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Microscopic examination of endometrial biopsies of retired sports mares: An explanation for the clinically observed subfertility?

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ABSTRACT

After their retirement from sports, performance mares often show a poor breeding success. The objective of this study was the microscopic evaluation of endometrial biopsies of retired sports mares (n = 189) to search for alterations that may explain subfertility. Mares of this study aged 3–23 years showed endometritis (30%) and endometrosis (77%); mild forms predominated. In regard to those mares biopsied during the breeding season (n = 99), 50% had glandular differentiation disorders, i.e. glandular inactivity (8%) or irregular glandular differentiation (42%). Compared to literature data retrieved from mainly non-performance mares, the sports mares of this study showed a similar prevalence of endometrosis and endometritis, but a much higher prevalence of glandular differentiation disorders. The most common cause of the latter is an ovarian dysfunction. Results of this study indicate an association between glandular maldifferentiation of the endometrium and the clinically observed reduced fertility of retired sports mares.

1. Introduction

The endometrial biopsy allows the microscopic detection of all – even subclinical – endometrial lesions that are associated with reduced fertility (Kenney, 1978; Kenney and Doig, 1986; Ricketts, 1975a, 1975b; Schoon et al., 1997; Snider et al., 2011). These include inflammatory and/or degenerative diseases (Kenney, 1978; Kenney and Doig, 1986; Ricketts, 1975a, 1975b; Schoon et al., 1997; Snider et al., 2011) as well as glandular differentiation disorders (Schoon et al., 1997, 2000; Snider et al., 2011).

Endometritis can be subclassified according to the degree of inflammation as well as the type(s) of involved inflammatory cells (Kenney, 1978; Ricketts, 1975a, 1975b; Schoon et al., 1997).

Acute endometritis is characterized by a predominance of neutrophils, whereas exclusively mononuclear cell infiltrates are observed in chronic endometritis (Kenney, 1978; Kenney and Doig, 1986; Ricketts, 1975a, 1975b). These two forms of endometritis are also named as acute suppurative endometritis and non-suppurative endometritis, respectively (Schoon et al., 1997). The diagnosis subacute suppurative endometritis is used, if the inflammatory cell infiltrate is composed of mononuclear cells and fewer neutrophils (Schoon et al., 1997; Schöniger et al., 2013). Endometritis eosinophilica represents an additional type of endometritis with the presence of mainly eosinophils (Schoon et al., 1997).

The most important degenerative disease is periglandular fibrosis that is associated with morphological and functional alterations of affected endometrial glands (Hoffmann et al., 2003; Kenney, 1978; Kenney and Doig, 1986; Lehmann et al., 2011; Ricketts, 1975a, 1975b; Schoon et al., 1992, 1997). This alteration was initially diagnosed as chronic degenerative endometritis (Ricketts, 1975a, 1975b). Subsequently, periglandular fibrosis was recognized as a primary non-inflammatory disease and named as chronic degenerative endometrial disease (Ricketts and Alonso, 1991) or endometrosis (Allen, 1993; Schoon et al., 1997). The term endometrosis was introduced by Kenney in 1992 (Allen, 1993). It was initially used as collective term for the diagnosis of different degenerative endometrial alterations (Allen, 1993) and confined to its current definition, i.e. periglandular fibrosis associated with alterations of affected glands, by Schoon et al. (1997).

Periglandular fibrosis can affect an individual gland branch or several gland branches (Kenney, 1978; Schoon et al., 1992; Snider et al., 2011). Hallmark of the destructive form is epithelial degeneration and necrosis of affected glands (Buczkowska et al., 2014; Hoffmann et al., 2009).

The endometrium is a target organ of estrogen and progesterone and therefore serves as a sensitive indicator of hormonal imbalances. The physiological changes in the serum levels of estrogen and progesterone during the reproductive cycle induce a cycle-synchronous differentiation of endometrial glands, i.e. a proliferative morphology during estrous and a secretory differentiation during interestrus (Kenney, 1978; Schoon et al., 2000). Alterations of the

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physiological serum concentrations of these hormones, for example in association with ovarian tumors, ovarian cysts, hormone treatment and/or during the transitional cycles, result in an asynchronous differentiation of endometrial glands, i.e. glandular maldifferentiation (Ellenberger et al., 2002; Schoon and Schoon, 2003; Schoon et al., 2000).

In case no endometrial alterations are detected by gynecological examination, one endometrial biopsy (size: $10 \times 3 \times 3$ mm) has been shown to be representative for the entire endometrium (Kenney and Doig, 1986; Schoon et al., 1992).

Veterinarians often observe a poor success in initial breeding attempts of performance mares that had been used for longer terms in high level tournament-sports (Burger et al., 2008). In addition, poor results are reported from embryo-transfers in athletic horses (Allen and Stout, 1999). Notably, once a retired performance mare has finally been bred successfully, it will commonly conceive the next time without difficulties (Davies Morel, 2008). Since the majority of performance mares never had foaled before the completion of their sports career in their teens, an association between maiden-status and subfertility is suspected (Pycock, 2006). Maiden mares often show cervical-dysfunction, poor myometrial contractility and intrauterine fluid accumulation (Pycock, 2006). In addition, ovarian inactivity is regarded as an important contributing factor (Aurich, 2005; Davies Morel, 2008; Kähn, 2004); therefore, it is advised to take mares out of competition in the beginning of the fall and to wait until the next breeding season before attempting to breed the mare (Aurich, 2005; Davies Morel, 2008; Kähn, 2004).

So far, only a few published studies – that focus on clinical aspects – investigated possible causes of subfertility in performance mares. Mortensen et al. (2009) demonstrated that mares exercised under hot-humid conditions ovulate smaller follicles and generate a lower number of embryos and embryos of a reduced vitality compared to those of a control group. Kelley (2009) observes prolonged ovulation intervals in mares under training conditions.

The objective of this study was the microscopic examination of endometrial biopsies of performance mares to search for findings that may explain the clinically observed – often temporary – subfertility of retired sports mares. To the best of the authors' knowledge, an investigation into the microscopic endometrial findings of retired sports mares has not been published previously.

2. Materials and methods

2.1. Animals

This retrospective study was performed on endometrial biopsies of 189 retired performance mares; the biopsies had been submitted to the Institute of Veterinary Pathology, University of Leipzig, for a routine microscopic investigation between 1994 and 2012. The performance mares of this study had frequently participated in high level tournament sports of classes M and S (Germany) or in international competitions, including show jumping, dressage and eventing.

Data retrieved from the data base of the Institute of Veterinary Pathology were the age of the mares (unknown for 10 mares), the length of barrenness (unknown for 25 mares), reported ovarian and/or uterine diseases, the foaling status prior to the sports career, the breeding/foaling status after completion of the sports career, the date of the biopsy retrieval and the length of the time period between the completion of the sports career and the biopsy retrieval (unknown for 4 mares). The latter information was available from the data base in the following time intervals: <1 year, approximately 1 year and ≥2 years.

According to the mares' breeding status after completion of their sports career, the retired performance mares of this study were divided into the following three groups: retired non-bred mares (n = 68), retired unsuccessfully bred mares (n = 84) and retired mares that had foaled (n = 37). In total, biopsies of 189 mares were examined; 99 mares were biopsied during the breeding season (15th March–15th September) and 90 mares during the transitional cycles. The 99 mares that were biopsied during the breeding season included 32 retired unbred performance mares, 51 unsuccessfully bred performance mares and 16 retired performance mares that had foaled. In regard to mares that had foaled after completion of their sports career, the time period between the last foaling and the biopsy collection was not known.

2.2. Histopathology

The endometrial biopsies were submitted fixed in 10% buffered formalin. They were embedded in paraplast and routinely processed. Each biopsy was stained with Hemalaun-Eosin and a Picro-Sirius Red stain.

All endometrial biopsies (n = 189) were examined microscopically to determine the presence of degenerative and inflammatory alterations, i.e. endometritis and periglandular fibrosis associated with alterations of affected glands (endometrosis). Endometritis and periglandular fibrosis were graded as mild, moderate and marked (Kenney, 1978; Kenney and Doig, 1986; Schoon et al., 1992). Endometritis was subclassified in acute suppurative, subacute suppurative and non-suppurative forms (Schöniger et al., 2013) as well as endometritis eosinophilica (Schoon et al., 1992, 1997). Endometrosis was subclassified into the destructive and the non-destructive subtypes (Hoffmann et al., 2003; Schoon et al., 1997); it was further evaluated if predominantly nests of glands or single glands were affected (Schoon et al., 1997).

All endometrial biopsies collected during the breeding season (n = 99) were examined for the presence of an irregular glandular differentiation (Schoon et al., 2000) or glandular inactivity (Kilgenstein et al., 2014). The irregular glandular differentiation was subdivided into the irregular proliferative, the irregular secretory and the completely irregular differentiation (Schoon et al., 2000).

Finally, endometrial biopsies (n = 164) were categorized according to the categorization system of Kenney and Doig (1986), modified by Schoon et al. (1992); in the remaining 25 mares the category could not be determined, since the time of barrenness was unknown.

2.3. Immunohistochemistry

Immunohistochemistry for the detection of estrogen and progesterone receptors (ER, PR) was performed on representative biopsies (n = 19), which were collected during the breeding season, as a confirmatory method to verify the presence of a regular or an irregular glandular differentiation (Schoon et al., 2000). By light microscopy 8 of these mares had a regular and 11 of these mares an irregular glandular differentiation. The peroxidase anti-peroxidase (PAP) method with 3,3′ diaminobenzidine-tetrahydrochloride (DAB) as chromogen was used; the following primary antibodies were applied: mouse anti-human estrogen receptor alpha (1:20, clone 6F11, citric buffer pretreatment, Novocastra Laboratories, Newcastle upon Tyne, UK; Aupperle et al., 2000) and mouse anti-human progesterone receptor (1:100, clone NCL-PGR-AB, citric buffer pretreatment, Newcastle upon Tyne). Appropriate positive and negative controls were performed.

2.4. Evaluation of the immunostaining

Previous investigations showed that – compared to regularly differentiated endometria – glands of irregularly differentiated endometria have a high variability in regard to the numbers of immunopositive nuclei as well as their staining intensity (Schoon et al., 2000).