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„Endometrial maldifferentiation" – A clinically significant diagnosis in equine reproduction?

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Summary

The present paper describes the functional morphology of endometrial maldifferentiation in mares according to the classification of *Schoon et al. (1998)*. The primary diagnosis is based on the investigation of routinely H.-E. stained slides, supplemented by immunohistological techniques (estrogen and progesterone-receptors, Ki-67 antigen, intermediate filaments, laminin) of formalin-fixed biopsy specimens. The morphological findings are interpreted as indicative for hormonal disturbances due to ovarian neoplasms and various ovarian dystrophies, or as a symptom of alterations affecting either the hypothalamo-pituitary-ovarian-endometrial axis or the local steroid hormone receptor status. Clinical symptoms are mostly absent in cases without ovarian abnormalities. No relationship can be identified between endometrial maldifferentiation and the age or parity of the mare or season. From the authors' point of view endometrial maldifferentiation should be taken into account in the assessment of endometrial biopsy specimens as one important parameter leading to temporary or permanent fertility problems. In a certain case, however, the definite prognosis with respect to reversibility remains unclear, because the aetiopathogenesis has not yet been identified except for those cases of endocrine active ovarian neoplasms. In this field an intensified co-operation between clinicians and pathologists is necessary. Especially systematic case control studies, including endocrinology and determination of foaling rates may reveal further understanding resulting in efficient plans of treatments.

Keywords: endometrium, maldifferentiation, steroid hormone receptors, fertility disorders, horse

„Endometriale Fehldifferenzierung" – Eine klinisch relevante Diagnose in der equinen Reproduktionsmedizin?

Entsprechend der Einteilung von *Schoon et al. (1998)* wird die funktionelle Morphologie einer endometrialen Fehldifferenzierung unter dem Bild einer „ungleichmäßigen endometrialen Differenzierung (UED)" und einer „irregulären endometrialen Differenzierung (IED)" beschrieben. Die primäre Diagnose basiert auf der Untersuchung von H.-E.-gefärbten histologischen Schnitten, sie wird ergänzt und präzisiert durch immunhistologische Techniken am formalinfixierten Biopsiematerial (Östrogen-, Progesteronrezeptorstatus, Expression von Ki-67 Antigen, Nachweis von Intermediärfilamenten und Laminin). Die histopathologischen Befunde werden, je nach Lage des Falles, als Hinweis für hormonelle Imbalancen bei Ovartumoren, eine ovarielle Dystrophie, eine Störung des Hypothalamus-Hypophysen-Ovar-Endometrium-Regelkreises bzw. als Ausdruck einer lokalen Störung des endometrialen Steroidhormonrezeptorstatus interpretiert. Hinweisende klinische Symptome mit Ausnahme derjenigen Stuten, die an klinisch manifesten Störungen der Ovarfunktion leiden, fehlen zumeist. Ein Zusammenhang konnte weder zum Alter der Patientinnen noch zum Reproduktionsstatus, der Parität oder der Jahreszeit hergestellt werden. Die Autoren halten eine Berücksichtigung des Befundes „endometriale Fehldifferenzierung" bei der Beurteilung von Endometriumbiopsien für dringend erforderlich, da dieses Erscheinungsbild nach eigenen Erfahrungen mit vorübergehenden oder permanenten Fertilitätsproblemen einhergeht. Die Ursache kann, mit Ausnahme des Vorliegens endokrin aktiver Ovartumoren, derzeit im Einzelfall noch nicht sicher ermittelt werden. Hierzu bedarf es einer intensiven Kooperation Klinik-Pathologie, insbesondere durch gezielte Verlaufsuntersuchungen unter Einbeziehung der Endokrinologie sowie der Abfohlergebnisse, um zukünftig einen dem Einzelfall angemessenen Therapieplan entwickeln zu können.

Schlüsselwörter: Endometrium, Fehldifferenzierung, Steroidhormonrezeptoren, Fertilitätsstörungen, Pferd

Introduction

For years it has been well-established in numerous reports that endometritis and endometrosis are important causes for fertility problems in the mare. Functional disturbances however, have so far played a minor role in equine diagnostics and research. In human gynaecopathology such alterations are well known as possible causes of infertility. Ovarian dysfunctions and tumours, central defects, hormone refractive endometria and hormonal treatments result in greatly varying but „specific" morphological changes in human beings *(Dallenbach-Hellweg, 1987)*.

During seasonal equine ovulatory cycles, the endometrium proliferates and differentiates in response to physiologic changes in estrogen and progesterone levels *(Brunckhorst et al., 1991)*. It reacts immediately to hormonal cyclical variations as well as to disturbances *(Schoon et al., 1999)* and hormonal treatment *(Klug et al., 1997)*.

In this way the endometrial biopsy serves as a distinct bioassay „measuring" the hormones at the tissue level. Therefore the estimation of the endometrial stage –"dating the endometrium" and the determination of endometrial differentiation as physiological or as pathological must be the first steps in the investigation of each biopsy sample *(Schoon et al., 1999)*.

Methods

In routine biopsy examination endometrial maldifferentiations are detectable by means of histopathology (HE, PAS-alcian-blue, Picro-Sirius Red stain). Additional investigations using newly established immunohistopathological methods, i.e. intermediate filaments (vimentin, desmin), lami-

nin, estrogen (ER) and progesterone receptors (PR) and the proliferation marker Ki-67 antigen *(Özgen et al., 1997; Schoon et al., 1997; Schoon et al., 1998; Aupperle et al., 1999)* are additionally helpful. These techniques are applicable to specimens fixed in neutral, buffered formalin, even in routine diagnostics. Bouin-fixative however, recommended for years *(Schoon et al., 1992)* whilst providing superior cellular details limits the possibilities of immunohistology. Therefore it should be avoided even for routine diagnostic purposes.

Classification

According to *Schoon et al. (1999)* two major features of endometrial maldifferentiation exist based on findings in H.-E.-stained slides: unequal and irregular endometrial differentiation. Endometrial maldifferentiations as described here are exclusively those which occur in endometrial areas without any periglandular fibrosis.

In cases of unequal differentiation in one biopsy sample two functional stages are obvious: areas differentiated in accordance with the stage of the ovarian cycle and multiple non fibrotic foci of glands deviated either in degree or quality of activity from the dominant physiological pattern.

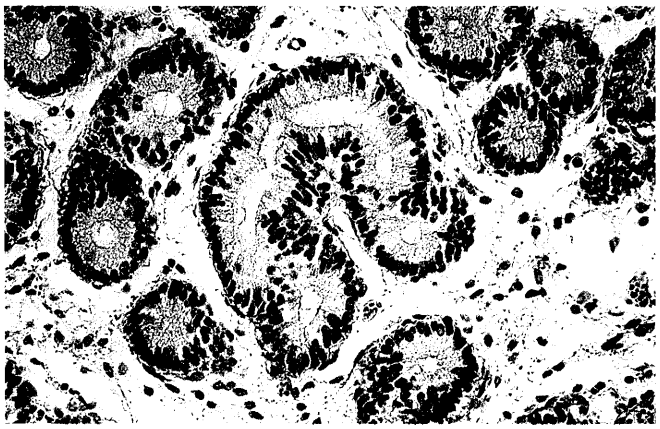

Fig. 1: Irregular proliferative endometrial differentiation. Polymorphic glandular epithelia show longish or oval, mostly hyperchromatic nuclei in basal, mid or apical positions and numerous mitoses. H.-E. stain, magnification 300x

Irregulär proliferative endometriale Differenzierung. Die Drüsenepithelien sind polymorph mit länglichen oder ovalen, meist hyperchromatischen Zellkernen in basaler, mittlerer oder apikaler Position. H.-E. Färbung, Gerätevergrößerung 300x

Frequently these glands are closely apposed forming non fibrotic nests, lacking distinct borders to the adjacent tissue. Epithelia as well as stromal cells express high values of estrogen receptors. Physiologically however, an intense expression in the epithelia is correlated with a weak stromal reaction and vice versa *(Aupperle et al., 1999)*. The labelling of the proliferation marker Ki-67 antigen is unusually low. The stromal cells show an unphysiological expression of desmin. It is reported that desmin-positive stromal cells occur in mares with pathologically inactive endometria *(Aupperle, 1997)*. The ER and PR reactivity of the unaffected glands resembles typical findings of the normal proliferation phase. In many glands the laminin labelling of the basal lamina is remarkably low and often discontinuous.

The irregular differentiation is characterised by a diffuse glandular alteration affecting all or at least most of the glands within a specimen. Abnormal proliferative or secretory activity may be present, in other cases patterns occur which cannot be appointed to any kind of physiological differentiation. Corresponding subdivisions were made based on the dominating signs of endometrial functional morphology.

The most obvious sign of irregular proliferative differentiation is the distinct polymorphism of the glandular epithelia. Within a single gland longish, oval and round, hyperchromatic and hypochromatic nuclei occur in basal, mid and apical positions (Fig. 1). Their lining has lost its orderly arrangement. Glands with hyperplastic epithelia and numerous mitoses may be present.

The epithelial expression of Ki-67 antigen is very intense and corresponds with high values of estrogen receptors of the glands and low levels in the stromal cells (Fig. 2). The expression of the progesterone receptor is pathologically low.

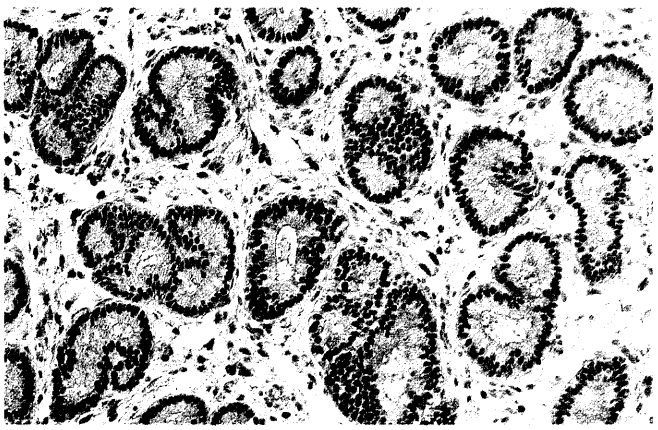

Fig 2: Irregular proliferative endometrial differentiation. The epithelial expression of ER is rather uniform and high, stromal cells show a weak expression. Immunohistology, estrogen receptor expression, Nomarski-interference-contrast, magnification 62.5x

Irregulär proliferative endometriale Differenzierung. Während die Epithelzellen eine hohe Expression von Östrogenrezeptoren zeigen, reagieren die Stromazellen nur schwach positiv. Immunhistologie, Östrogenrezeptornachweis, Nomarski Interferenz Kontrast. Gerätevergrößerung 62,5x

In cases of an irregular secretory differentiation, large and highly secretory glands dominate. The glandular epithelia are of enormous height, the pale cytoplasm contains numerous vacuoles. Large, round and hypochromatic nuclei are intermingled with depolarised nuclei of various shape and chromatin density (Fig. 3). Although a secretory morphology is obvious, an intense epithelial estrogen receptor expression and proliferation activity is present (Fig. 4).

rentiation did not occur. The teratoma and the benign serous cystadenoma did not affect the endometrial differentiation. Different types of non tumorouss ovarian dystrophies (Tab. 1) result in various features of IED too.

Tab. 1: Histopathological type of endometrial maldifferentiation occurring spontaneously or in the course of ovarian diseases.

Histopathologische Charakterisierung der endometrialen Fehldifferenzierung bei Spontanfällen und im Verlauf ovarieller Erkrankungen.

Morphological classification	Spontaneous cases (n)	Ovarian neoplasms (n)	Ovarian dystrophy (n)
Normal endometrial differentiation	–	2	–
Unequal endometrial differentiation (UED)	11	–	–
Irregular endometrial differentiation (IED)			
– *proliferative*	13	2	1
– *secretory*	19	3	–
– *completely irregular*	11	5	2

In the cases where a second biopsy after ovariectomy was available after 2 to 5 weeks (n=10) a distinct tendency to a more regular endometrial differentiation was obvious, interpreted as indicative for a possible reversibility of these hormonally mediated alterations of functional endometrial morphology.

Clinical significance

Equine endometrial functional disturbances are first described by our group as a phenomenon occurring spontaneously *(Schoon et al., 1998)* or in the course of hormonal active ovarian neoplasms *(Bartmann et al., 1998)*. The diagnosis is possible by the investigation of routinely H.-E.-stained slides and confirmed by immunohistological methods. In all cases investigated at least one and mostly all of the immunohistological parameters differ from normal expression patterns.

The endometrial differentiation is a highly sensitive indicator of the hypothalamo-pituitary-ovarian-endometrial axis. A physiological equine estrous cycle results in a uniform and synchronous functional morphology of the endometrium *(Brunckhorst et al., 1991; Strankmeyer, 1993; Raila et al., 1997)* Disorders may lead to permanent or temporary subfertility possibly due to an altered uterine environment, e.g. in a disturbed uterine secretory protein pattern as described in endometrosis *(Bader et al., 1997)*. While these alterations have been well known in human gynecopathology for years and described in detail indicating a „specific" aetiology in a certain case *(Dallenbach-Hellweg, 1987)*, comparable investigations are lacking in the horse. There is little information about endometrial differentiation disorders. Endometrial atrophy during the late physiological breeding season as a result of hormonal refractive endometrium *(Schoon et al., 1997)* and endometrial hypoplasia and hyperplasia are reported *(Ricketts, 1975; Kenney, 1978)*. Thus these descriptions do not correspond with the phenomena of unequal or irregular endometrial differentiation as defined here. Focal hypertrophy and atrophy occur in glands affected by endometrotic lesions *(Kenney, 1978; Schoon et al., 1997)*. These glands are known to undergo processes of maldifferentiation as well, however, due to other pathomechanisms *(Aupperle et al., 1997)*.

It is supposed that diffuse irregular endometrial maldifferentiations are results of hormonal disturbances including those resulting from hormonally active ovarian neoplasms and dystrophy as described in women *(Dallenbach-Hellweg, 1987)*. In cases of unequal differentiation a partial hormone refractive endometrium is more likely (Fig. 6).

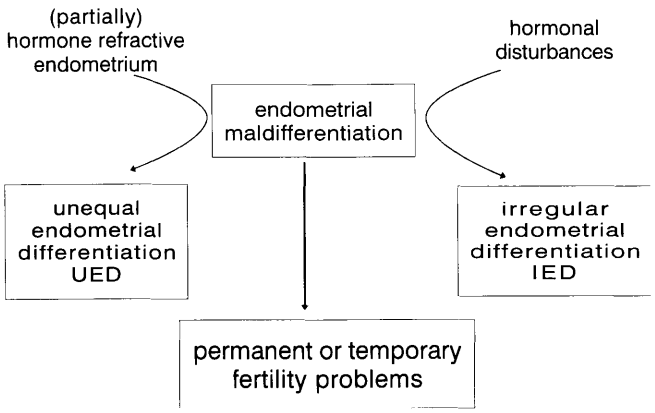

Fig. 6: Causes and features of endometrial maldifferentiation.

Ursachen und Erscheinungsformen endometrialer Fehldifferenzierungen.

No conclusive statement regarding the aetiopathogenesis can be made in cases where ovarian disorders have been excluded. The condition is related neither to age nor to parity of the mare nor to the annual season. Most of the mares have been barren for one or several years. The latter fact indeed indicates that endometrial maldifferentiation is a diagnosis of clinical importance which should be included in the assessment of endometrial biopsy diagnosis. More detailed case control studies including the estimation of the endocrine status are necessary for the understanding and prognostic assessment of this lesion. The aim of our current work is to correlate classical histopothological methods, immunohistopathology and clinical symptoms including endocrinology to infer a causal therapeutic concept from a certain feature of endometrial maldifferentiation.

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