ANTICANCER RESEARCH *25*: 1725-1730 (2005)

Expression of Inhibin/Activin Subunits, Sialyl-Lewis A (CA 19-9, sLea) and Sialyl-Lewis X (sLex) Carbohydrate Antigens in a Hydatidiform Mole with Persistent Polymorphic Trophoblastic Hyperplasia

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Abstract. *The persistence of polymorphic trophoblastic hyperplasia in a hydatidiform mole is an extremely rare condition. Its early diagnosis is essential since such cases can transform into invasive tumours. Materials and Methods: The paraffin-embedded biopsies were routinely stained with HE. Immunohistochemical staining reactions were performed with monoclonal antibodies against inhibin-α, inhibin-βA and inhibin-βB subunits. Additional immunohistochemical reaction was performed with, Sialyl-Lewis A and Sialyl-Lewis X and glycodelin. Results: Large villi and hydatidiform villi with ranging syncyctio- and cytotrophoblasts were seen. Intervillous proliferating trophoblasts showed cell- and nuclear polymorphy with invasion of the myometrium wall. The immunohistochemistry exhibited strong positivity for inhibin-α, inhibin-βA and inhibin-βB subunits in trophoblastic tissue, while the decidua was negative. Sialyl-Lewis A and Sialyl-Lewis X showed no or minimal focal immunohistochemical reaction. Conclusion: A complete hydatidiform mole with hyperplasia and proliferation presents a high risk of developing a persistent (eventually metastatic) trophoblastic disorder and, in up to 15% of the cases, an invasive mole. In 2.5 % of the cases it can transform into a choriocarcinoma. Since the inhibin/activin subunits reacted positively with trophoblastic tissue, they might be a useful diagnostic marker for hydatidiform mole with persistence of polymorphic trophoblastic hyperplasia.*

Inhibins are dimeric glycoproteins, composed of an α-subunit and one of two possible β-subunits (β_A or β_B), that were initially isolated from the gonads and identified as modulators of FSH production from the anterior pituitary gland (1-3). Two forms of inhibin, namely inhibin-A and inhibin-B, exist. These proteins were shown to be disulphide-linked dimers which have a common α-subunit but just one of two β-subunits, differentiated as inhibin-A (α-βA) and in inhibin-B (α-βB).

The persistence of polymorphic trophoblastic hyperplasia in a hydatidiform mole is an extremely rare condition. Its early recognition is essential since it can transform into an invasive tumour. Increased levels of circulating inhibin have previously been reported in molar pregnancy, and it has been proposed that serum inhibin concentrations may be of value in the management of trophoblastic disease (4). Increased concentrations of inhibin can also be found in hydatidiform mole (5). Recently, it has been suggested that serum inhibin-A and activin-A measurements might be of value in the diagnosis and short-term follow-up of molar pregnancy (6). Inhibin-alpha and -beta subunits are consistently co-expressed immunohistochemically in syncytiotrophoblast in complete and partial moles, suggesting that these glycoproteins might be useful tissue markers in the differential diagnosis of trophoblastic lesions (7).

Tumour cells can produce and secrete large amounts of several antigens, which differ in their glycosylation from antigens produced by normal cells. Therefore, unique carbohydrate epitopes and larger portions of the inner nuclear region, which are not accessible to antibodies due to the numerous branched carbohydrate chains, can be demonstrated. Several of such structures such as the MUC1 core protein,

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Key Words: Invasive mole, gestational trophoblastic disease, inhibin, activin, Sialyl-Lewis A, glycodelin, immunohistochemistry.

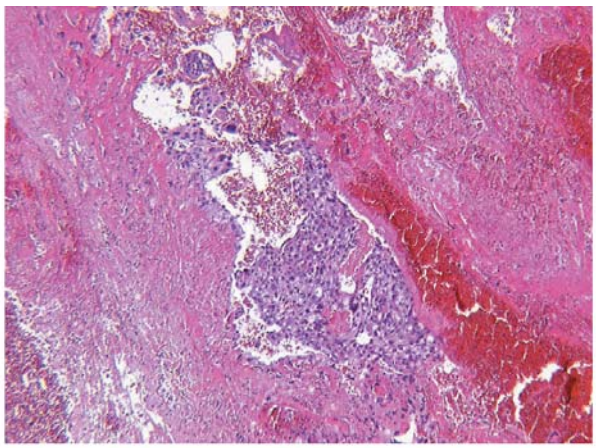
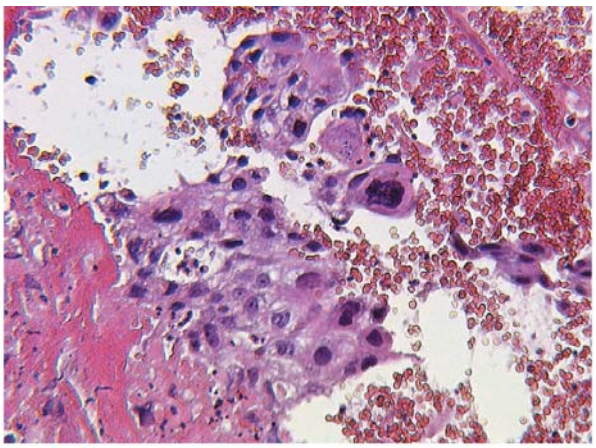


Fig. 1



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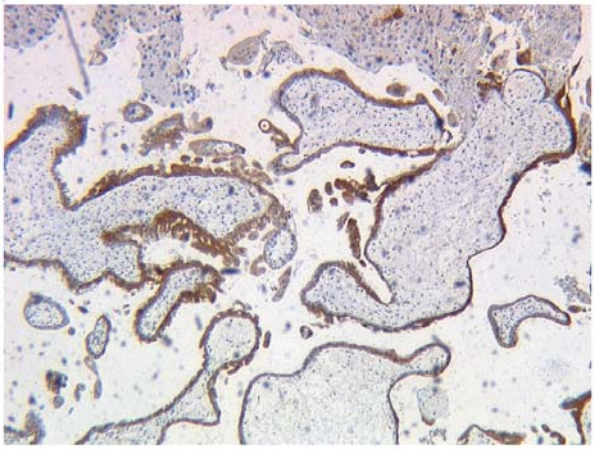

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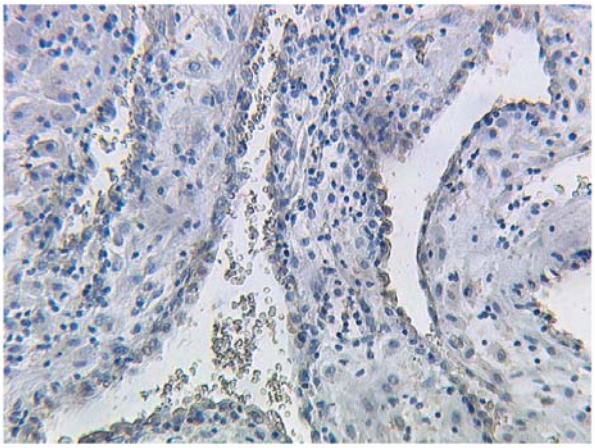

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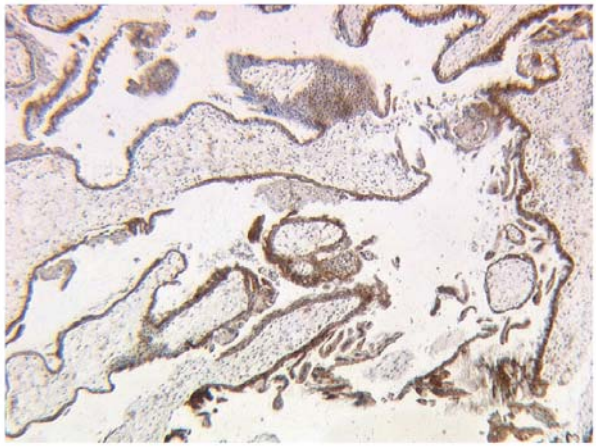

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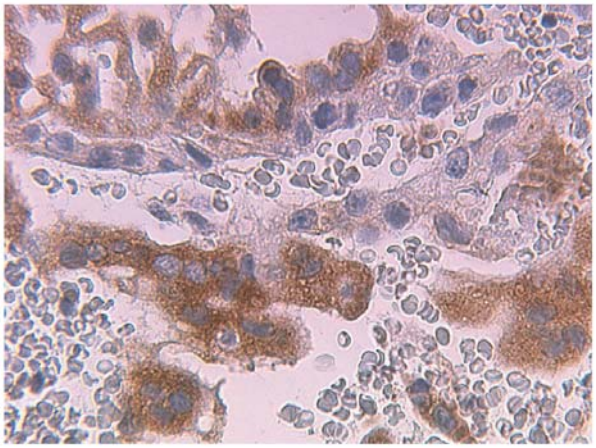


Fig. 6

Figures 1-2. *Haematoxylin staining reaction. Extravillous trophoblast with cellular and nuclear polymorphy (Figure 1 x100; Figure 2 x250).*

Figure 3. *Inhibin-alpha staining reaction (Figure 3 x100; Figure 4 x250). Fig. 3: villous trophoblast. Fig. 4: extravillous trophoblast.*

Figures 5-6. *Inhibin-beta immunohistochemical reaction (Figure 5 x100; Figure 6 x250). Fig. 5: villous trophoblast. Fig. 6: extravillous trophoblast.*

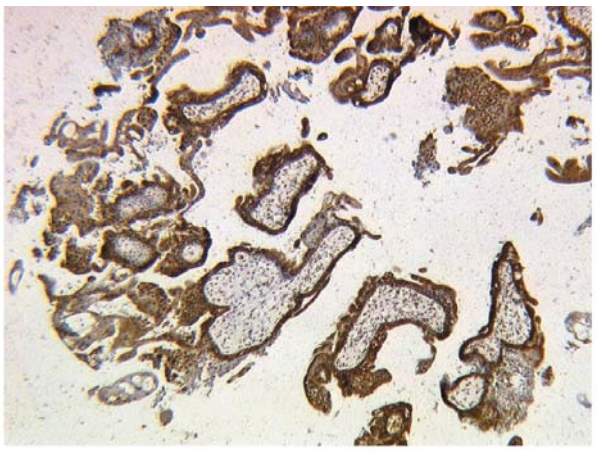
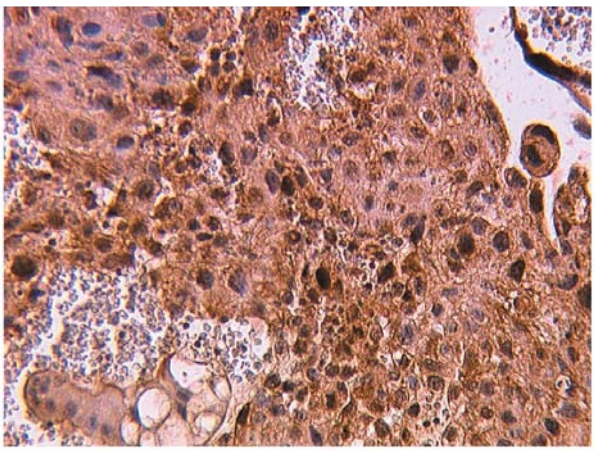


Fig. 7



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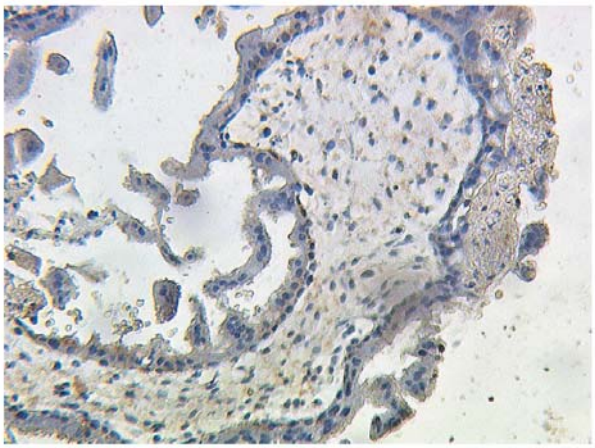

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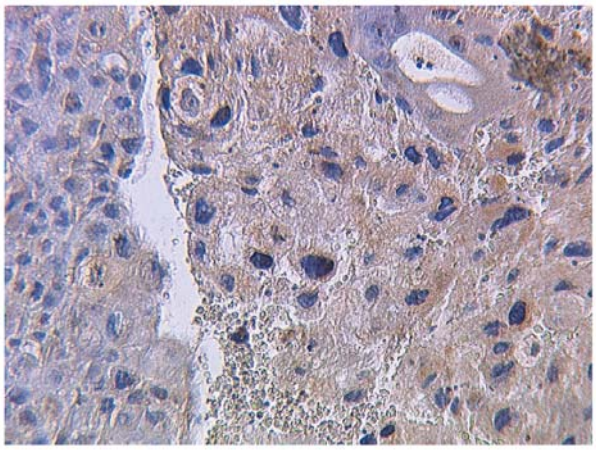

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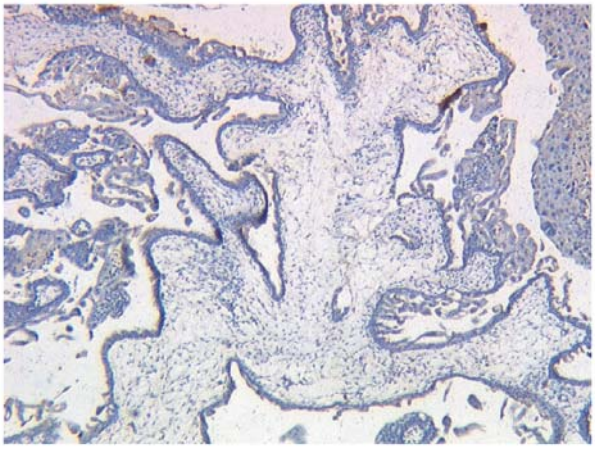

Fig. 11

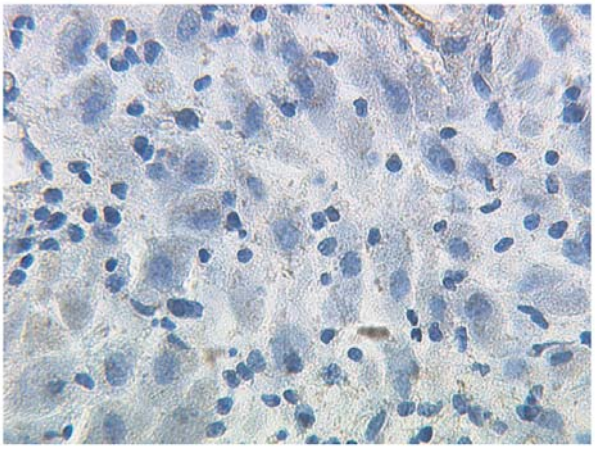

Fig. 12

Figures 7-8. *Inhibin-betaB immunohistochemical reaction (Figure 7 x100; Figure 8 x250). Fig. 7: villous trophoblast. Fig. 8: extravillous trophoblast.*

Figures 9-10. *Sialyl-Lewis A immunohistochemical reaction (Figure 9 x150; Figure 10 x400). Fig. 9: villous trophoblast. Fig. 10: extravillous trophoblast.*

Figures 11-12. *Sialyl-Lewis X immunohistochemical reaction (Figure 11 x100; Figure 12 x400). Fig. 11: villous trophoblast. Fig. 12: extravillous trophoblast.*

Table I. *Antibodies used for immunohistochemical characterisation.*

Antibody	Clone	Isotype	Dilution	Source
Inhibin-α	R1	mouse IgG_{2a}	1:50	Serotec, Oxford, United Kingdom
Inhibin-βA	E4	mouse IgG_{2b}	1:50	Serotec, Oxford, United Kingdom
Inhibin-βB	C5	mouse IgG_{2a}	1:10	Serotec, Oxford, United Kingdom
Sialyl-Lewis A	KM 231	mouse IgM	1:60	Calbiochem, San Diego, U.S.A.
Sialyl-Lewis X	KM 92	mouse IgM	1:100	Calbiochem, San Diego, U.S.A.

Thomsen-Friedenreich (TF) epitope, CA19-9 and CA50 can be demonstrated in malignant cells, while their expression is limited in normal tissue. The biologically relevant ligands for selectins are diverse and complex macromolecules that share in common certain types of anionic carbohydrate structures. Most, but not all, ligands carry sialylated, sulfated and/or fucosylated sequences normally found at the non-reducing termini of N-linked or O-linked oligosaccharides, or on glycosphingolipids. The Sialyl-Lewis A (CA 19-9) and Sialyl-Lewis X (sLex) carbohydrate antigens are expressed during pregnancy (8) and are the main ligands for selectins (9), which are thought to be involved in tumour spreading and metastasis (10).

The aim of this study was to analyse a rare case of persistence of polymorphic trophoblastic hyperplasia in a hydatidiform mole with inhibin/activin subunits. Additionally we evaluated the immunohistochemical expression of Sialyl-Lewis A and Sialyl-Lewis X carbohydrate antigens.

Case Report

A 25-year-old G1 PO woman was diagnosed during routine ultrasound examination with a deformed amniotic sac without any fetal compounds. The last menses had occurred four weeks previously. A therapeutic curettage was performed. Pathological analysis revealed a non-malignant, non-invasive embryonic mole. Subsequent serum beta-hCG controls showed a rapid increase, so a re-curettage was performed. Pathological analysis revealed a hydatidiform mole with persistent, polymorphic trophoblast cell hyperplasia. Therapy with methotrexate showed a decrease of serum beta-hCG. Screening procedures showed metastatic focuses in the left and the right lung (thoracic CT-scan). An abdominal CT-scan demonstrated an enlarged uterus with suspect hypodense structure of approximately 4.5 cm on the left uterine fundus. No focal hepatic lesions and no significant lymphadenoma were noted. Staging by MRI of the pelvis showed an approximately 3-cm-large focus at the uterine wall on the left fundus with an intensive heterogeneous signal. Suspicious infiltration into perifocal latero-caudal adipose tissue was also noted and a presumption diagnosis of hydatidiform mole or choriocarcinoma was suggested. Combined chemotherapy with the EMACO-scheme was then initiated. Our case showed a recurrence-free patient by radiological and laboratory means (beta-hCG-< 1.0 IU/ml).

Materials and Methods

The paraffin-embedded tissue was routinely analysed by haematoxylin-eosin staining. Immunohistochemistry was performed using a combination of microwave-oven heating and the standard streptavidin-biotin-peroxidase complex, using the mouse-IgG-Vectastain Elite ABC kit (Vector Laboratories, Burlingame, California, USA) for staining. Mouse monoclonal antibodies used for the experiments are listed in Table I. Briefly, paraffin tissue sections were dewaxed using xylol for 15 min, rehydrated in an alcohol row, and subjected to antigen retrieval on a high setting for 10 min in a pressure cooker in sodium citrate buffer (pH 6.0), containing citrate acid 0.1 M and sodium citrate 0.1 M in distilled water. After cooling, the slides were washed twice in phosphate-buffered saline (PBS). Endogenous peroxidase activity was quenched by immersion in 3% hydrogen peroxide (Merck) in methanol for 20 min. Non-specific binding of the primary antibodies was blocked by incubating the sections with "diluted normal serum" for 20 min at room temperature. Sections were then incubated at room temperature for 60 min with the primary antibodies. The antibodies were diluted in Dako-dilution medium (Dako, Glostrup, Denmark). After washing with PBS, the slides were incubated in "diluted biotinylated serum" for another 30 min at room temperature. After incubation with the avidin-biotin peroxidase complex (reagent ABC) for another 30 min and a repeat washing step with PBS, visualisation was performed with substrate and chromogen 3,3'-diaminobenzidine (DAB; Dako) for 8-10 min. The slides were counterstained further with Mayer's acidic haematoxylin and washed in an alcohol row (50-98%). After xylol treatment, the slides were covered. Negative controls were performed by replacing the primary antibody. Positive cells showed a brownish colour and negative controls as well as unstained cells were blue.

Results

Histological results. The following observations were made: large villi and hydatidiform villi with ranging syncytio- and cytotrophoblasts and hyperplasia of the cytotrophoblast; several villi with non-polar proliferation of the epithelial layer; extensive solid extravillous trophoblast cell aggregation with strong cell and nuclear polymorphy; focal haemorrhagic necrosis of villus tissue, extravillus trophoblast and decidua with a small wall invasion of the myometrium (Figures 1-2).

Immunohistochemical results. Inhibin-alpha showed a strong expression in syncytiotrophoblastic villi and a low expression in the extravillous trophoblast (Figures 3-4) Inhibin-betaA expression was noted in the cells of the solid trophoblast formation, while a stong reaction was noted in the villi (Figures 5-6) The strongest immunohistochemical reaction was observed for inhibin-betaB in polymorphic trophoblastic and villous tissue (Figures 7-8). Sialyl-Lewis A expression showed minimal focal expression in villous tissue and the extravillous trophoblast (Figures 9-10), while Sialyl-Lewis X showed focal expression in villous tissue with a negative staining reaction in extravillous trophoblast (Figures 11-12).

Discussion

In regulation of the human menstrual cycle, inhibin and estradiol (E_2) seem to be the negative controls of FSH secretion, that disappear at the time of luteal regression (11). The expression of inhibin/activin subunits in different female tissue suggests diverse functions, such as paracrine modulators of reproductive function (3, 12, 13) and gonadal tumorigenesis (14, 15). Inhibin has been demonstrated by immunohistochemical means in baboon and human endometrium (16-18). In early stages, the α- and β-subunits were observed in human endometrium, primarily in glandular epithelial cells (17-20). Previous immunohistochemical analysis showed a higher expression during the late secretory phase (16, 17, 21, 22), while other investigators could either not detect the inhibin-α subunit in human endometrium (19) or demonstrate an expression without a significant variation across the normal menstrual cycle (18). We could demonstrate a significantly higher inhibin-α expression in the secretory phase than in the proliferative phase, confirming previous results (16, 17, 21). Recent data also indicate that high concentrations of activin-A are secreted by stromal cells after *in vitro* decidualization (23, 24), equivalent to levels detected in maternal serum during the third trimester of pregnancy (25).

The inhibin/activin subunits are also localized in trophoblast (26) and trophoblastic tumours (27, 28). The immunohistochemically detectable inhibin-alpha subunit in placental tissue was mainly localized within the syncytiotrophoblast with positive staining reaction of the decidua (26). Production of inhibin by these cells may account for raised serum levels during pregnancy. Inhibin can also be demonstrated in choriocarcinoma and in non-gestational trophoblastic tissue. The detection by immunohistochemistry of inhibin/activin subunits has been recently proposed, in association with beta-hCG, as a useful marker of trophoblastic neoplasia (27, 28). It has been suggested that measurements of serum inhibin A and activin A might be more useful in diagnosing and following up molar pregnancies than hCG (6). However, other research groups demonstrated that serum molecular forms of inhibins (inhibins-A and -B) might not be of relevance in the biological survey of patients with gestational trophoblastic diseases (29, 30). Additional immunohistochemical and serological studies are still needed to evaluate the measurement of inhibin/activin subunits in the clinical setting regarding trophoblastic lesions.

This case report also demonstrates an increased risk of persistent or metastatic trophoblastic disease. Approximately 15% of trophoblastic diseases can transform into an invasive mole, with a subsequent development of choriocarcinoma in 2.5% of such cases (31). Choriocarcinomas and related trophoblast diseases are primarily characterized by their origin from chorion tissue, their high production of beta-hCG and a high curability with chemotherapy (31). However, such diseases have a ten-fold risk of recurrence and development of an invasive mole at following pregnancies. The expression of beta-hCG, hPL, CK 18 and MIB1 in normal placenta and in partial and complete hydatidiform mole are well known and confirmed in our case (32). With the Mucl-cor antibody and an antibody against the Thomsen-Friedenreich antigen, a strong staining reaction was recently observed in proliferative trophoblast at the surface of hydatidiform villi (32), while the strongest immunohistochemical reaction was observed with the Muc1 antibody, especially at proliferative and extravillous trophoblasts (32). Immunohistochemical analysis with Muc1, Muc1-cor and Thomsen-Friedenreich antigen showed a strong and intense staining reaction compared to normal placenta in the first and second trimesters, especially in the extravillous trophoblast compartment and the surface of the hydatidiform villi (33). However, Sialyl-Lewis A (CA 19-9) and Sialyl-Lewis X (sLex) carbohydrate antigens showed just focal minimal expression, and their immunohistochemical expression can be assumed to be negative. The proliferation marker Ki67 and the expression of p53 are more intensive in gestational trophoblastic diseases than in normal placenta after spontaneous abortion (34, 35). Recently, significant differences in the expression of cyclin E in placentae with hydropic changes, partial and complete moles, as well as choriocarcinomas, were also found (36).

In conclusion, an early recognition of gestational trophoblastic diseases is of extreme importance for the course of disease as well as an intensive cooperation between gynaecologists, radiologists, pathologists and oncologists. Inhibin is a sensitive marker of syncytiotrophoblast (26), which may be helpful in the diagnosis of hydatidiform mole with persistence of polymorphic trophoblastic hyperplasia.

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Received August 2, 2004
Accepted February 8, 2005