Acta vet. scand. 1997, *38*, 69-78.

Intrauterine Fluid Accumulation in Oestrous Mares

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Reilas, T., T. Katila, O. Mäkelä, M. Huhtinen and E. Koskinen: Intrauterine fluid accumulation in oestrous mares. Acta vet. scand. 1997, 38, 69-78. – Intrauterine fluid (IUF) was collected using a tampon from mid-oestrous mares (n = 57) with and without ultrasonically detectable accumulations of free intraluminal fluid. Bacteria were cultured and neutrophils counted from all samples (n = 57). Total protein concentration, trypsin-inhibitor capacity (TIC), and plasmin, ß-glucuronidase (B-Gase) and N-acetyl-ß-D-glucosaminidase (NAGase) activities were determined in 27 IUF samples. The motility of spermatozoa in the presence of IUF, IUF extended with Kenney's medium (1:1) and Kenney's medium alone was analysed in 9 samples using a Hamilton-Thorn motility analyser. Thirty-five mares were inseminated immediately after collection of IUF, and every second day until ovulation. Embryos were recovered nonsurgically 6 days after ovulation. After embryo transfer, fluid accumulations were recorded during oestrus and an endometrial biopsy specimen taken (n = 53).

In the beginning of oestrus, fluid accumulations were detected in 39% (22/57) of mares, while on the day when IUF was collected, fluid accumulations were observed in 26% (15/57) of mares. The fluid was anechogenic, and in 80% of the mares located in the uterine body. None of the mares exhibited cytological or bacteriological evidence of acute endometritis. Total protein concentrations, TIC and B-Gase activities in IUF were statistically significantly lower in mares with fluid accumulations (n = 14) than in mares without fluid accumulations (n = 13) ($p<0.01$). The addition of undiluted IUF to extended semen significantly reduced total and progressive motilities, path velocities and percentages of rapid spermatozoa ($p<0.05$) in vitro. On endometrial biopsy, fibrosis was found to be more prominent ($p=0.025$) in mares with fluid accumulations (n = 9) than in mares without (n = 44). It was concluded that anechogenic fluid accumulations during oestrus were associated with compositional changes in IUF. Although IUF had negative effects on spermatozoal motility in vitro, the presence of fluid accumulations at the time of insemination did not affect embryo recovery rates.

protein; trypsin-inhibitor capacity; plasmin; NAGase; ß-glucuronidase; spermatozoal motility; endometrial biopsy.

Introduction

Detection of intraluminal uterine fluid accumulations in mares by rectal palpation of ventral dilations of the uterus was first reported by *Knudsen* (1964). Only large fluid accumulations can be diagnosed by rectal palpation. Nowadays, transrectal ultrasonography allows detection of even small fluid accumulations (*Ginther & Pierson* 1984). However, the presence of a thin layer of intrauterine fluid (IUF) on the uterine mucosa cannot be identified by ultrasonography.

Fluid accumulation during dioestrus indicates an inflammatory process that reduces pregnancy rates and increases embryonic loss (*Adams et al.* 1987). Detection of fluid accumulations one or 2 days after ovulation or during

dioestrus has been found to be associated with higher than normal embryonic death rates (*McKinnon et al.* 1987). Although dioestral fluid accumulation is considered harmful, the significance of its occurrence in oestrus is not clear. Liu (*review by Allen* 1993) has suggested that impaired uterine drainage through the cervix may be a reason for excessive fluid accumulation and that the initially sterile fluid may provide an ideal medium for bacterial growth. It has been proposed that fluid reduces fertility by a direct effect on spermatozoa (*Squires et al.* 1989). Ultrasonography is valuable for estimation of quality of IUF because the echogenicity of the latter is related to amount of debris and neutrophils (*McKinnon et al.* 1988). Accumulated IUF can not only be of inflammatory origin, it can be transudate from vessels. The permeability of the endothelium of uterine vessels increases during oestrogen treatment (*McRae* 1988). Little is known about the composition of clear, anechogenic IUF. Measurements of plasmin, the proteolytic enzyme formed from blood-derived plasminogen, and trypsin-inhibitor capacity (TIC), mainly provided by blood-derived α1-proteinase-inhibitor (α1-PI), could reflect permeability changes in the uterus, as they do in bovine mastitis (*Honkanen-Buzalski & Sandholm* 1981, *Kaartinen et al.* 1988). In addition, uterine secretions contain proteins and lysosomal enzymes synthesized and secreted by uterine cells. N-acetyl-ß-D-glucosaminidase (NAGase) is, for example, secreted by the equine endometrium (*Hansen et al.* 1985). Lysosomal enzymes are also released from cells following tissue damage, and during phagocytosis (*Gleisner* 1979). In bovine mastitis, both NAGase and ß-glucuronidase (B-Gase) have been found to be useful as marker enzymes allowing detection of abnormal udder secretions (*Nagahata et al.* 1987). Activities of these lysosomal hydrolases in equine leukocytes are relatively high (*Healy* 1982).

The objective of the study reported here was to measure and compare neutrophil numbers, total protein concentrations, proteolytic and antiproteolytic activities, and various lysosomal enzyme activities in IUF from mares with and without ultrasonically detectable fluid accumulations. Potential adverse effects of IUF on spermatozoa were studied by in vitro motility measurements. Embryo recovery rates were taken as indicators of the effect of fluid accumulation on fertility.

Materials and methods

Animals

Fifty-seven cyclic, nonlactating horse mares of light breeds were used in the study. Their ages varied from 3 to 18 years (mean 8.7 years). The mares were either maiden (n = 32) or they had foaled from 1 to 9 times (n = 25). They had no history of reproductive failure and were clinically normal. Weekly serum progesterone measurements were started at the beginning of February. Progesterone was determined using a direct RIA method (Farmos Diagnostika, Turku, Finland). Two subsequent samples with elevated (>10 nmol/l) progesterone concentrations were considered as the onset of cycling. The study was conducted from March to May 1994. During the experimental period, mares were used as embryo donors or recipients in another research project.

Examinations and sample collection

In 21 mares, oestrus cycles were synchronized by injecting 7.5 mg of luprostiol (Prosolvin, Intervet International B.V., Boxmeer, The Netherlands) i.m. during dioestrus. In other mares, examinations were started after 2 high serum progesterone values had been obtained. Rectal palpation and ultrasonography (Aloka SSD-210 Dx, 5 MHz probe) were performed every second day at the beginning of oestrus (serum progesterone <3 nmol/l), daily after detection

of a follicle ≥35 mm, and on the second day after ovulation. Ultrasonically visible free fluid within the uterus, termed fluid accumulation, was recorded until sampling and on the second day after ovulation. The maximum depth of free fluid was measured in 3 places (left and right uterine horns, uterine body). The sums of the measurements were used in the analyses. The appearance of the fluid was also evaluated: anechogenic (black), hypoechogenic (dark fluid with white spots) and hyperechogenic (light grey to white). The degree of endometrial oedema was scored from 0 (no oedema) to 3 (marked oedema).

When a mature follicle was detected, a tampon was placed into the uterus using a double-glove technique and left there for 15 min. The technique has been described by *Katila et al.* (1990). The degree of cervical opening was scored from 0 to 3 during sampling according to the number of fingers that could easily be passed through the cervix.

Handling of samples

After removal, each tampon was placed inside a 20-ml syringe, and the IUF absorbed by the tampon squeezed out into a sterile plastic tube. Microbiological and cytological analyses were performed on fresh samples. The remaining fluid was stored at -20 °C for later protein and enzyme analysis and in vitro semen motility studies.

Bacteriological and cytological examination

One drop of fluid was cultured on blood agar and incubated at 37 °C for 48 h. Plates were examined at 24 and 48 h. Bacterial growth was considered significant when more than 10 colonies of potential pathogens (ß-haemolytic streptococcus, *Staphylococcus aureus,* haemolytic *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa*) were recovered per plate. Polymorphonuclear neutrophils (PMN) were counted using a haemocytometer (25 µl sample + 475 µl 0.8% NaCl). The detection limit was 100000 cells/ml. If no fluid was obtained from the tampon, blood agar was touched with the tip of the tampon and further streaking with a loop undertaken. A smear for cytological examination was made by rotating the side of a tampon on a slide. The slide was stained with May-Grünewald-Giemsa. Cytological examination results were considered indicative of inflammation when >1 neutrophil was seen per high power field (hpf, 400X) or more than 100000 PMN/ml were recovered from the uterine fluid.

Protein and enzyme analysis

Protein concentrations (g/l) in IUF were measured using a dye-binding Bio-Rad protein assay (Bio-Rad Laboratories, California, USA) (*Bradford* 1976) and a microtitration plate reader (Titertek Multiskan photometer, Labsystems, Finland). TIC was measured using colorimetric assay (*Mattila et al.* 1985). The assay expresses antitrypsin activity in relative units, where a value of 1.0 represents the trypsin-inhibitor capacity of a standard bovine milk sample. The α_1-proteinase inhibitor content of this standard was 9 mg/l. A fluorogenic coumaryl peptide substrate was used for determinations of plasmin (*Richardson & Pearce* 1981). The determinations were performed on microtitration trays (*Mattila & Sandholm* 1986). Plasmin activity was expressed as µmol product/min/l at room temperature (RT). NAGase activity was determined using a method developed for microtitration plate fluorometry (*Linko-Löppönen & Mäkinen* 1985). Ten µl of IUF were mixed with 50 µl of substrate solution (2.25 mM 4-methyl-umbelliferyl-N-acetyl-ß-D-glucosaminide (Sigma M-2133) in 0.25 M citrate buffer, pH 4.6). After 15 min of incubation in the dark at RT, the reaction was stopped by adding 100 µl of 0.2 M glycine-NaOH buffer (pH 10.7). The fluorescence of the released 4-me-

thyl-umbelliferone (4-MU) was measured (excitation 355 nm, emission 480 nm) using a Fluoroskan-1 fluorometer (Labsystems, Finland). NAGase activities of IUF (μmol product/min/l at RT) were interpolated from a standard curve obtained using serial dilutions of 4-MU (Sigma M-1381) in 0.2 M glycine-NaOH buffer (pH 10.7). The method for determining B-Gase activity, a modification of the procedure described by *Nagahata et al.* (1987), was essentially the same as that used for determining NAGase activity. Four-methyl-umbelliferyl-ß-glucuronide (Sigma M-9130) was used as substrate, and the incubation period was increased to 1 h.

In vitro-motility studies of spermatozoa

Uterine fluid from 9 mares and semen from 1 stallion were used to investigate the effects of IUF on spermatozoal motion. Semen was collected using an open-ended artificial vagina. Spermatozoal concentration was determined, and the semen was extended 1:1 with Kenney's extender (*Kenney et al.* 1975). The extended semen was added to test tubes containing a) 0.4 ml of IUF (n = 9), b) 0.2 ml of IUF + 0.2 ml of Kenney's extender (n = 9) and c) 0.4 ml of Kenney's extender (n = 9, control). The final sperm concentration in each tube was 40 x 10^6/ml. The tubes were transferred into a water bath (37 °C). Spermatozoal motility was measured at 15 min, 2h15 min and 5h15 min. The motion characteristics of spermatozoa were measured using a Hamilton-Thorn motility analyser (HTM-S version 7.2) using Makler chambers. Five fields/sample were recorded on videotape for 10 sec/field. The tapes were then analysed and approximately 200 cells counted. The basic parameter settings had been determined earlier (*Andersson & Katila* 1992). Minimum contrast was adjusted to 6, minimum size to 8 and low/high intensity gates to 0.9/1.4. These values were determined by examining the specimen with the "playback" screen, ensuring that only sperm cells were included in the analysis. Total motility (MOT), progressive motility (PMOT), average path velocity (VAP), and percentage of rapid cells were used in the analysis of results. Possible spermatoxic effects of the tampon were tested by incubating pieces of tampon in semen for 2 h. MOT, PMOT, VAP and percentage of rapid cells were the same in semen incubated with tampon material as compared to control semen.

Artificial insemination and embryo recovery

The donor mares (n = 35) were inseminated with 500 x 10^6 progressively motile spermatozoa extended in Kenney's medium every second day until ovulation, starting immediately after (n = 24) or within 2 (n = 9) or 3 to 5 days (n = 2) of sampling. Twenty-four out of 57 mares were given 2500 IU of hCG i.v. to synchronize ovulation. Embryos were recovered nonsurgically 6 days after detection of ovulation. Immediately after embryo recovery, the donor mares were given 7.5 mg of luprostiol i.m.

Endometrial biopsy

An endometrial biopsy specimen was taken during induced (25 mares) or natural (28 mares) oestrus after completion of the embryo transfer programme. Four mares were not biopsied. The presence of fluid accumulation was determined by ultrasonography before taking the biopsy. Biopsy specimens were obtained and processed according to *Kenney & Doig* (1986). Quantities of PMN and mononuclear cells were scored as follows: none (0), slight diffuse infiltration in the stratum compactum (SC) (1), moderate diffuse infiltration in SC possibly associated with occasional small focal infiltrations in the stratum spongiosum (SS) (2), and diffuse, moderate or heavy infiltration in SC and focal infiltrations in SS, either frequently occurring or of a moderate size (3). Foci of gland nests or cystically distended

Table 1. Protein concentrations and enzyme activities in uterine fluid in mares with and without intrauterine fluid accumulation detected using ultrasonography.

	No fluid detected (n = 13)	Fluid detected (n = 14)	P value
Total protein (g/l)	25.2 ± 12.9	8.2 ± 8.7	0.0005
TIC (relat. units)	71.4 ± 72.8	9.4 ± 14.7	0.0045
Plasmin (U/l)	0.89 ± 0.84	1.39 ± 1.40	0.2701
NAGase (U/l)	57.4 ± 43.5	98.8 ± 147.4	0.3405
ß-glucuronidase (U/l)	4.66 ± 2.94	1.89 ± 0.99	0.0047

Values are expressed as means ± SD.
TIC : trypsin-inhibitor capacity.
NAGase : N-acetyl-ß-D-glucosaminidase.
U : µmol product/min.

glands surrounded by periglandular fibrosis were counted in at least 4 linear fields of 5.5 mm and the average calculated. Fibrosis was scored as follows: none (0), <1 focus/field (1), 1 to 3 foci/field (2), 3.1 to 5 foci/field (3), and >5 foci/field (4). The number of periglandular fibrocyte layers ranged from 2 to 4. Lymphatic lacunae (LL) were scored as follows: none (0), low numbers of small LL (1), moderate numbers of small or medium LL (2), high numbers of small to medium LL or small or moderate numbers of large LL (3), and LL covered a substantial area in the biopsy specimen (4).

Statistical analysis

Chi-square analysis or Fisher's exact test was used to assess significance of associations between fluid accumulation and clinical findings (endometrial oedema, scores 0-1 vs. 2-3; degree of cervical opening, scores 0-1 vs. 2-3; age of mares, over 7 years vs. younger), and fluid accumulation and endometrial biopsies (neutrophils, scores 0 vs. 1-2; mononuclear cells, scores 0-1 vs. 2-3; fibrosis, scores 0-1 vs. 2-4 and lymphatic lacunae, scores 0-2 vs. 3-4). Differences in the concentration of total protein and the activities of TIC, plasmin, NAGase and B-Gase in IUF were analysed using general linear model procedures for analysis of variance (*SAS Institute Inc.* 1988). Analysis of variance was used to determine the significances of effects of IUF on spermatozoal motility.

Results

Fluid accumulations were detected before sampling on one or more occasions in 39% of mares (22/57). No mare exhibited fluid accumulation 2 days after ovulation. Fifteen mares (26%) exhibited accumulation of anechogenic fluid on the sampling day. In these mares, the sums of maximal depths of fluid at 3 locations were 1 to 15 mm (n = 9), 16 to 30 mm (n = 4) and >30 mm (n = 2). In 80% of cases fluid was located only in the uterine body. Forty-two mares (74%) were classed as mares without fluid accumulation, although 7 had exhibited fluid accumulation before the sampling day. The average time ± SD between sampling and ovulation was 2.8 ± 1.6 days (range 1 to 9 days).

Six of the 15 mares with fluid accumulation (40%) and 14 of the 42 mares without fluid accumulation (33%) had moderate to marked oedema of endometrial folds (scores 2 to 3) at the time of sampling. The degree of cervical opening was mostly 1 in both groups. Mares ≤7 years (4.8 ± 1.4 years) exhibited fluid accumulation almost as often (4/24 vs. 11/33, p=0.16)) as mares >7 years (11.5 ± 2.7 years).

IUF was recovered from 14/15 (93%) and 13/42

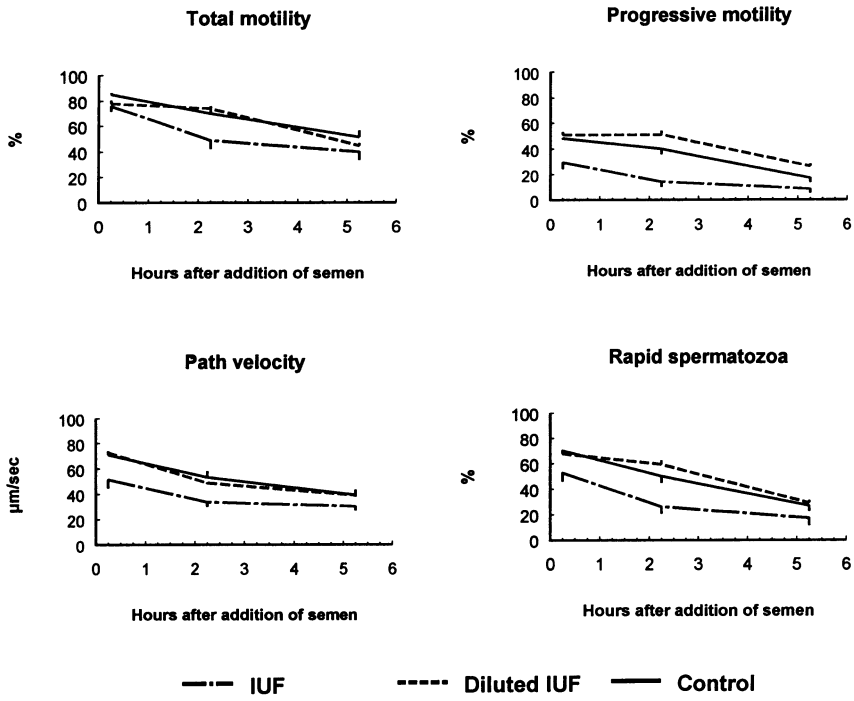

Figure 1. Motion characteristics (mean ± SEM) of spermatozoa extended with Kenney's medium, and incubated with uterine fluid (IUF, n = 9), diluted uterine fluid (1:1 dilution IUF/Kenney's medium, n = 9) and Kenney's medium only (control, n = 9).

(31%) tampons from mares with and without fluid accumulations, respectively. The average volume of IUF recovered was 1.6 ml (range 0.6 to 4 ml) and 0.5 ml (range 0.25 to 1 ml) in mares with and without fluid accumulation, respectively. Nine out of 15 (60%) and 22 of 42 (52%) tampons from mares with and without fluid accumulations, respectively, were bacteriologically positive, but growth was considered insignificant. Twenty-four samples yielded 1 to 10 colonies. Seven yielded more than 10 colonies, but had either mixed growths or nonpathogenic organisms. No neutrophils were recovered from uterine fluid (n = 27). A few neutrophils were found in 3 out of 30 smears, but the mean number of PMN per hpf in each case was <1.

Total protein concentration, TIC and B-Gase activity in IUF were statistically significantly lower in mares with fluid accumulation than in mares without fluid accumulation ($p<0.01$) (Table 1.). No difference in plasmin ($p=0.27$) or NAGase ($p=0.34$) activities was found between the two groups.

The effects of IUF on spermatozoal motion are shown in Figure 1. The addition of undiluted IUF to extended semen significantly reduced PMOT, VAP and percentage of rapid spermatozoa ($p<0.01$). The effect on MOT was less pronounced ($p=0.03$). The addition of diluted ute-

rine fluid to extended semen had no effect on MOT, PMOT, VAP or percentage of rapid spermatozoa.

Embryo recovery rate was 6/9 (67%) and 17/26 (65%) in mares with and without fluid accumulation, respectively. Twenty-five embryos (including 2 sets of twins) were recovered. On endometrial biopsy, fibrosis was more prominent ($p=0.03$) in mares with fluid accumulation ($n = 9$) than in mares without fluid accumulation ($n = 44$), and in mares >7 years ($n = 30$) than in mares ≤7 years of age ($n = 23$; $p=0.046$). Neutrophils, mononuclear cells, and lymphatic lacunae were not associated with fluid accumulation ($p>0.05$).

Discussion

The collection of IUF with a tampon proved satisfactory in mares with fluid accumulation. However, in mares without fluid accumulation, the fluid absorbed into the tampon was not always sufficient for all analyses. A higher pressure system for squeezing the fluid from the tampon would need to be used to improve recovery rates from the tampon. In recent decades, uterine secretions have been obtained by uterine lavage (*Zavy et al.* 1978). This technique results in dilution of native secretions, to unknown extents. Observations on components of undiluted uterine secretions from normal cyclic mares are scarce. The biochemistry of endometrial secretions during oestrus is of great interest because spermatozoa are exposed to this environment on their way to the oviduct. The results of the study reported here provide reference data on various proteins and enzymes in native uterine secretions during oestrus. Furthermore, the low protein content suggests that anechogenic IUF during oestrus resembles a transudate. When fluid was not detected on ultrasonography, the protein content of IUF was much higher than when fluid accumulations were detected. IUF may also have originated from watery endometrial gland secretions, as suggested by Liu (*review by Allen* 1993) or mucus from epithelial cells in response to hormonal or irritative/inflammatory stimuli (*Freeman et al.* 1990).

The mean TIC of uterine fluid was lower in mares with fluid accumulations than in mares without, but variation among mares was high. Alpha-1-proteinase-inhibitor, also known as α1-antitrypsin, accounts for 90% of the TIC of human serum (*review by Berninger* 1986). *Scudamore et al.* (1994) have shown that α1-PI in the mare is not produced in the endometrium but leaks from the blood into the uterine lumen. An enhanced accumulation of α1-PI is expected under vascular conditions of increased permeability. Consequently, in the study of *Scudamore et al.* (1994), the mean concentration of α1-PI relative to total protein was higher in uterine flushings collected during oestrus than in flushings collected during dioestrus.

The proteolytic activity of the uterine fluid in the form of plasmin did not differ between groups. Plasmin is a serine proteinase which digests fibrin, fibrinogen, and many other proteins (*Dodds* 1980). In the present study, the techniques for TIC and plasmin analyses detected only functional antiproteinase and proteinase. Because of antiproteinase-proteinase complex formation or proteolytic inactivation, results of these analyses may not be reliable measures of vascular leakage of α1-PI and plasminogen.

Mean NAGase activities did not differ in mares with and without fluid accumulation. B-Gase activities were lower in mares with fluid accumulation. Although NAGase and B-Gase are both lysosomal enzymes, their origin, secretion rate or inactivation rate may have been different from each other.

A few bacteria were frequently cultured from tampons. They probably represented contamination because none of the cultures were accompanied by neutrophilia. A non-guarded

tampon is likely to become contaminated especially during withdrawal through a vaginal speculum. *Pycock & Newcombe* (1996) have reported that nonechogenic fluid frequently accumulates in the absence of microbiological and cytological signs of acute inflammation. These studies indicate that anechogenic IUF during oestrus is generally not of inflammatory origin.

Uterine fluid is usually graded from 1 (white) to 4 (black), according to the echogenicity (*McKinnon et al.* 1988). It would seem that fluid quality has a greater influence than fluid quantity on fertility. In the study of *Squires et al.* (1989), small volumes of grade 1 or grade 2 fluid greatly reduced embryo recovery rate. In our study, anechogenic fluid (grade 4) during oestrus did not affect embryo recovery rates. In the study of McKinnon et al. (1987), embryo recovery rates were unaffected when the estimated fluid quantity was <100 ml. In the above study, detection of grade 1 or grade 2 fluid was rare. Only large quantities of fluid during oestrus affected embryo recovery rates. Although anechogenic fluid during oestrus does not affect fertilization significantly, it could have negative effects on later embryo development, and thus on pregnancy rates. Significantly higher pregnancy rates were reported for mares with no visible fluid just before mating (115/185, 62%) than for mares with 1 to 20 mm of fluid (4/15, 27%) and those with >20 mm of fluid (1/8, 13%) (*Pycock & Newcombe* 1996).

Squires et al. (1989) have shown that all grades of undiluted IUF (grades 1 to 4) suppress spermatozoal motility in vitro and that the negative effect disappears with dilution of the IUF. Similarly, MOT, PMOT, VAP and percentage of rapid spermatozoa were reduced by the addition of undiluted IUF in our study. It is, however, not clear what consequences these observations might have in vivo. Sensitivity of stallion semen to adverse environmental effects is extremely variable. Semen from only one stallion was tested in our study. Different results might have been obtained, if several stallions had been included in the study. Although small amounts of grade 1 or grade 2 fluid obviously decrease fertility by damaging spermatozoa prior to fertilization (*Squires et al.* 1989), embryo recovery rates in our study indicate that fertilizing spermatozoa reach the oviduct undamaged when small amounts of grade 4 fluid are present in the uterus.

In the study reported here, fluid accumulation was associated with increased fibrosis of the endometrium. Endometrial fibrosis has been suspected to interfere with uterine drainage through the lymph vessels. *LeBlanc* (1994) has suggested that mares susceptible to endometritis or accumulation of fluid after mating may have problems with lymphatic drainage. In our study, lymphatic lacunae were a common finding in the endometrial biopsy specimens obtained during oestrus, and were not associated with fluid accumulation.

Our conclusion is that anechogenic IUF of non-inflammatory origin is commonly seen during ultrasonography of the uterus of normal oestrous mares. Accumulation of anechogenic fluid is associated with compositional changes in the uterine secretions. Although IUF may have negative effects on spermatozoal motion, small accumulations of non-inflammatory IUF at the time of insemination do not decrease conception rates.

Acknowledgements

The authors thank the personnel at the Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Helsinki, Finland for facilities for protein and enzyme analyses.

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Sammanfattning

Ansamling av vätska i livmodern hos brunstiga ston.

Sekret ur livmodern hos brunstiga ston (n = 57) uppsamlades med tampon för att jämföra sådan vätska som ansamlats i livmodern (intrauterine fluid = IUF) då vätska diagnosticerats genom ultraljudsundersökning, med fall där ansamling inte kunnat ses med ultraljud. Prov av alla IUF (n = 57) togs för bakteriekultur och räkning av antalet neutrofila celler. Dessutom bestämdes den totala proteinmängden, trypsininhibitionskapaciteten (TIC) samt aktiviteten av ß-glukuronidas (B-Gase), N-acetyl-ß-D-glukosaminidas (NAGase) och plasmin ur 27 prov. Nio av dessa prov analyserades med en Hamilton-Thorn motilitetsanalysator i avsikt att utröna rörligheten av spermier i sädesvätska som utspätts enbart med IUF, med IUF och Kenney's medium (1:1) och enbart med Kenney's medium. De ston som var avsedda för embryodonation inseminerades omedelbart efter o.a. provtagning och varannan dag därefter ända tills ovulation skedde. Embryona återvanns på icke-kirurgisk väg 6 dygn efter ovulationen. Under följande brunst, registrerades förekomsten av IUF i livmodern och en biopsi av livmoderslemhinnan togs (n = 53). Ansamling av IUF förelåg före provtagningen hos 22 av 57 ston (39%) och på dagen för provtagningen hos 15 av 57 ston (26%). Vätskan var inte eckogen och i 80% av fallen befann den sig i corpus uteri. I inget fall förelåg cytologiska eller bakteriologiska tecken på akut endometrit. Den totala proteinkoncentrationen, TIC och B-Gase var statistiskt signifikant lägre i IUF från ston med sonografiskt påvisbar IUF (n = 14) än från ston utan sonografiskt påvisbar IUF (n = 13) ($p<0.01$). Då outspädd IUF tillsattes till utspädd sädesvätska, sjönk spermiernas totala och progressiva motilitet och hastighet och även andelen snabba spermier reducerades in vitro ($p<0.05$). Endometriebiopsierna uppvisade mer markant fibros bland ston med IUF ansamling ($p=0.025$, n = 9) än bland ston utan IUF ansamling (n = 44).
Sammanfattningsvis kan konstateras att ansamlingen av icke eckogen vätska under brunsten var förenad med förändringar i sammansättningen av IUF. Trots att IUF inverkade menligt på motiliteten av spermier in vitro, påverkade inte förekomsten av IUF vid tidpunkten för insemination återvinningen av embryon.

(Received February 27, 1996; accepted November 15, 1996).

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