

The Category I mare (Kenney and Doig 1986): Expected foaling rate 80-90% - fact or fiction?

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Summary

The subject of the present paper is a critical reflection on the categorization system of Kenney and Doig (1986) which is until now accepted internationally in the histopathological investigation of equine endometrial biopsies. Selected aspects of progress in equine gynecopathology made since 1986 are described and discussed under the view of clinical and prognostic relevance. It was concluded that the categorization neglects important histopathological findings (e.g. angioses, endometrial maldifferentiation) and is therefore incomplete. Beyond this schematized grading which means a reduction of the different findings to an arithmetical code, the biological complexity of etiology, reversibility, and possible interactions of the various alterations is more or less ignored. From the authors' point of view this is unacceptable from both medical and forensic aspects, especially under the conditions of the new German mercantile law.

Keywords: Equine, mare, endometrial biopsy, categorization

Die „Kategorie-I-Stute“ nach Kenney und Doig (1986) - Abfohlwahrscheinlichkeit 80-90%. Tatsache oder Fiktion?

Die vorliegende Arbeit unterzieht das für die Untersuchung von Endometriumbiopsien international anerkannte Kategorisierungsschema von Kenney und Doig (1986) angesichts der in den zurückliegenden 15 Jahren erzielten Fortschritte in der equinen klinischen Gynäkopathologie einer kritischen Bewertung. Es wird der Schluss gezogen, dass dieses Kategorisierungsschema wesentliche Befunde (z.B. Fehldifferenzierungen, Angiosen) unberücksichtigt lässt und deshalb nach heutigem Wissensstand unvollständig ist. Aufgrund der Komplexität bezüglich Ursache, Reversibilität und möglicher Addition und Interaktion der Krankheitsbilder erscheint eine schematisierte Kategorisierung, d.h. die Reduzierung der Befunde auf einen verschlüsselten Zahlenwert, weder medizinisch für das Individuum Stute heute noch gerechtfertigt noch unter forensischen Gesichtspunkten vertretbar, insbesondere unter Berücksichtigung des neuen deutschen Tierkaufsrechts.

Schlüsselwörter: Pferd, Stute, Endometriumbiopsie, Kategorisierung

The Categorization

For decades the value of endometrial biopsy in the evaluation of sub- and infertility in mares is accepted internationally. Especially in mares with no clinically apparent reason for temporary or persistent barrenness, the histopathological investigation has shown to be of diagnostic and prognostic importance.

Ricketts (1975) and Kenney (1978) were the first to summarize and classify the different histopathological findings in endometrial biopsies. Taking the quantity of inflammation and degeneration (periglandular fibrosis) as well as „lymphatic lacunae“ into account, Kenney (1978) established the first categorization system (Cat I, II, III). Based on the investigation and statistical analysis of 244 mares, these categories were associated to an expected foaling rate. To predict the foaling rate more accurately Kenney and Doig (1986) improved this system by a more subtilized differentiation of the lesions mentioned by Kenney (1978), adding the diagnosis „atrophy in the late physiological breeding season“ and including the duration of barrenness. The well-known subdivision in Cat I, Cat IIA, Cat IIB and Cat III was established.

The categorization of Kenney and Doig (1986) has been accepted internationally for years, because it is based on clear principles which are easy to understand. The reduction of the different histopathological findings to one numerical code (Category) leads to results even comparable by statistical methods. On the other hand this grading obliterates impor-

tant differences (e.g. reversibility/irreversibility and interactions) of the basic diagnoses (Schoon et al. 1997). As demanded already by Kenney (1978) the first step in writing the epicrisis is considering the stage of the cycle. The coincidence of behavioural, physical, and histological findings is an essential prerequisite for fertility. The fact that this important condition is neglected in the categorization system of Kenney and Doig (1986) and the progresses in research since 1986 has stimulated the authors to reflect on this categorization system.

The actual problem – selected aspects of progress in research since 1986

Endometrial Maldifferentiation

On the contrary to comparable findings in women (Dallenbach-Hellweg 1987), functional disturbances have so far played a minor role in equine gynecopathology. The endometrium reacts immediately to hormonal cyclical variations (Brunckhorst et al. 1991, Aupperle et al. 2000) as well as to disturbances (Schoon et al. 1999, 2000, Häfner et al. 2001, Ellenberger et al. 2002) and hormonal treatments (Klug et al. 1997). Disorders may lead to permanent or temporary infertility – depending on the individual cause –, possibly resulting from an altered uterine environment, for example a disturbed uterine secretory protein pattern, as described in endometrio-

sis (Bader et al. 1997, Hein 2000, Hoffmann et al. 2003). Even the conventional endometrial biopsy technique (HE-stain) serves as a distinct bio-assay by measuring the hormones at the tissue level. These findings can be completed and defined by immunohistochemistry, analyzing the endometrial steroid-hormone-receptor status and the glandular protein secretions (Schoon et al. 1997). Endometrial maldifferentiation occurs in about 7% of the cases in routine diagnostic investigations (Schoon et al. 2000). The findings can be summarized as follows:

Irregular endometrial differentiation

All or most of the glands in a biopsy specimen are affected, showing abnormal proliferative, secretory activity, inactivity or patterns that cannot be appointed to any kind of physiological differentiation (Schoon et al. 2000). Possible causes are tumorous or non-tumorous ovarian disorders (Ellenberger et al. 2002), hormonal treatment (Klug et al. 1997) or idiopathic cases without clinically obvious ovarian disorders (Schoon et al. 2000, Häfner et al. 2001)

Unequal endometrial differentiation

Within a biopsy sample two functional stages are obvious: areas differentiated in accordance with the stage of the ovarian cycle and multifocal non-fibrotic areas of glands, deviating from this dominant physiological pattern (Schoon et al. 2000). Possible causes are idiopathic local endometrial alterations affecting the steroid-hormone receptor expression (Häfner et al. 2001).

Beside these phenomena, Ricketts (1975) and Kenney (1978) describe an endometrial atrophy related to senility and ovarian inactivity in debilitated mares. Hypoplasia has been associated with delayed endometrial maturity in young mares, erratic oestrus cycles, and chromosomal mosaicism (Ricketts 1975, Kenney 1978, Ricketts and Barrelet 1997). Hyperplasia is a result of a delayed endometrial involution after fetal resorption or abortion (Ricketts 1975). These three conditions as well as unequal and irregular endometrial differentiation are with no doubt associated with a poor probability of successful mating at the time of diagnosis.

Nevertheless, strictly following the guidelines of the categorization by Kenney and Doig (1986) these mares have to be graded as Cat I mares, unless there are signs of endometrosis, endometritis or atrophy during the late physiological breeding season. This, however, would be a neglectation of the present state of the art in equine gynecopathology. A mare suffering from a severe endometrial maldifferentiation will not get pregnant as long as these alterations persist, even if other histopathological findings are completely absent. Additionally, these phenomena occur in endometria with concomitant endometritis, endometrosis, and angiomas (Cat IIA, Cat IIB, Cat III) as well (Schoon et al. 2000, Häfner et al. 2001, Ellenberger et al. 2002). The precise prognosis of these lesions is very difficult to determine, and depends on the aetiology in an individual case. From our point of view endometrial maldifferentiation at the time of diagnosis is a reason for infertility, because of a non sufficient uterine micro-environment due to a disturbed endometrial secretory protein pattern, comparable to the condition described in endometrosis (Bader et al. 1997, Hein 2000, Hoffmann et al. 2003). In principle these alterations have to be considered as reversible as shown in endocrine active ovarian neoplasms or cysts after

ovariectomy (Ellenberger et al. 2002) and after ending a hormonal treatment (Klug et al. 1997). In some cases however the definite pathogenesis remains unclear (Schoon et al. 2000, Häfner et al. 2001). Therefore, an all-embracing prognostic assessment of endometrial maldifferentiation is not possible, especially in cases of the coexistence of inflammatory, degenerative, and functional alterations as seen often in routine biopsy investigations.

A special case of equine endometrial malfunction is the frequently occurring equine hydromucometra, which is defined as a non-inflammatory intrauterine fluid accumulation occurring periovulatory. The fluid of watery to mucous consistency appears in ultrasonography as a sharply-contoured non echogenic area (Özgen et al. 1997, Özgen et al. 2002). Serial investigations support the hypothesis, that the shortened lengths of the interovulatory intervals in these mares are due primarily to luteal insufficiency rather than uterine-induced luteolysis. Probably due to the lacking of luteal progesterone in the affected mares, the progesterone mediated suppression of the steroid hormone receptors is impeded and glandular cell differentiation shifts to asynchronous secretion (Özgen et al. 2002). The diagnosis of this phenomenon in an individual mare by one biopsy specimen, investigated conventionally by HE-staining is not possible. The minimum requirements necessary for diagnosis are clinical ultrasonographic specifications and a specialized laboratory well versed in equine steroid hormone receptor analysis. Only by combining these methods and the exclusion of inflammatory causes a reliable tentative diagnosis dealing with the pathogenesis is possible. As shown by many investigations (for references see Özgen et al. 1997, 2002) fertility prognosis of affected mares is poor, however, this condition is not included in the categorization of Kenney and Doig (1986).

In mares with periparturient disturbances, often a temporary unphysiological delay in glandular re-differentiation occurs, visible as an asynchronous persistence of endometrial secretion morphology during foal heat (Steiger et al. 2002). This phenomenon which is not detectable by clinical methods, results in an endometrial maladaptation for the requirements of a new pregnancy and a poor pregnancy prognosis during foal heat. This even includes cases of a clinically undisturbed uterine involution and the absence of puerperal endometritis in the biopsy.

Dating of a certain biopsy specimen during the time of the physiological breeding season and the diagnosis of endometrial maldifferentiation, especially when conventional methods of histopathology are supported and supplied by immunohistochemistry does not create any problems for a well versed and equipped laboratory. However, it has to be taken into consideration that physiological deviations occur during the transition cycles in early fall and spring (Kenney and Doig 1986, Schoon et al. 1997). In the time of winter anoestrus the relations between endometrial functional morphology, steroid hormone receptor status, and clinical findings are confusing, and even the basic mechanisms are poorly understood until now (Aupperle et al. 2003).

Persistent endometrial inactivity at the beginning of the breeding season leads to an impaired pregnancy prognosis for the following 60 days (Kenney and Doig 1986). For the definite diagnosis of this condition, the special climate characteristics of a certain year must be considered. In this sense, the spring biopsies serve as sensitive seasonal chronobiological indicators as well (Schoon et al. 1997)

Angiopathies

Perivasculitis and Vasculitis

Perivasculitis of endometrial blood vessels is present in about 10 to 20% of the biopsy specimens (Schoon et al. 1997). There is no relationship between the age and the reproductive status of the mares. Concomitant endometritis occurred in only 50% of these specimens. Perivascular infiltration is most frequently encountered in small venous vessels, but it is also noticed in arterioles, arteries, and veins. Vasculitis, represented by moderate numbers of lymphocytes, macrophages, and fibrinoid necrosis within the vascular wall, is seldom. The aetiology of inflammatory vascular changes remains unknown. In case of multifocal perivasculitis there is a significant negative influence on fertility, even in biopsies without any other histopathological lesions (Kriesten 1995).

Degenerative Angiopathies

Angioses are detectable, varying in quality and quantity, in nearly 80% of broodmares (Schoon et al. 1999). Only younger maiden mares have intact vessels, older maiden mares show mostly a mild sclerosis within the intima and especially the adventitia. In multiparous mares the vascular alterations affect all layers of the vascular walls and resemble the so called pregnancy sclerosis in other species. Incidence and degree increase with the number of foals delivered, statistically independent of the age of the mare. At least two factors, age and parity, have to be considered as possible causes of degenerative angiopathies. There is an obvious relationship between angioses, age, and barrenness. The incidence of severe angioses decreases with the time interval from the last foaling, but only in younger or middle-aged mares. The vessel wall is able to regenerate, a capacity which is reduced in mares older than 17 years (Schoon et al. 1999).

There seems to be no obviously direct but an indirect negative effect on fertility of the mare, because angioses result in a reduced endometrial perfusion (arteries) and drainage (veins) and the alterations are intimately related to endometrosis (Schoon et al. 1997). Fibrosis of moderate or severe degree in the A. uterina is associated with an elevated vascular resistance as described by Blaich et al. (1999) and Ludwig et al. (2002) in a Doppler ultrasonographic study.

Drainage disturbances are interpreted as the sequela of an impaired venous circulation (Schoon et al. 1999). This may result in a persisting oestrus edema (pathological asynchronic edema), lymphangiectasia, and lymphatic cysts. These two different types of lymphatic ectasia (Schoon et al. 1993) are summarized as „lymphatic lacunae“ (Kenney and Doig 1986). Only if these lesions are palpable or result in a jelly-like consistence are they considered in the categorization of Kenney and Doig (1986).

Additionally, there is a distinct correlation between the degree of endometrial, myometrial, and extrauterine (Ludwig et al. 2001) as well as extraovarian and ovarian (Aupperle et al. 2003) angiopathies. Histopathological findings in endometrial vessels are reliable markers for inferring the status of the extrauterine arteries. Older multiparous mares with moderate to severe angioses diagnosed in endometrial biopsies can be identified as risk patients for fatal ruptures of extrauterine vessels during late pregnancy or birth (Ludwig et al. 2001). Ovarian angiopathies may lead to an impaired perfusion, resulting in disturbed follicular and luteal development as well as

endocrine insufficiency causing embryonic death in the mare (Allen 2001). Using Doppler ultrasonography Wein et al. (2002) verify that the ovarian perfusion is significantly lower in mares with uterine angiopathies compared to mares without pathological vascular findings.

Endometrial angioses have a negative effect on fetal placental development. Mares suffering from degenerative angiopathies show a lower density and branching of the chorionic villi as calculated by the villus-index. The villus-index is also negatively correlated with the age of the mares and the degree of endometrosis (Kersten 2000).

The age of the mare

Even in mares with unaltered endometria (Cat I) there is a statistically significant correlation between age and foaling prognosis (Schoon et al. 1997). Aged mares are not as able to carry a foal to term as younger mares.

Endometrosis is one of the most important causes of infertility in the mare, integrated in the categorization system. The disease occurs age-related and shows a progressive character (Ricketts and Alonso 1991, Schoon et al. 1997). The authors emphasize that the condition is independent of the number of previous foals. Endometrosis may lead to endometrial atrophy, eventually resulting in gynecological senility (Ricketts and Barrelett 1997). A supposed correlation to repeated inflammation could be excluded (Hoffmann et al. 2003).

Conclusions

As shown above, since 1986, the year of publication of the categorization system of Kenney and Doig, many clinical pictures and conditions have been described in equine clinical gynecopathology, affecting the breeding prognosis of a certain mare. Even in cases of the absence of any diagnosis included in the categorization system (Cat I, expected foaling rate 80-90%, Kenney and Doig 1986), many alterations with more or less characteristic histopathological findings may occur, leading to permanent or temporary sub- or infertility. Based on the own experiences, a mare suffering from, for example, a severe maldifferentiation, resulting in a complete irregularity of the glandular functional morphology will not become pregnant as long as this condition persists. These phenomena may appear alone (e.g. angiopathies, maldifferentiation), coexist, or are combined with the classical diagnoses endometritis and endometrosis within the Cat IIA, IIB, and III in younger and older mares. Contrary to the latter diagnoses, the new syndromes cannot be calculated exactly by statistical methods concerning the breeding prognosis in a certain case. Interactions with endometritis and endometrosis are not completely understood and reversibility depends on many factors (e.g. age, parity, reproduction status, clinical aspects, reasons for endocrinological abnormalities).

Consequently and taking forensic aspects of the new German laws into account, the „classical“ Kenney-categorization has to be declared as not „up-to-date“ nowadays. This must be considered in the use of routine biopsy investigation and the scientific literature as well, because many essential diagnoses, relevant for breeding prognosis, are missing.

As firstly mentioned by *Ricketts* (1975) and *Kenney* (1978) we need an individual assessment of biopsy findings and clinical aspects for every single mare. Composing a complete epicrisis necessitates taking into consideration the actual status, the age of the mare, and the reversibility of the findings. Making prognoses should enable the owner of the mare and the veterinarian to decide whether the mare is worth the effort (*Kenney* 1978).

However, concrete numerical data/codes in an individual, imagining diagnostic safety, should be avoided in future; such statements are arbitrary taking the state of the art in gynecopathology into account. Nevertheless, the endometrial biopsy is a potential tool of raising importance in the course of continuously advancing knowledge about functional endometrial morphology. Only this method enables the clinician to gather information about reasons for clinical soundless sub- or infertility (*Schoon et al.* 1997) and includes the possibility to get an imagination dealing with quality and quantity of lesions associated with a given clinical symptom under the view of reversibility, therapy, and prognosis. Summarizing these aspects, the endometrial biopsy has to be required as an integral clinical method to investigate the health of the female genital organs, especially the endometrium.

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