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CASE REPORT

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Gynandroblastoma of the ovary: a case report with an immunohistochemical and ultrastructural study

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Abstract An ovarian gynandroblastoma in a 60-year-old woman is described. The cut-surface of the right ovary showed multiple macrofollicles separated by white fibrous tissues and multiple ill-defined yellowish nodules. The tumour consisted of substantial amount of a granulosa cell element and a Sertoli cell element with intermingled Leydig cells. Immunohistochemically, the tumour cells in both the granulosa cell and Sertoli cell elements were positive for cytokeratin CAM5.2. The granulòsa cell element showed strong membrane staining of Ewing's sarcoma antigen 013 and the Sertoli cell element was focally positive. Vimentin was observed in both the Sertoli cell element and the granulosa cells. Both elements and the Leydig cells were uniformly negative for epithelial membrane antigen, muscle specific actin, CD31 and CD34. The tumour was aneuploid by flow cytometry. The patient was well with no evidence of tumour five months after surgery.

Key words Gynandroblastoma · Granulosa cell tumour · Sertoli cell tumour · Leydig cell · Crystal of Reinke

Introduction

The term "gynandroblastoma" was first coined by Meyer [16] in 1930 to describe a tumour which had features of both androblastoma and granulosa cell tumour. The World Health Organization (WHO) has suggested that the diagnosis of gynandroblastoma be reserved for those lesions containing substantial amounts of recognizable ovarian and testicular elements that are intermingled [20]. Gynandroblastoma is an extremely rare tumour [4, 5, 9, 10, 14, 15, 18, 19] and Fox and Langley [8], in a review of the literature in 1976, found only eight unequivocal examples. Since then only a few isolated cases have been reported [2, 3, 11, 13, 17, 21, 23]. We describe a case of gynandroblastoma of the ovary and discuss the histogenesis of this neoplasm in this report.

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Clinical history

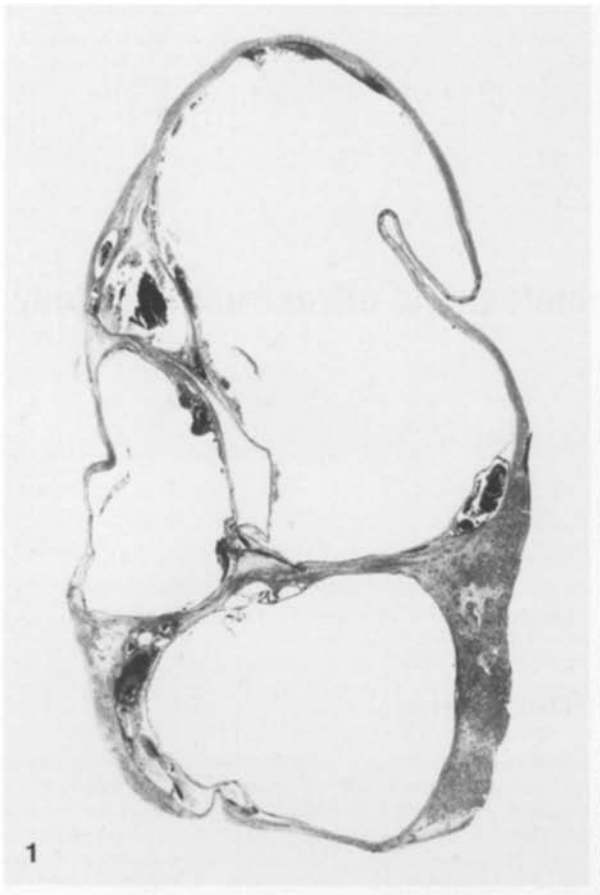
A 60-year-old postmenopausal Japanese woman (gravida 3, para 1) had a 6-month history of lower abdominal swelling. Pelvic examination showed a first-sized uterine tumour and cystic changes of the right ovary measuring 4 cm. There was no evidence of virilism. Preoperative serum hormonal assays were not done. Laboratory data showed no abnormalities. A total hysterectomy and a right salpingo-oophorectomy were performed in January 1996. The patient was well with no sign of recurrence 5 months after diagnosis.

Materials and methods

The resected tissues were fixed in 10% buffered formalin, routinely processed and paraffin embedded. Sections were examined with haematoxylin and eosin, periodic acid-Schiff with and without diastase, Masson trichrome stain, reticulin stain and Sudan III stain for fat.

Tissue sections were evaluated immunohistochemical, using the peroxidase-antiperoxidase technique or avidin-biotin peroxidase complex (ABC) technique with ABC kit (Vector Laboratories, Burlingame, Calif., USA). The PAP technique was used for S-100 protein (polyclonal, Dakopatts, Glostrup, Denmark, dilution; 1:200), and the ABC technique for CAM 5.2 (monoclonal, Becton Dickinson, San Jose, Calif., USA; 1:1), epithelial membrane antigen (EMA; monoclonal, Dakopatts; 1:100), vimentin (monoclonal, Amersham, Little Chalfont, UK; 1:40), alpha-smooth muscle actin (monoclonal, Dakopatts; 1:50), muscle specific actin (HHF35; monoclonal, Enzo Diagnostics, New York, N.Y., USA; 1:50), laminin (monoclonal, Boehringer Mannheim Biochemica, Mannheim, Germany; 1:50), CD31 (monoclonal, Dakopatts; 1:20), CD34 (monoclonal, Becton Dickinson; 1:25), and Ewing's sarcoma antigen (monoclonal, Signet Laboratories, Dedham, Mass., USA; dilution 1:100). Appropriate positive and negative controls were used.

Samples for electron microscopy were taken from the formalin-fixed specimen. They were re-fixed with 1.2% glutaraldehyde and post-fixed with 1% osmium tetroxide. After dehydration in a graded ethanol series, they were embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate and were observed at 50 kV with a Hitachi HS-9 electron microscope.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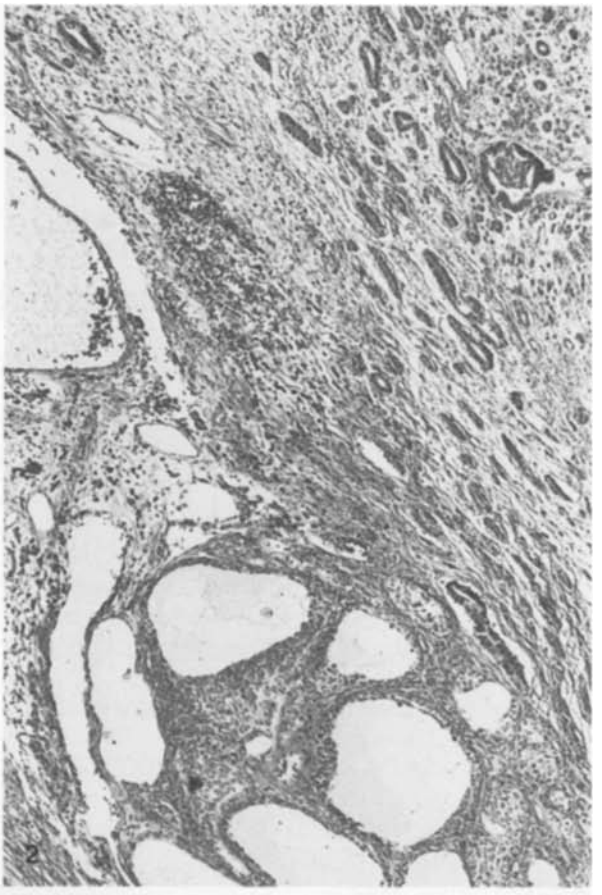
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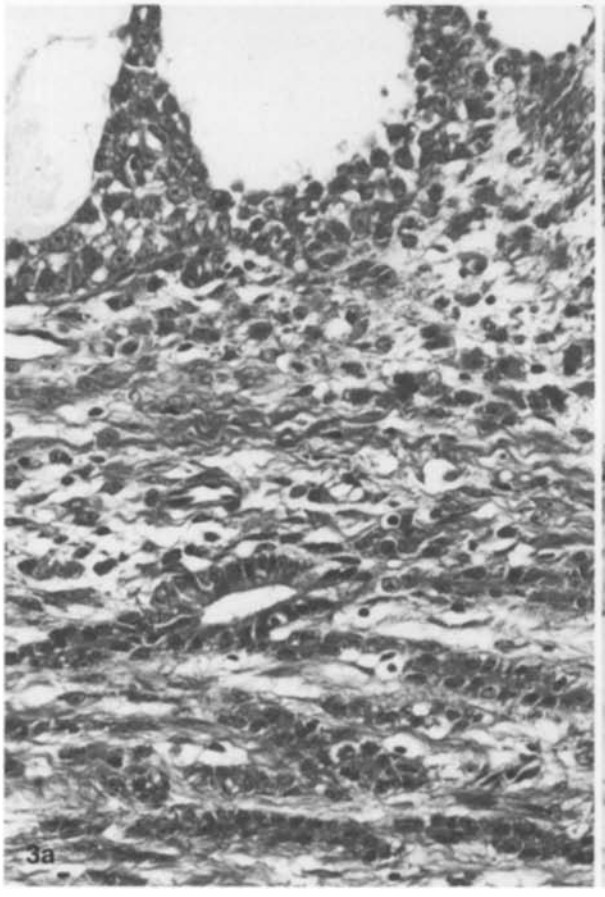
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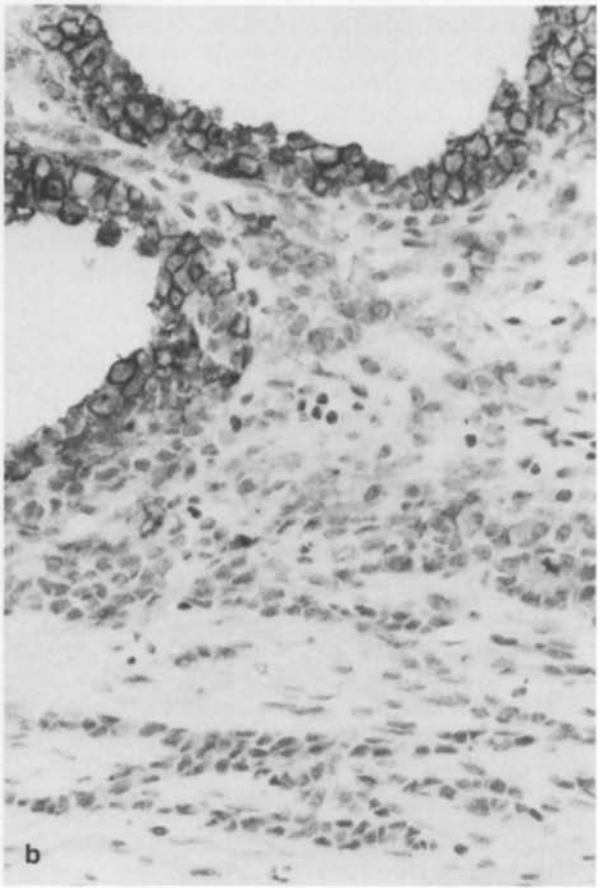
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Flow cytometry was performed on the formalin-fixed, paraffin-embedded tissue block. The technique of Hedley et al. [10] was used for DNA analysis, with some minor modifications. The methods used have been previously described [6].

Pathological findings

Macroscopically, the right ovarian tumour was firm-to-hard with a smooth capsule, measuring 3.5×3.0×2.5 cm. The cut-surface showed multiple macrofollicles of various sized separated by white fibrous tissue and multiple ill-defined yellowish nodules measuring up to 0.8 cm (Fig. 1). There was neither necrosis nor haemorrhage. The uterus, which was enlarged measuring 13.0×7.0×5.0 cm, showed a 5.0 cm intramural leiomyoma in the body.

Microscopically, the ovarian tumour was composed of a granulosa cell element which comprised 30% of the tumour and a Sertoli cell element of around 70%. These two elements were intimately mixed (Fig. 2). The macrofollicles were surrounded by granulosa cells. The areas containing granulosa cells showed macro- and microfollicular and trabecular patterns. These tumour cells were characterized by ovoid or round nuclei with single small nucleoli and pale abundant cytoplasm (Fig. 3). In some areas luteinized granulosa cells predominated (Fig. 4). Call-Exner bodies were observed occasionally. Some cells had nuclear grooves. A transition from the granulosa cell element to a Sertoli cell element was observed (Fig. 4). In the Sertoli cell areas, hollow tubular and cord-like structures were prominent (Fig. 5). The Sertoli cells were taller and cotained abundant foamy cytoplasm, with clear vesicular nuclei (Figs. 4, 6). Mitotic figures were uncommon in both elements. In the fibrous stroma there were many luteinized stromal cells (Leydig cells) which had round nuclei with small nucleoli and abundant eosinophilic cytoplasm. Crystals of Reinke were observed light-microscopically in some of these cells (Fig. 6). Some stromal cells had small round nuclei and abundant vacuolated cytoplasm. Reticulin fibres surrounded groups of tumour cells in the granulosa cell areas. Sudan III staining showed lipid droplets in the tumour cells of both Sertoli cell and granulosa cell elements and in some Leydig cells.

The uterine tumour was a leiomyoma. The endometrium showed simple endometrial hyperplasia without atypia (Fig. 7). Eosinophilic and ciliated cell metaplasia was also observed.

Immunohistochemically, both granulosa cell and Sertoli cell elements were diffusely and strongly positive for CAM 5.2. The granulosa cell element showed diffuse and strong membrane staining of 013 (Fig. 3b) and focal staining of vimentin, S-100 protein and alpha-smooth muscle actin while the Sertoli cell element was focally positive for 013 and negative for vimentin. Leydig cells were focally positive for vimentin and 013. The basement membrane of both granulosa cell and Sertoli cell elements showed laminin staining. The granulosa cell, Sertoli cell and Leydig cell elements were uniformly negative for HHF35, EMA, CD34 and CD31.

The ultrastructural specimens contained only Sertoli cell and stromal cell elements. The Sertoli cells were columnar with long axis oriented perpendicular to the lumen. The nuclei were deeply indented with focal condensation of chromatin peripherally and nucleoli were conspicuous. Well developed desmosomes were present. The cytoplasm contained a moderate amount of rough endoplasmic reticulum, mitochondria, lipid droplets and intermediate filaments (Fig. 8). Luteinized stromal cells had rounded nuclei with finely dispersed chromatin and inconspicous nucleoli, abundant rough endoplasmic reticulum, mitochondria and secretory granules (Fig. 9). Crystals of Reinke were not observed.

The ovarian tumour was aneuploid with a DNA index of 1.14 and S-phase fraction of 3.3%. The coefficient of variation was 2.7%.

Discussion

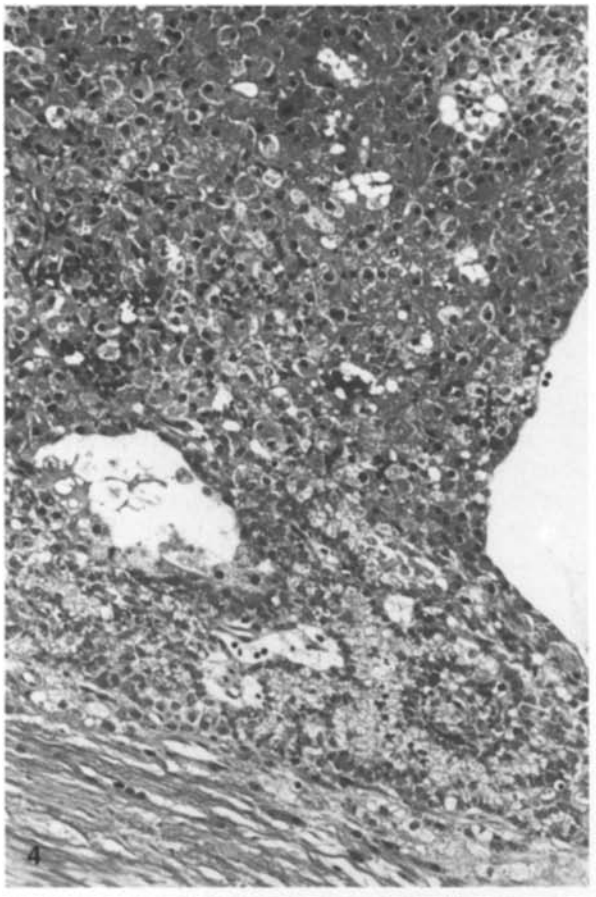
The current ovarian neoplasm was identical histologically to the previously reported cases of gynandroblastoma [1–5, 9, 11, 13–15, 18, 19] although hormonal studies were not performed and no hormonal symptoms were present. Substantial amounts of recognizable ovarian (granulosa cell tumour) element and testicular (Sertoli cell tumour with Leydig cells) elements were intermingled. The diagnosis of gynandroblastoma depends on histological examination, rather than the clinical and laboratory findings [7]. Occasional foci of female-type cells in an otherwise typical Sertoli-Leydig cell tumour, or of male cell types in granulosa-stromal cell tumour, do not constitute sufficient evidence for this neoplasm. Gynandroblastoma matching the WHO definition [20] strictly is very rare and there have been probably no more than 20 acceptable documented cases.

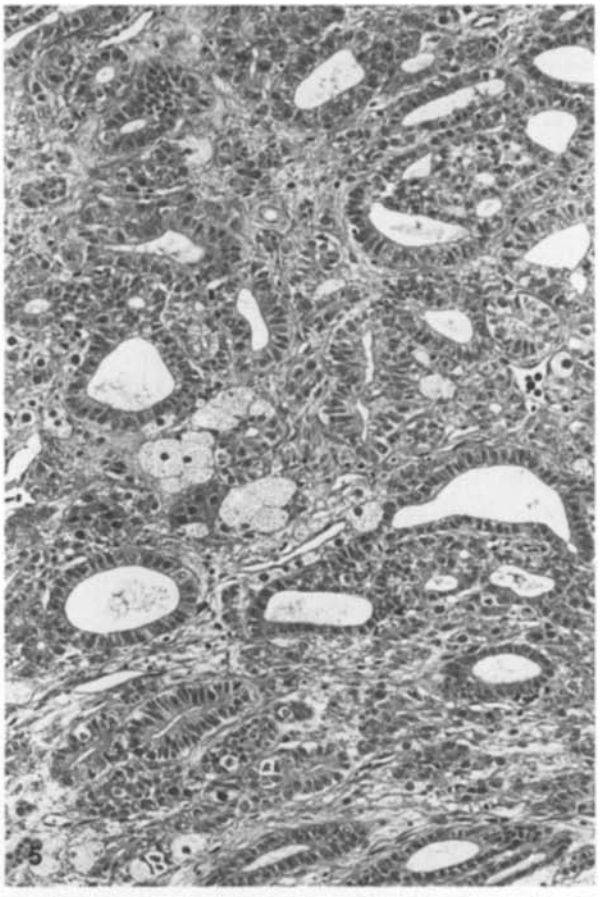
Gynandroblastomas may produce clinical evidence of androgenic or oestrogenic function. In our case the endometrial hyperplasia observed might be due to the oestrogenic effect by the tumour. In a review of ten gynandroblastomas by Novak [18], virilism was the most common symptom; this was present in seven cases and there was one case each of amenorrhoea and endometrial cystic hyperplasia while the remaining case showed no endocrine disturbance. Novak mentioned that the androgenic stimuli overrode the oestrogenic stimuli as far as the general pattern was concerned, so when both were functional, masculinizing manifestations were usually seen. This lack of correlation between histology and biological function in gonadal stromal tumours is not usual [18].

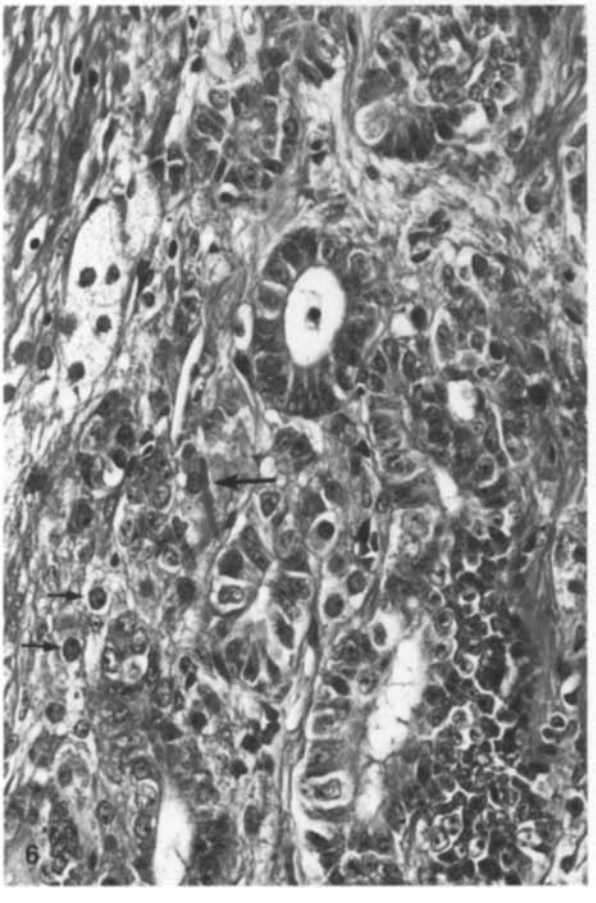
Fig. 1 Tumour showing multiple macrofollicles of various sizes separated by fibrous tissue forming multiple ill-defined nodules

Fig. 2 Note macrofollicles surrounded by granulosa cells and tubular structures of sertoli cells in the upperright field

Fig. 3 **a** Macrofollicles composed of granulosa cells (*top*), stromal cells (*middle*) and a Sertoli cell element (*bottom*). **b** The granulosa cell element shows membrane staining of 013 while Sertoli cell elements shows no reactivity (immunostaining)







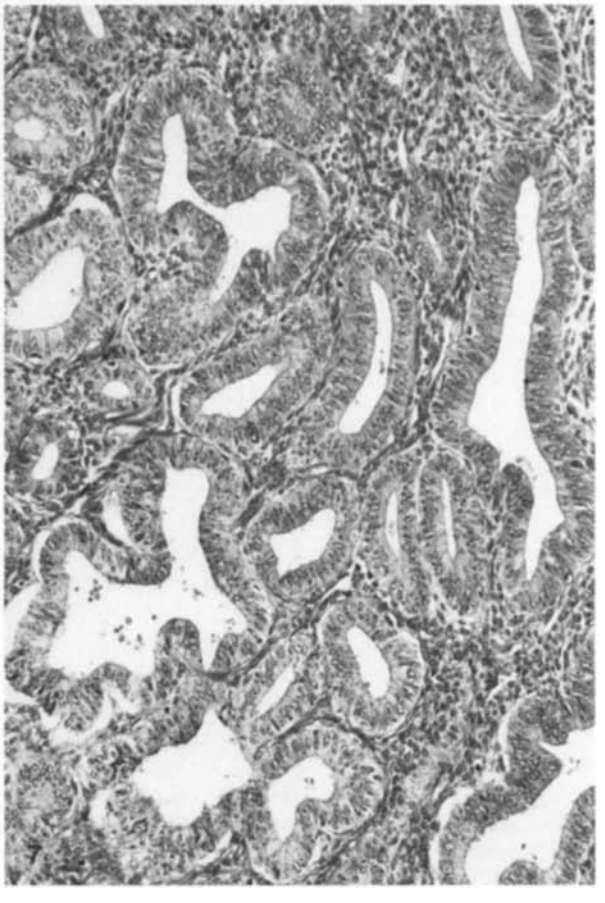
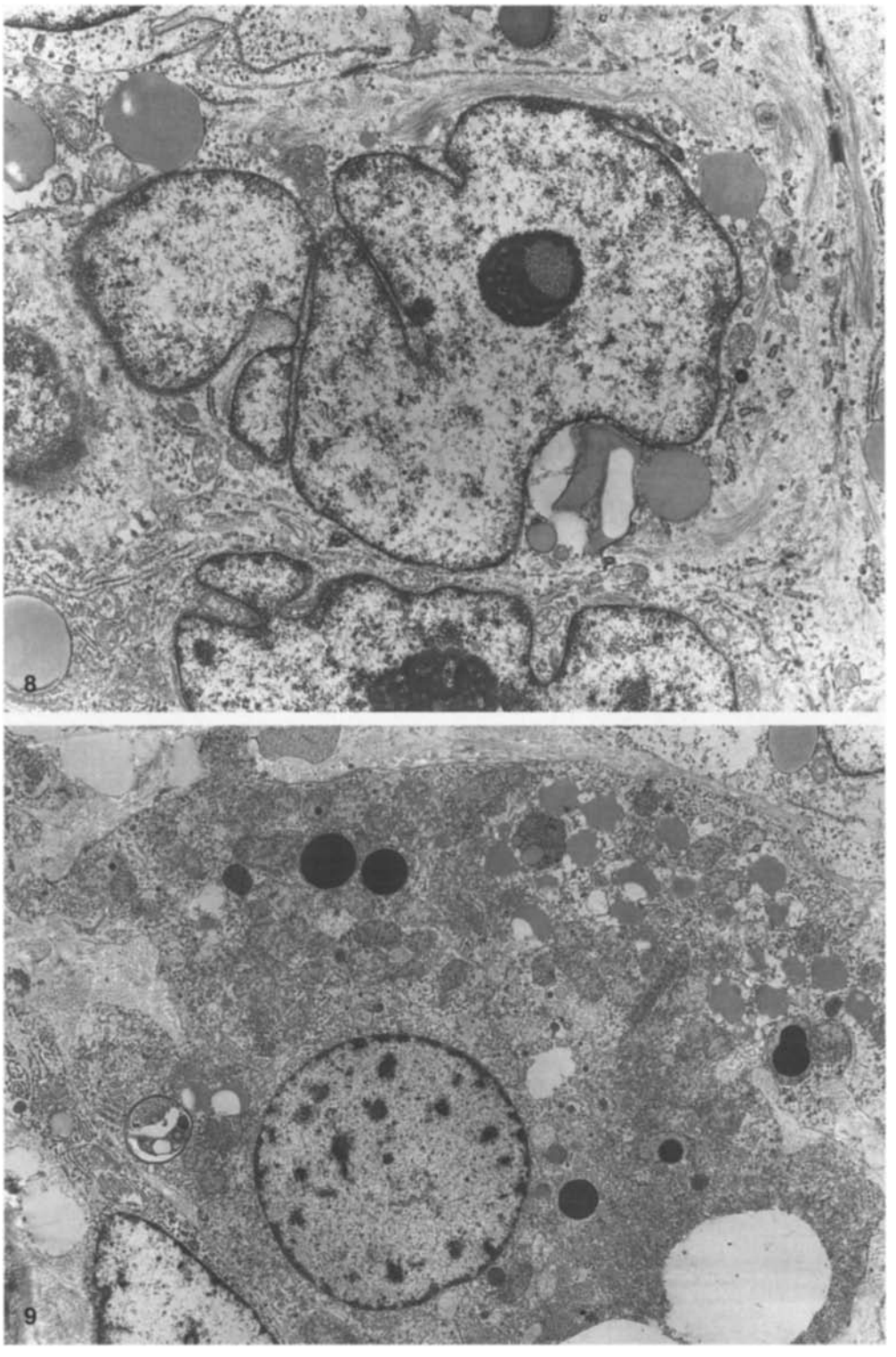


Fig. 8 Electron micrograph of a Sertoli cell showing deeply indented nucleus, desmosomes, a moderate amount of rough endoplasmic reticulum, mitochondria, lipid droplets and intermediate filaments (×7,800

Fig. 9 Electron micrograph of a luteinized stromal cell showing rounded nucleus, abundant rough endoplasmic reticulum, mitochondria and secretory granules (×4,200)



◀ **Fig. 4** A transition from the luteinized granulosa cell element (*top*) to a lipid-rich Sertoli cell element (*bottom*)

Fig. 5 In the Sertoli cell area, tumour cells form hollow tubular and cord-like structures and there are many stromal cells (Leydig cells). Some stromal cells have small round nuclei and abundant vacuolated cytoplasm

Fig. 6 Sertoli cell element and luteinized stromal cells (*small arrows*). Note a crystal of Reinke in a Leydig cell (*large arrow*)

Fig. 7 The endometrium revealed simple endometrial hyperplasia without atypia

In this case the granulosa cell element showed various histological features, including macro- and microfollicular, trabecular and solid patterns with luteinization. In a limited area it was difficult to discern granulosa cells from Sertoli cells. In the Sertoli cell element, an hollow tubular arrangement was dominant. Immunohistochemically, vimentin and 013 were useful in distinguishing granulosa cell elements from Sertoli cell elements in this case. The granulosa cell element was positive for vimentin and 013 while the Sertoli cell element was negative for vimentin and focally positive for 013. Loo et al. [12] reported immunohistochemical staining of ovarian granulosa cell tumours with 013.

Only three cases of ovarian gynandroblastoma have been examined ultrastructurally [3, 11]. Our ultrastructural specimens, containing only the Sertoli cell and stromal cell element, showed the typical features Sertoli and luteinized stromal cells. The presence of intermediate filaments in the Sertoli cells was consistent with CAM5.2 immunoreactivity. Chalvardjin and Derzko [3] reported that tubular components resembled those of the rete ovarii more closely than they did Sertoli cells.

In the reported cases, only three gynandroblastomas [8, 19], including our case, have contained crystals of Reinke. In this case there were many luteinized stromal and a few Leydig cells. These cells, as well as granulosa cells and Sertoli cells, were sudanophilic. It is possible that these stromal cells may represent an indigenous non-neoplastic cell population that is in turn induced to differentiate by neoplastic sex-cord elements into cells active in steroid production.

There has been no flow cytometric study of gynandroblastomas. The current case had an aneuploid DNA content although it lacked frank histological evidence of malignancy; further studies need to clarify the significance of ploidy status in gynandroblastomas.

The histogenesis of gynandroblastoma remains unclear. Meyer [16] originally postulated a hermaphroditic tumour in which indifferent germinal epithelium developed into male and female cells. Because of the presence of both elements of granulosa cells and Sertoli cells, it has been assumed that such a growth arises from undifferentiated gonadal mesenchyme [4, 14, 19]. Our findings of apparent female and male cells closely mixed together supports the suggestion of divergent differentiation from a common precursor. Taylor et al. [22] supported the versatility of cells of the primitive undifferentiated type and believed that they had the potential to develop into either ovarian or testicular tissue in the formation of tumours. Mechler and Black [15] suggested a teratomatous origin.

None of the previously reported gynandroblastomas has manifested evidence of malignant behaviour. This neoplasm is considered to be well differentiated and its malignant potential is probably low. Conservative therapy would, therefore, seem to be justified.

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