

Aetiopathogenesis and Therapy of Equine Hydromucometra: Preliminary Data

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Introduction

Hydromucometra is the term used here to describe intrauterine fluid accumulation of watery to mucous consistency which appears in ultrasonography as a sharply-contoured non-echogenic area. Intraluminal fluid accumulation in both oestrous and dioestrous mares is associated with reduced pregnancy rates or poor fertility in general (Adams *et al.* 1987; Allen and Pycock 1988; McKinnon *et al.* 1988; Pycock and Newcombe 1996).

Mares that accumulate fluid in oestrus are considered to be susceptible to endometritis (Allen and Pycock 1988).

The cause most frequently given is an impaired clearance through the cervix (Evans *et al.* 1986; Adams *et al.* 1987; Allen and Pycock 1988; LeBlanc *et al.* 1987; Troedsson and Liu 1991; Liu 1992), but reduced lymphatic drainage caused by weaker myometrial contractions (LeBlanc 1994) is also discussed as a reason for uterine fluid accumulation.

Liu (1992) supposes that the fluid originates from the excess and watery endometrial gland secretion.

Results of previous studies (Rasch 1994, Kropp 1996)

The aetiopathogenesis of hydromucometra was studied comparing clinical observations, bacteriologic and cytologic data as well as pathohistologic and histomorphologic evaluations of 80 oestrous mares accumulating uterine fluid.

Statistical analysis found significant correlations between the amount of uterine fluid and increasing years of age of the mare and years of barrenness.

With increasing amount of free uterine fluid, detected ultrasonographically, more commonly bacteria were cultured and more mares showed histopathologically evidence of chronic endometritis and endometrosis.

Histomorphologic measurements of endometrial structures (e.g. height of luminal epithelial cells, glandular density, diameter of glands) determined significant differences between mares with uterine fluid accumulation and those without any apparent uterine abnormalities.

Plasma progesterone concentrations obtained four to twelve hours after ovulation showed values three times higher in the group of mares with a large amount of uterine fluid accumulation than in the control group.

Morphologic and endocrinologic evaluations indicated that hydromucometra may correlate with hormonal dysregulation caused by delayed or incomplete luteal regression or a premature progesterone secretion and which results in glandular hypersecretion.

Intravenous oxytocin treatment of mares with uterine fluid accumulation caused increased pregnancy rates of 64 % (n = 39) compared to nontreated mares with 40 % (n = 10) and 70 % (n = 10) in the control group. Although, in a second study, a positive effect on uterine drainage could not be detected.

Periovalutary treatment with prostaglandin also had no positive effect on fertility.

As fundamental aspects of the aetiopathogenesis of intraluminal fluid accumulation still remained open, more detailed investigations seemed to be necessary.

Materials and methods

Eight mares with fluid accumulation and seven mares without any apparent uterine abnormalities were monitored throughout an entire oestrous cycle.

During dioestrus, blood samples were taken from the mares every two to three days and during oestrus every day in order to determine oestradiol and progesterone plasma concentrations. In addition, endometrial biopsies were obtained on day 0 (day of ovulation), 5, 10, 13, 16, 19 and 21 of the oestrous cycle.

Endometrial samples for microbiological evaluation were taken before first biopsy.

Endometrial biopsies were evaluated histopathologically according to Kenney and Doig (1986), and endometrial structural elements, e.g. glandular and glandular luminal diameters, were measured morphometrically.

Receptors for estrogen and progesterone were stained with the peroxidase-anti-peroxidase-technique to determine receptor status in epithelial and stromal structures during the different phases of the cycle.

For the precise assessment of the immunohistologic reaction, Remmele and Stegner's (1987) method of evaluating oestrogen receptors in carcinoma of the human breast was modified and adjusted to the special requirements of the equine endometrium. The immunohistologic reaction is given an IRS (Immuno Reactive Score), calculated with the formula described below (fig. 1).

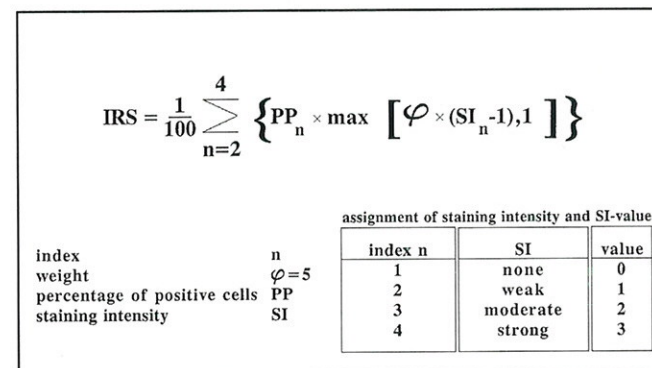


Fig. 1: Calculation of IRS in endometrial oestrogen and progesterone receptors.

Prostaglandin F_{2α}-concentrations were evaluated in endometrial tissues (CAYMAN CHEMICAL Enzyme Immunoassay Kit).

Results of recent studies

Daily examinations using transrectal ultrasonography showed that in most cases uterine free fluid appeared with the end of the luteal phase and disappeared after ovulation, but in cases of large amounts of intrauterine fluid, it persisted throughout the entire oestrous cycle (n=2).

Endometrial swabs of one mare (*Pseudomonas* sp.) of the control group (n = 7) and of four mares (β -haemolytic streptococci) of the hydromucometral group (n = 8) were classified as bacteriologically positive.

Pathohistologic evaluations of formalin fixed and paraffin embedded biopsies showed no signs of acute endometritis. Slight infiltrations of lymphocytes, plasma cells and eosinophiles in the stratum compactum and spongiosum were detected in both groups.

In three out of seven mares in the control group, light endometrial fibrosis was observed. In the hydromucometral group, severe and extensive fibrotic changes were observed in three cases, and slight changes in five cases.

Plasma progesterone concentrations showed statistically significant differences between the groups during dioestrus. Progesterone values in mares accumulating fluid declined earlier in the cycle and mares exhibited short interovulatory intervals.

Progesterone plasma values obtained on days 3, 5 and 8 of the oestrous cycle reached average values of only 1.18, 2.29 and 1.76 ng/ml in the hydromucometral group compared to 3.8, 5.83 and 5.07 ng/ml in the control group and differed significantly ($p < 0.05$, $p < 0.01$, $p < 0.01$) between the groups.

Immunohistologic evaluations of oestrogen receptors in endometrial samples of mares without any apparent uterine abnormalities and mares accumulating intrauterine fluid resulted in different patterns for epithelial and stromal cells throughout the oestrous cycle. Peak immunoreactivity of glandular epithelia was reached in early dioestrus. Then the intensity of reaction declined until preoestrus to rise slowly in early oestrus. In contrast to epithelial reaction, oestrogen receptors in stromal cells could scarcely be demonstrated during the luteal phase. Immunoreactivity became more intense at preoestrus and stayed high until the end of oestrus.

Oestrogen receptor immunoreactivity in mares with uterine fluid accumulation was greater during all phases of oestrous cycle compared to the control group. During oestrus ($p < 0.05$), early dioestrus ($p < 0.001$) and late dioestrus ($p < 0.05$) IRS values were significantly higher in the hydromucometra group. Stromal cells also tended to show higher immunoreactivity in the hydromucometra group (statistically significant during early oestrus $p < 0.05$).

Immunohistologic studies showed a distribution pattern in progesterone receptors similar to that in oestrogen receptors, but with less intense reactions.

During oestrous cycle, in glandular epithelial cells immunoreactivity in progesterone receptors was higher in the hydromucometra group. Immuno Reactive Scores differed significantly during late interoestrus ($p < 0.05$).

Investigations of endometrial tissues for PGF_{2 α} concentrations showed higher values in the hydromucometra group during late dioestrus and preoestrus.

Morphometric measurements of surface epithelium height showed no significant difference between the groups. Diameters of glands and glandular lumina in hydromucometra group were

greater during all phases of oestrus cycle, especially in dioestrus and oestrus (both $p < 0.05$). Mares with uterine fluid accumulation also showed higher values of basal and apical glandular epithelium height during the entire cycle, significantly during preoestrus ($p < 0.001$) and early oestrus ($p < 0.001$).

Conclusions

Endocrinologic immunohistologic and histomorphologic results indicate that mares with uterine fluid accumulation suffer from luteal insufficiency. Lacking progesterone influence during the luteal phase leads to excessive expression of oestrogen and progesterone receptors as a result of missing receptor down regulation.

Hormonal dysregulation might lead to hypersecretory activity of endometrial glandular structures causing intrauterine fluid accumulation.

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