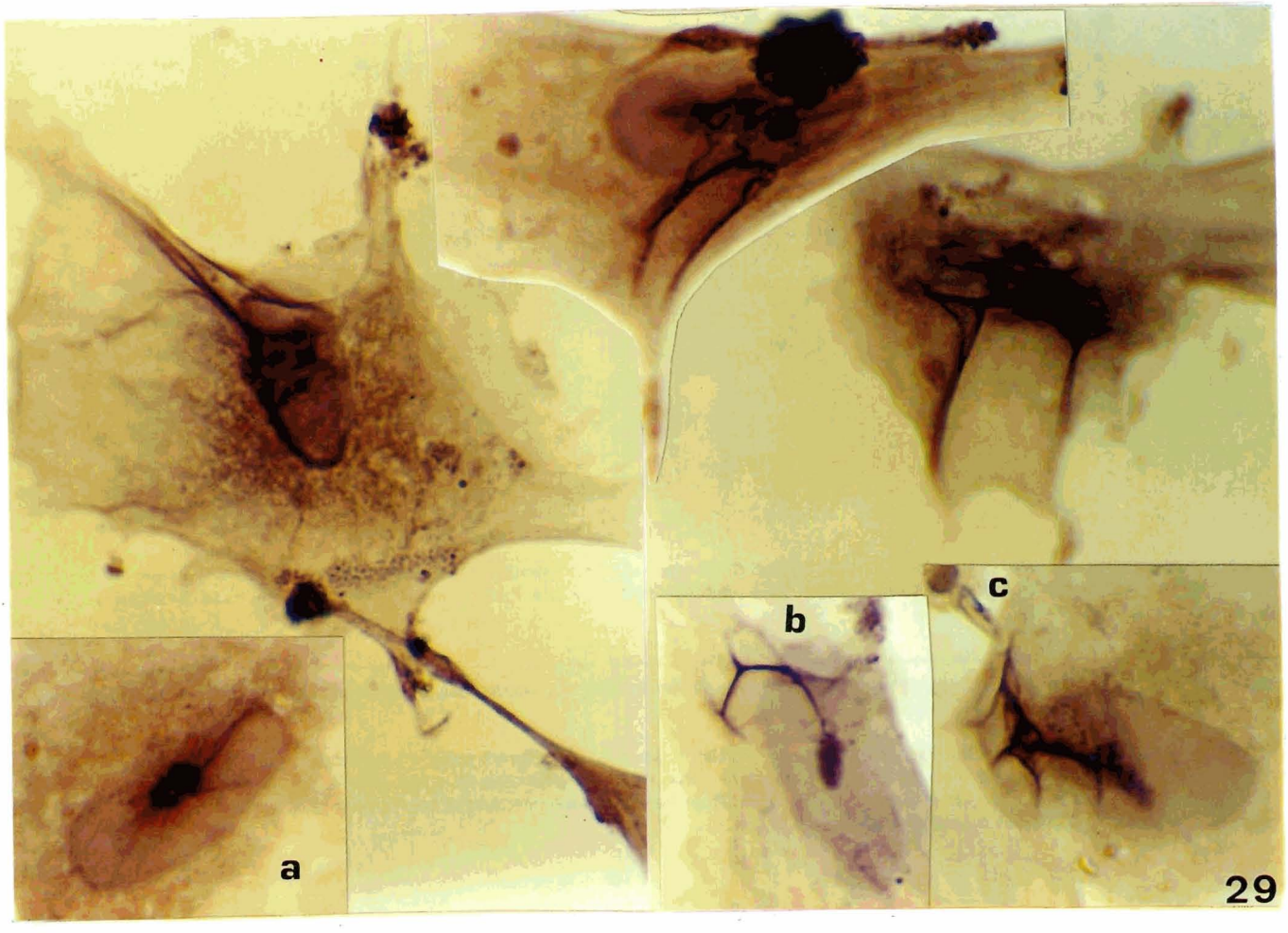
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nuclei and/or cytoplasm. NuVs have previously been reported during early embryogenesis at the stage of hatched blastocyst (Logothetou-Rella, 1993); thus NVs transfer and implant chromosomes (DNA) and NuVs RNA into host cells. These observations have previously been documented in characterizing meiosis of malignant cells and sufficient evidence has been provided to exclude artefacts (Logothetou-Rella, 1994b).

The present observations lead to the embryonic cell life cycle, identical to that of malignant cells as follows:

Zygote —meiosis→ NVs and NuVs —invasion→ recipient host

cells —nuclear fusion / metaphase fusion→ hybrid cells → organogenesis

→ mitosis

NV invasion into host cells documents the parasitic property of embryonic NVs and continuance of

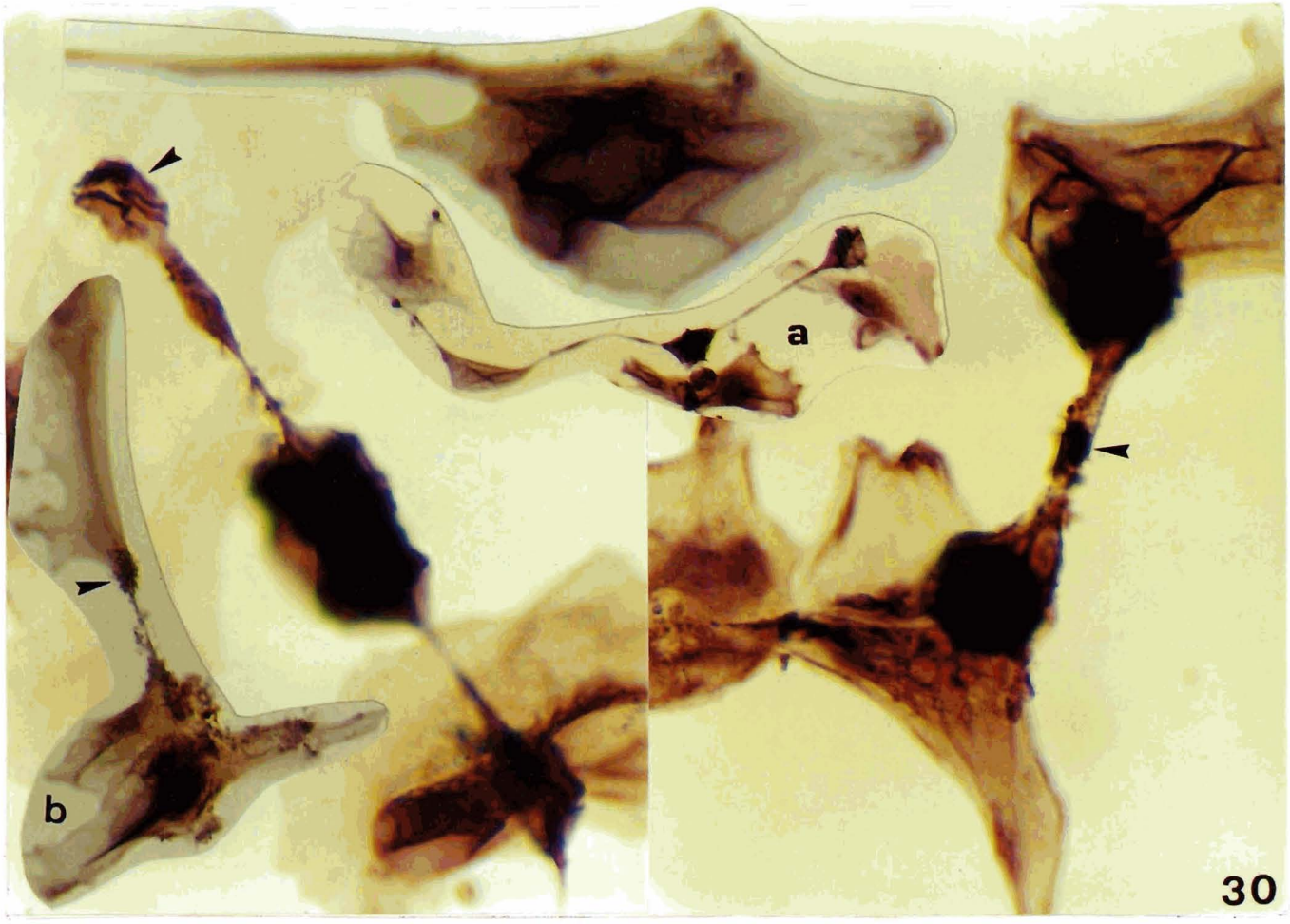
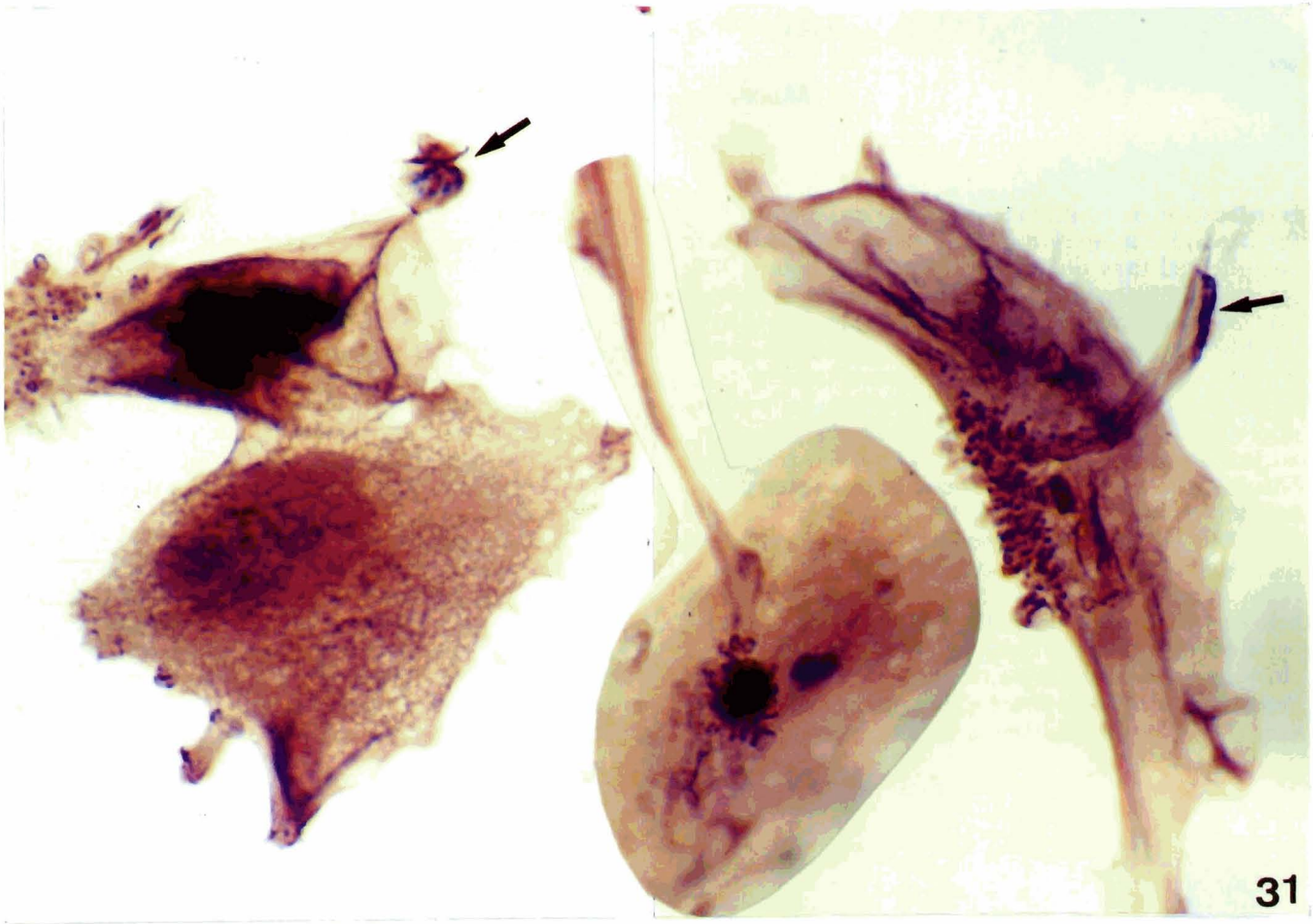


Fig. 30. Strong immunoreactivity of NV-heads, nuclear content of NBs (arrow) and condensed chromosomes (arrow). HT-a_1-antichymotrypsin. x 1,000. **Inset a:** Nuclear and cytoplasmic bridges during meiosis. HT-a_1-antichymotrypsin. x 200. **Inset b:** Immunoreactivity of NV head chromosomes (arrow). HT-a_1-antichymotrypsin. x 400

Fig. 31. Strong immunoreactivity associated with metaphase chromosomes, NBs and condensed chromosomes transfered via NBs. Arrows point at condensed chromosomes. HT-a_1-antichymotrypsin. x 1,000

Fig. 32. Inset a: Immunoreactive dense perinuclear granules in an NV head. HT-a_1-antichymotrypsin. x 1,000. **Inset b:** An NV extruding and implanting immunoreactive condensed chromosomes in the cytoplasm of a host cell. HT-a_1-antichymotrypsin. x 400. **Inset c:** An NV with homogeneously diffused immunoreactivity in the head. HT-a_1-antichymotrypsin. x 1,000. **Other Insets:** Immunoreactivity of two or three cells connected by NBs. Arrows point out condensed chromosomes. HT-a_1-antichymotrypsin x 1,000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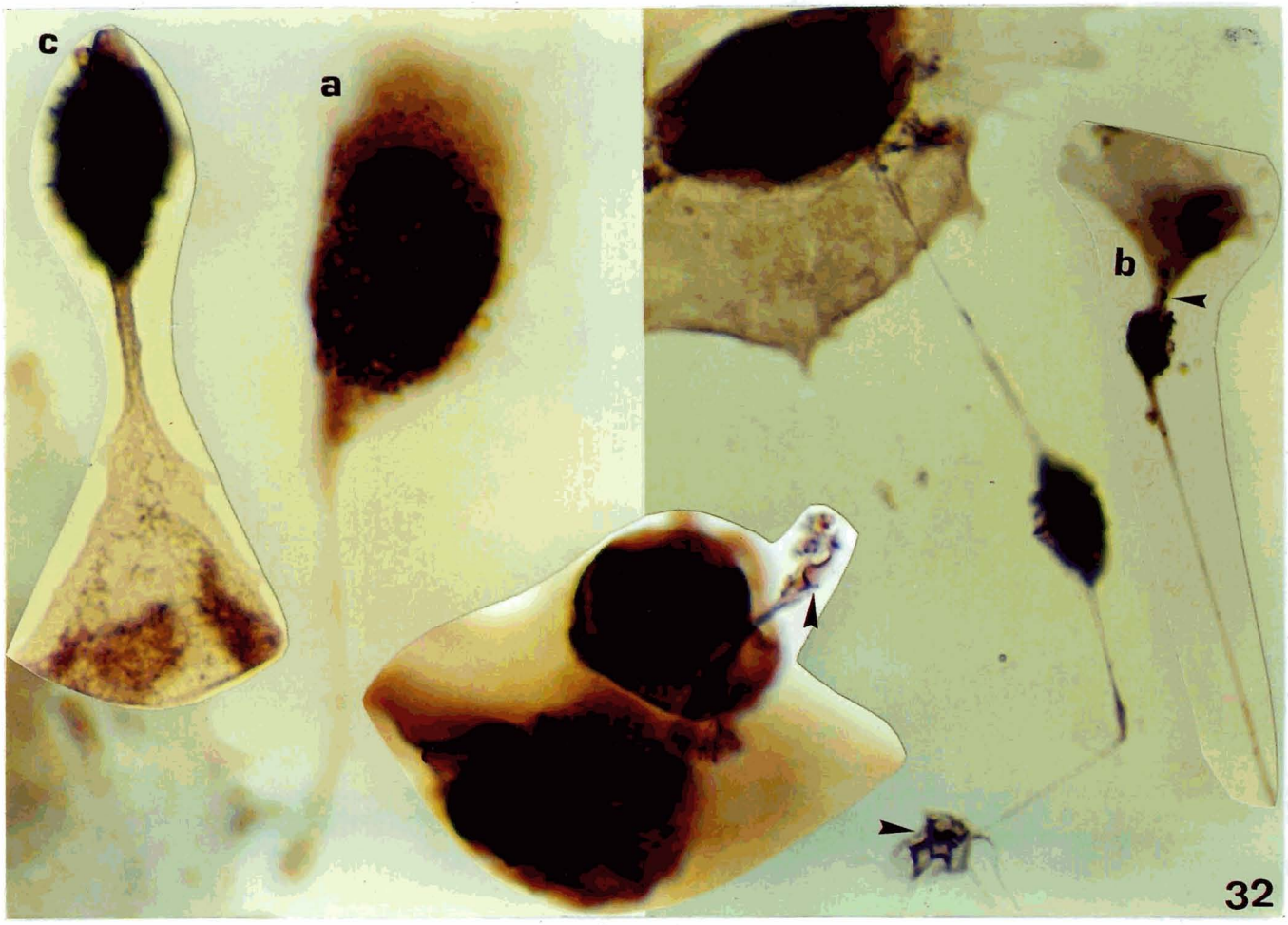
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fertilization during embryonic development. This fertilization appears more anarchical and is more complicated than oocyte fertilization by sperm. The capability of embryonic cell nucleus to fuse with another nucleus while producing an NV in metaphase as well as the different size of cells and nuclei within the same cell population, characterize the high degree of anarchy.

Chromosomal (DNA) and RNA transfer from nucleus to nucleus is accomplished via proteases, nuclear and nucleolar bridges. There were two types of NBs identified. One type is formed between mother and daughter cell during meiotic NV formation. This NB eventually breaks upon separation of cells and comprises the tail of NVs. The other type of NBs was identified between cells in interphase. More than one NB runs between two embryonic cells. The same NB connects cytologically different types of cells. Identification of chromosomes along NBs, strongly documents that genetic material is transfered and exchanged among cells via NBs. Considering that each chromosome carries about 10,000 genes, genetic transfer involves thousands of genes. Protoplasmic bridges have previously been reported as sizable communications between daughter cells during meiosis of male germ cells (Bloom and Fawcett, 1982). Thus, NBs or protoplasmic bridges constitute a characteristic feature of meiotic cells. The best method to visualize intact NBs is hypotonic treatment of cells for 10 min and fixation with 4% formaldehyde in PBS. Longer hypotonic cell treatment causes distortion of NBs and only remnants can be seen. NBs identified in malignant cells are presently under investigation.

The lumen of NBs is full of CFS. This study evidences that CFS is at least composed of GSG-bound protease. Meiotic chromosomes, NVs and GSG showed strong immunoreactivity against a_1-chymotrypsin antiserum. However, the antiserum against a_1-chymotrypsin has cross reacted with calcium activated neutral proteinase (CANP) and since embryonic cells have been sensitive to the inhibitor of CANP

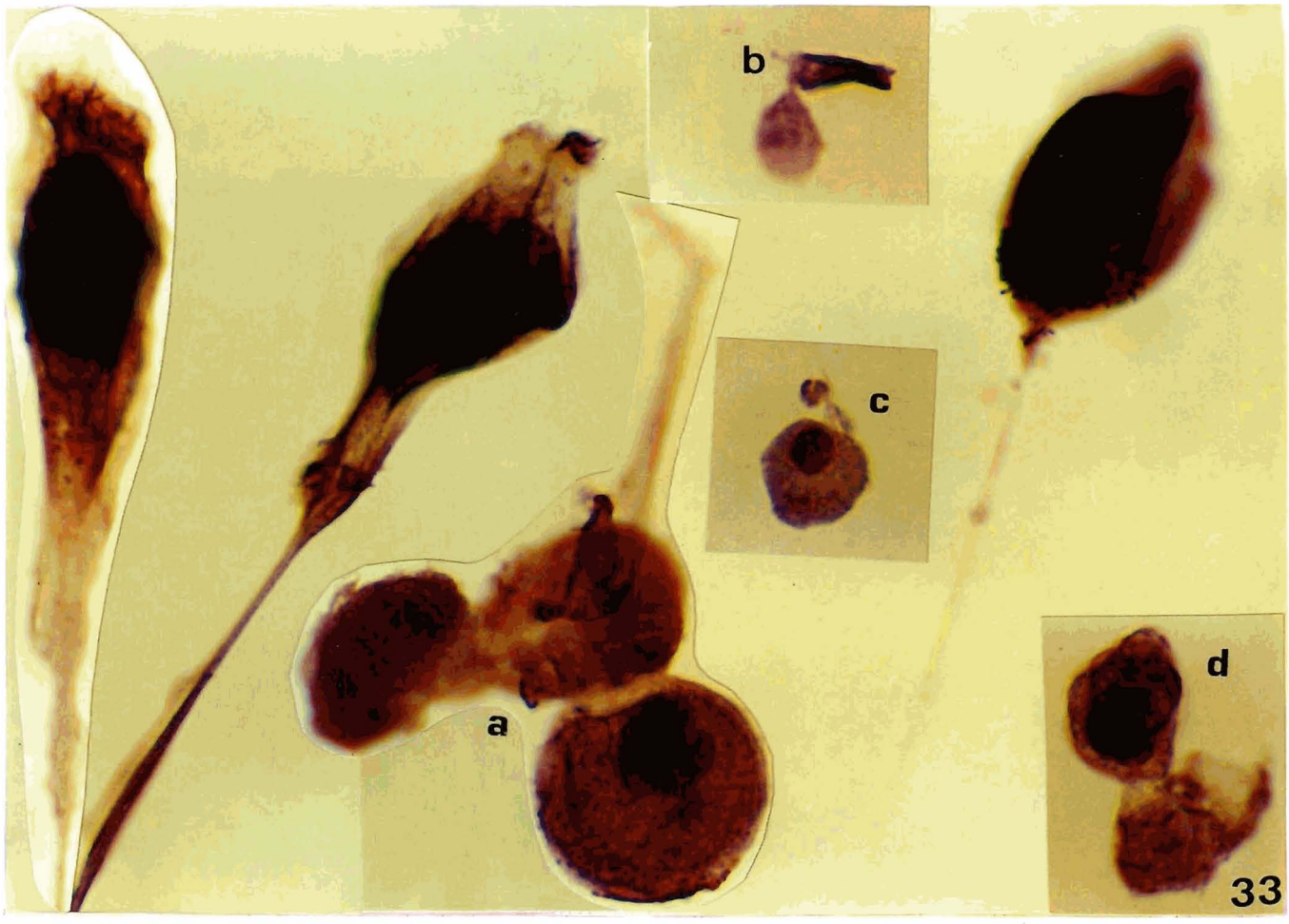

Fig. 33. Free NVs extruding immunoreactive condensed chromosomes. NV tails show different intensity of immunoreactivity. HT-a_1-antichymotrypsin. x 1,000. **Insets a, b, c, d:** Immunoreactivity of amniotic cells, prepared by the chromosomal spreading technique, associated with NBs, condensed chromosomes, nucleolus and nuclear vacuoles. HT-a_1-antichymotrypsin. x 1,000

(Logothetou-Rella et al., 1992, 1994c), the protease associated with meiosis is CANP (Logothetou-Rella, 1995). The antiserum against a_1-chymotrypsin was used in this study to demonstrate protease activity, due to the unavailability of antiserum against CANP.

The ECM of embryonic cells is identical to that of tumor cells (Logothetou-Rella et al., 1989, 1992, 1994d). GSG-sacs and the network of GSG-rivulets provide a communicative cytoplasmic system and support for embryonic cells. A recent report has documented that GSG-sacs, rivulets and GSG-bound protease are formed by cell to cell invasion and interaction (Logothetou-Rella, 1994d) and are responsible for cell nodule formation in trophoblastic cells (Logothetou-Rella et al., 1989). GSGs are also responsible for cell nodule formation in the amniotic cells (late embryonic cells) of this study.

The best method to study cytogenetic morphology and chromosomal content of embryonic NVs is the in situ technique because it maintains intact the head and tail shape of NVs, not always kept by the chromosomal spreading technique. The chromosomal spreading technique provides better chromosomal spreading for karyotyping and cytogenetic morphology useful in exfoliated cytology and genetics.

Cytogenetic analysis of trophoblastic cells documents that early embryonic cells exhibit either higher meiotic and lower mitotic activity, or higher asymmetrical and lower symmetrical meiotic activity than late amniotic cells. The fact that the high ratio of aneuploidy:diploidy is reversed in the late amniotic cells can be evaluated that, embryonic cells shift towards mitosis or symmetrical meiosis along with establishment of organogenesis. However, besides mitosis, symmetrical meiosis also results in diploidy, as diploid metaphases and symmetrical metaphases of meiotic chromosomal morphology have been observed in this study. Hence, it has been difficult to distinguish meiotic from mitotic diploidy in this study. The high sensitivity of diploid and aneuploid cells to CANP-I (Logothetou-Rella, 1994c), indicates that embryonic diploidy (from 9- to 17-week-old embryonic cells) is meiotic since CANP-I does not affect mitotic cells (Logothetou-Rella, 1994c, 1995). Exact correlation of diploidy:aneuploidy with embryonic age is not presented in this study. Cytogenetic analysis and aneuploidy of trophoblastic tissue cells was evaluated in order to avoid possible cytogenetic artefact and aneuploidy due to cultivation of cells. Aneuploidy, NVs and the characteristic events of meiosis are also exhibited by very early mice embryos at the morula stage (preliminary results), and the following events have been reported in the literature: presence of multinucleated blastomeres in 69% of 4-8 cell human embryos, after normal monospermic fertilization (Plachot, 1985); extrusion of pseudonuclei and chromatin aggregates to the extracellular space (Tesarik et al., 1987); and co-existence of nucleus, subnucleus and dispersed chromosomes in the cytoplasm of the same blastomere in a 2-cell embryo (Plachot et al., 1987b). However, nuclear fragmentation and extrusion, chromosomal extrusion and multinucleation are events of meiosis (Logothetou-Rella, 1994b) and by no means define mitosis. Human embryos, from IVF program, displaying normal diploid chromosome number (Plachot et al., 1986) may derive from symmetrical meiosis of the zygote.

Aneuploidy caused by meiosis is not new (Goodenough and Levine, 1974). Aneuploidy caused by meiosis in embryonic cell development is the main new observation of this study.

The identical life cycle and anarchy, sensitive to CANP-I, of malignant and embryonic cells documents that undifferentiated cells divide by meiosis. It is well known that differentiated cells divide by mitosis. Embryonic cells are undifferentiated cells which slowly differentiate and organize into different tissues, organs and functions, reaching establishment of mitotic division. How can a fertilized egg be transformed into a multicellular organism? Transfer of genetic material via NBs, NVs, as well as nuclear, metaphase fusion, all events of meiosis lead to formation of hybrid cells, which is the answer to this question. Zygote development into different types of cells from the relatively simple red blood cells (no nucleus) to the extremely complex brain cells is favoured by hybrid cell formation.

The present observations indicate that cell differentiation involves a shift from meiosis to mitosis (embryogenesis, organogenesis) while cell de-differentiation involves a shift from mitosis to meiosis (neoplasia, viral infections). What impels the many meiotic divisions observed in the embyro to reach their complete and ultimate form? The forces which control differentiation are now restricted between mitosis and meiosis. What causes a mitotic differentiated cell to reverse to a meiotic undifferentiated stage? Is the genetically controlled CANP-CANP-I system, the control system of undifferentiation ⟷ differentiation?

More study is in progress in order to answer these questions.

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