FACTORS AFFECTING THE CERVICAL MUCUS CRYSTALLIZATION, THE SPERM SURVIVAL IN CERVICAL MUCUS, AND PREGNANCY RATES OF HOLSTEIN COWS

UKAZATELE OVLIVŇUJÍCÍ KRYSTALIZACI CERVIKÁLNÍHO HLENU, PŘEŽITELNOST SPERMIÍ V CERVIKÁLNÍM HLENU A ZABŘEZÁVÁNÍ KRAV HOLŠTÝNSKÉHO PLEMENE

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Manuscript received: December 18, 2006; Reviewed: July 2, 2008; Accepted for publication: July 16, 2008

ABSTRACT

The objective of this study was to determine the relationship between calving year and season, parity, number of AI, day of lactation, milk production in the 1st 100 lactation days or diseases occurrence (retained placenta, endometritis or cysts), sperm motility (SM) during 30, 60 and 90 minutes of the cervical mucus survival test, cervical mucus crystallization (CMC) and their influence on days to first insemination (interval), open days (SP), inseminations number for pregnancy (index), and pregnancy rates (PR) in Holstein cows (n=284). Significant differences of interval, SP and index were detected also in relation to number of AI and day of lactation ($P < 0.001$). Cows without reproduction diseases (healthy) had better results of interval, SP ($P < 0.01$), index and PR. Pregnancy rate of healthy cows was by 11.43% higher, but without statistical significance. The higher results of PR (62.74%) were discovered in relation to ferny-like crystallization of cervical mucus ($P < 0.001$). CMC affected results of cervical mucus survival test, the highest motility of sperms after the 60 and 90 minutes was assumed in the case of club moss – ferny (14.80% and 7.96%) and ferny-like crystallization (13.82% and 8.47%) with statistical significance ($P < 0.05$). The choice of these characteristics and definition of their relations allows assuming their using for detailed study and determination of the cows´ biological ability to conceive, the one of the main components of efficiency of cows´ reproduction.

Keywords: Holstein cows, reproduction, milk production, health, sperm motility, cervical mucus crystallization

ABSTRAKT

Cílem práce bylo vyhodnotit vztahy mezi rokem a ročním obdobím otelení, pořadím laktace, dnem laktace, mléčnou užitkovostí za sto dní laktace, výskytem reprodukčních poruch (zadržení lůžka, endometritida, ovariální cysty), aktivitou spermií během testu přežitelnosti v cervikálním hlenu, krystalizaci cervikálního hlenu a vliv těchto faktorů na délku inseminačního intervalu, servis periodu, inseminační index a zabřezávání ve stádě holštýnských dojnic (n=284). Vzorky cervikálního hlenu (CM) byly získávány sterilní pipetou rektovaginální metodou v den inseminace u skupiny krav během března 2002 až prosince 2005. Arborizace CM byla hodnocena pod mikroskopem po rozředí a zaschnutí na podložním sklíčku. Mikroskopicky byl posuzován podíl spermií s progresivním pohybem (aktivita) během testu přežitelnosti po 30, 60 a 90 minutách inkubace v cervikálním hlenu ve vodní lázni při teplotě 38±1°C. Byl zjištěn statisticky významný ($P<0,001$) vliv pořadí inseminace a počtu laktačních dní na délku intervalu a SP. Zdravé plemenice dosahovaly lepších výsledků v délce intervalu (rozdíl 8,82 dne), SP (rozdíl 42,72 dní, $P<0,01$), indexu (rrozdíl 0,44) i zabřezávání (rozdíl 9,44%) v porovnání s kravami s reprodukčními poruchami zjištěnými po otelení. Nejlepších výsledků ($P<0,001$) v zabřezávání (62,74%) dosahovaly dojnice, u nichž v testu arborizace CM byla zajištěny kapraďovitá krystalizace (tzn. optimální doba pro inseminaci). Nejvyšší aktivita spermií ($P<0,05$) byla zjišťována v případě plavuňovito-kapraďovité a kapraďovité krystalizace CM v čase 60 (14,8 a 13,82%) a 90 (7,96 a 8,47%) minut. Testy arborizace byly zvoleny pro posouzení vhodnosti doby inseminace a testy přežitelnosti v CM pro posouzení schopnosti oplození, resp. pro predikci zabřeznutí. K hodnocení výsledků byl využit statistický program SAS STAT8,0 GLM, lineární model.

Klíčová slova: Holštýnské dojnice, reprodukce, mléčná užitkovost, zdraví, aktivita spermií, krystalizace cervikálního hlenu

INTRODUCTION

Reproductive management, use of particular timely oestrus detection, is important for profitable dairy production. Several factors influence reproductive performance, they may be caused by problems related to the modern high yielding dairy cow herself (postpartum endocrinopathies leading to ovarian disturbances), or may reflect serious shortcomings in the management (inadequate heat detection) according to [21], [13]. These main factors are days from calving, breed, parity, signs of behavioural oestrus, insemination dates, pregnancy determinations, energy status, body fat status, milk urea content, and reproductive disorders associated with calving [16], [17]. Further, there has been a substantial decline in reproductive performance in recent decades and associated with genetic selection for higher milk production and correlated increases in usage of body reserves [18], [7]. There has been a general increasing trend in herd size with an associated increase in the number of cows per husbandry person. This fact frequently resulted in less time and attention is devoted to each cow.

There already exist a number of systems for estrus detection, based on behavioural or physiological measures, these factors suggest that an improved management tools especially an automated one, to detect oestrus and other reproductive states would be valuable [6].

Therefore, the relationship between various estrus characteristics and time of ovulation was studying to investigate for possibility of ovulation time prediction [15]. The crystallization pattern (arborisation) of cervical mucus could be used to detect oestrus. The cervical mucus during estrus shows a fern pattern of crystallization [8].

The objective of this study was to determine relations between calving year, season, parity, lactation days, milk production in the 1st 100 days of lactation, health status in lactation and their influence on fertility traits and results of cervical mucus arborisation or sperms motility during survival test in Holstein cows.

METHODS

Production traits (calving year and season, parity, milk production in the 100 days of lactation) and fertility traits (calving to first insemination interval – interval, open days – service period, pregnancy rate and services per conception – index) were analysed in herd of high production Holstein cows.

Data and samples were collected at the university dairy farm with the 423 purebred Holstein cows. Cervical mucus samples were collected and analyzed in a group of reproduction problematic cows (n=284) calved

Table 1: Relations of year of calving, number of lactations and inseminations to fertility indicators
Tabulka 1:Vliv roku otelení, pořadí laktace a počtu inseminací na ukazatele plodnosti

		n	Interval			SP			Index			PR		
			μ+α	SE	P	μ+α	SE	P	μ+α	SE	P	μ+α	SE	P
Year of calving	1.(2002)	48	82.00	4.07	1-2*	198.34	12.16		3.58	0.23		38.16	7,39	
	2. (2004)	49	85.61	5.57		203.74	17.08		3.05	0.33		54.96	9,78	
	3. (2005)	187	93.87	2.53		209.93	7.59		3.27	3.27		35.02	4,49	
Number of lactations	1st	107	87.94	4.24		200.14	12.98		3.48	0.25		41,03	7,44	
	2nd	70	84.38	4.06		195.91	12.31		3.11	0.24		45,37	7,19	
	3rd and next	107	89.17	3.46		209.98	10.55		3.30	0.20		41,74	5,93	
Number of insemination	1st	127	123.59	3.55	1-2-3***, 2-3***	188.98	10.46	1,2-3*	2,02	0.20	1,2-3***, 2-3***	50,93	7,14	
	2nd	69	77.19	4.00		190.20	11.73		3,09	0.23		36,61	6,93	
	3rd and next	88	60.71	4.22		226.83	12.18		4,79	0.24		40,6	6,29	

from March 2002 to December 2005. The cows with reproduction disorders were treated with Ovsynch method to heat synchronization and ovular dysfunction therapy in interval of 14 days. Cows´ health disorders such as retained placenta, endometritis, and ovarian cysts occurrence before evaluation were obtained from the herd personnel or the herd veterinarian's farm records.

Samples of cervical mucus for laboratory testing of arborisation and survival test were sampled in this group of cows. The cervical mucus was drained with a sterile pipette by recto-vaginal method at the time of insemination. Samples were transferred at 4°C temperature to the university laboratory within 2 hours in. The arborisation test (crystallization) of the cervical mucus for assessment of insemination time suitability and the test of sperm survival in cervical mucus for assessment of fertilization ability were realized. The microscopy method for evaluation of crystallization (levels: club moss-like patterns, club moss-fern patterns, fern-like patterns, fern-like patterns decomposition, none or dotted structures) of cervical mucus dried on glass slide was used. A sperm survival test in the cervical mucus for assessment of cows' ability to conceive was performed. Motility of the bulls' sperms in cervical mucus was evaluated by microscopic examination. The percentage rate of progressively moving sperms was evaluated using a microscope with phase contrast. Frozen insemination doses of five Holstein bulls were used in the tests. Sperm motility after thawing was 40% on the average. The motility values were detected after 30, 60, and 90 minutes of the test duration in a water bath at a temperature of 38 ± 1^{o}C. Sperm motility in the cervical mucus survival test was estimated on the following scale: 0%-without, 1%-sporadic, 10%, 20%, 30%, 40%, and 50%. Control insemination doses were evaluated by the same procedure, but without cervical mucus. The average motility of the control measuring was 50, 40 and 30% respectively during the course of the test.

The statistical program SAS STAT 8.0- GLM, by the general linear model, analyzed data:

$$Y_{ijklmno} = \mu+A_i+B_j+ C_k+D_l + F_m + G_n + e_{ijklmno},$$

where:

$Y_{ijklmno}$ … observed value of the trait as a dependent variable (pregnancy rate, days to first insemination, open days, number of inseminations, cervical mucus arborisation or sperms motility in %),

μ … average value of dependent variable

A_i … effect of i-calving year (i = 2002, 2004, 2005)

B_j … effect of j-calving period (j = 1 - from January to March, 2 – from April to June, 3 – from July to September, 4 – from October to December)

$C_{k\ ....}$ effect of k-number of lactation (k = 1st , 2nd , 3rd and next lactations)

D_l … effect l-AI number/order (l = 1st, 2nd, 3rd and next AI)

F_m … effect of m-group of lactation day in AI (m = 1 - ≤72 days, 2 – 73-96 days, 3 – 97-120 days, 4 – 121-144 days, 5 – 145-168 days, 6 – 169-216 days, 7 – 217 days ≥)

G_n … effect of n-group of milk production during the first 100 lactation days (n = 1 - < $\bar{x}$ - s_d, 2 – from $\bar{x}$ - s_d to $\bar{x}$ - 0.25 s_d, 3 – from $\bar{x}$ - 0.25 s_d to $\bar{x}$ + 0.25 s_d, 4 – from $\bar{x}$ + 0.25 s_d to $\bar{x}$ + s_d, 5 - $\bar{x}$ + s_d >

$e_{ijklmno}$ …residual effects

The evaluation of the effect of health disorders was based on the same general linear model supplemented with H_o … effect of o-occurrence of retained placenta or endometritis or ovarian cysts or ovarian cyst frequency (o = 1 – without disorder or 1x ovarian cyst occurrence, 2 – disorder occurrence or multiple ovarian cysts occurrence).

Differences between dependent variables were tested on the levels of significance $P < 0.05$ (*); $P < 0.01$ (**) a $P < 0.001$ (***).

RESULTS AND DISCUSSION

Sperm motility was determined in the 284 cervical mucus samples of Holstein cows after 30, 60 and 90 minutes of a survival test. Cows calved from March 2002 to December 2005 for the first to the eighth lactation, with the average parity 2.28. The average milk production in the first month of lactation was 33.82 kg with a protein content of 3.27%, 3612.1 milk kg with a protein content of 3.19% in the first 100 days of lactation, and 9 855.1 milk kg with protein content of 3.30% in 305 days of lactation. Cows in the evaluated group had reproduction failures, were treated with Ovsynch for estrus synchronization and bred by timed AI. The average values of reproduction parameters were 3.00 AI services per conception, 106.4 days of interval, and 208.6 open days (SP). Average sperm motility in cervical mucus samples were 19.78%, 12.37% and 7.69% at times of 30, 60 and 90 minutes during the survival test

The results of reproduction characteristics (calving to first insemination period-interval, open days - SP, number of insemination for pregnancy - index and pregnancy rate) are expressed in relation to calving year, lactation number, order of inseminations in Table 1 and in relation to calving season, lactation days, and milk production in the 100 days of lactation in Table 2.

The best results of interval (82 days, $P < 0.05$) and SP

Table 2: Relations of calving season, days of lactation and 100 days milk production to fertility indicators
Tabulka 2: Vliv ročního období otelení, dne laktace a mléčné užitkovosti ve 100 dnech laktace na ukazatele plodnosti.

		n	Interval			SP			Index			PR		
			μ+α	SE	P	μ+α	SE	P	μ+α	SE	P	μ+α	SE	P
Calving season	1. (January - March)	65	90.57	4.57		217.96	14.31		3.33	0.28		48,27	7,91	
	2. (April - June)	46	88.37	4.76		191.08	14.39		3.46	0.28		47,38	8,28	
	3. (July- September)	97	83.40	3.30		200.44	9.62		3.30	0.19		41,98	5,91	
	4. (October -December)	76	86.30	3.24		198.54	10.08		3.10	0.19		33,22	6,1	
Days of lactation	1. (≤ 72 days)	16	37.59	7.94	1-3,4,5,6,7***, 2-3,4,5,6,7***, 3-5,6,7***, 4-5*,4-6,7***,5-6,7***	161.09	22.92	1-5*,1-6**,1-7***,2-5*,2-6,7***, 3-6*,3-7***,4-6,7***, 5-7***	3.35	0.44		6,49	17,64	1-3**, 1-4,5*, 1-6,7*, 2-3*
	2. (73-96 days)	40	45.71	5.13		151.72	14.88		3.29	0.29		16,57	12,59	
	3. (97-120 days)	27	77.65	6.16		185.66	17.66		3.29	0.34		47,11	12,56	
	4. (121-144 days)	58	84.90	4.54		179.09	13.80		3.18	0.27		34,82	9,68	
	5. (145-168 days)	34	100.26	4.70		210.79	13.77		3.69	0.27		42,40	9,19	
	6. (169-216 days)	31	126.85	5.07		250.18	15.07		3.24	0.29		39,81	9,90	
	7. (217 days ≥)	78	137.16	3.95		275.49	11.97		3.05	0.23		47,85	7,93	
100 days milk production	1. (≤ 2742 kg)	46	85.32	4.95		204.57	15.45	2-3*,3-5**,4-5*	2.99	0.30	1-3*,2-3***,2-4,5*	46,13	9,18	
	2. (2743-3395 kg)	80	88.78	4.11		189.82	12.43		2.69	0.24		51,48	7,25	
	3. (3396-3830 kg)	53	91.74	4.67		230.31	13.55		3.85	0.26		45,38	8,18	
	4. (3831-4483 kg)	55	84.24	4.35		209.53	13.24		3.52	0.26		35,45	7,81	
	5. (4484 kg ≥)	50	85.73	4.45		176.08	14.22		3.44	0.27		35,12	7,99	

(198.34 days) were found in 2002. The lower index (3.05) and pregnancy rate (54.96%) was detected in 2004, but with no statistical significance.

Number of inseminations had statistical significant effect ($P < 0.001$, $P < 0.05$) on interval, SP, index and PR. The lowest SP (188.98 days), index (2.02) and highest PR (50.93%) was detected after first insemination; the shortest interval (60.71 days) was detected in cows after more than 3rd insemination, on the contrary. [9] stated a decline of conception rate, when number of inseminations increased.

There was not found significant trend in fertility traits in relation to calving season, in our study. The best pregnancy rate was detected in cows calved during year periods January - March (48.27%) and April – June (47.38%), lower SP (191.08 days) was detected in April - June. [23] mentioned that fertility might be more affected by milk production than hot weather. [4] described lower pregnancy rates during summer vs. winter insemination or natural service (9.0% vs. 17.9%). According to [1] estrus behaviour and reproduction results are affected by heat stress in warm-summer months.

Lactation number affected reproduction traits without statistical significances; the lowest interval, SP, index and highest PR was assumed in cows in second lactation. [2] found that primiparous cows were more likely to become pregnant than multiparous cows, while [9] introduced opposite results. Cows on the second lactation achieved the best reproduction (interval 84.38 days, SP 195.91 days, index 3.11 and pregnancy rate 45.37%) in our study. Primiparous cows as well as cows on 3rd and subsequent lactation achieved worse results in every indicator.

The lactation day (stage of lactation) significantly ($P < 0.05$, $P < 0.01$, $P < 0.001$) affected interval, SP and PR. Differences of production parameters in relation to milk production in the 100 days of lactation was assumed. Differences with statistical significance were found in SP ($P < 0.05$, $P<0.01$) and index ($P < 0.05$, $P < 0.001$). The lowest SP (173.08 days) had cows with the 100 days milk production higher than 4 484 kg, cows with milk production from 2 743 to 3 395 kg had lowest index (2.69). Research of [2] described the better reproduction results of cows with higher milk production during evaluation.

The monitoring of the chosen health disorders and their effect for the indices of milking efficiency, respective of the fertility, is more common [20]. Table 3 includes effect of cows´ health (healthy, retained placenta, metritis, ovarian cysts and their frequency) on fertility traits. Health affects observed fertility traits. Healthy cows had better result of interval (84.61 days vs. 93.43 days), SP (172.12 days vs. 214.87 days), and index (2.99 vs. 3.43);

Table 3: Relations of health and reproductive disorders to fertility indicators
Tabulka 3: Vliv zdravotního stavu a reprodukčních poruch na ukazatele plodnosti

		n	Interval			SP			Index			PR		
			μ+α	SE	P	μ+α	SE	P	μ+α	SE	P	μ+α	SE	P
Health	1. (no)	242	84.61	2.93		214.87	8.60		3.43	0.17		44,67	4,57	
	2. (yes)	42	93.43	4.35		172.12	12.36	1-2**	2.99	0.24		35,23	8,30	
Retained placenta	1. (no)	264	87.37	2.62		203.29	7.73		3.30	0.15		43,99	4,25	
	2. (yes)	20	84.54	6.33		182.11	20.99		3.35	0.41		26,69	11,74	
Uterus wash	1. (no)	253	86.56	2.60		202.29	7.75		3.26	0.15		44,59	4,24	1-2*
	2. (yes)	31	95.67	5.80		203.99	18.60		3.97	0.36	1-2*	23,26	10,23	
Ovarian cyst	1. (no)	46	92.35	4.08		173.41	11.73		2.99	0.23		36,08	7,85	
	2. (yes)	238	84.59	3.00		217.04	8.78	1-2**	3.46	0.17		44,66	4,62	
Frequency of cysts´ occurrence	1. (1x)	121	86.88	3.29		199.41	10.68		3.11	0.21		62,77	5,85	1-2***
	2. (2x ≥)	115	82.74	3.46		237.53	11.49	1-2**	3.79	0.23	1-2**	33,82	6,05	

Table 4: Relations of sperm motility during survival test (30, 60, 90 minutes) to fertility indicators
Tabulka 4: Vliv aktivity spermií během testu přežitelnosti (30, 60, 90 minut) na ukazatele plodnosti

			Interval			SP			Index			PR		
		n	μ+α	SE	P	μ+α	SE	P	μ+α	SE	P	μ+α	SE	P
test30	1. (≤1%)	48	84.89	4.42		190.37	14.33		3.39	0.27	4-5*	34,13	8,02	
	2. (10%)	56	79.11	4.53		201.01	13.88		3.31	0.26		42,20	7,90	
	3. (20%)	68	89.03	4.11		193.92	12.62		3.18	0.24		43,39	7,29	
	4. (30%)	85	88.69	3.61		210.55	10.29		3.50	0.19		49,89	6,44	
	5. (40 % ≥)	27	90.72	5.43	2-3,4*	182.68	15.39		2.83	0.29		50,79	50,79	
test60	1. (≤ 1%)	118	84.23	3.28		188.28	10.14		3.21	0.19		43,80	5,68	
	2. (10%)	59	86.00	4.18		197.34	12.90		3.17	0.25		39,40	7,34	
	3. (20%)	47	89.78	4.56		209.17	12.79		3.57	0.24		40,67	8,51	
	4. (30% ≥)	60	91.47	4.14		206.61	11.72		3.26	0.22		53,71	7,52	
test90	1. (≤ 1%)	176	85.00	2.91		194.61	8.77		3.25	0.17		42,55	4,96	
	2. (10%)	40	89.09	4.83		189.39	13.96		3.23	0.27		51,38	8,65	
	3. (20%)	37	96.79	5.00		215.62	14.12		3.29	0.27		48.00	9,26	
	4. (30% ≥)	31	87.58	5.51	1-3*	211.57	15.48		3.47	0.30		52,55	10,03	

statistical significance was detected in SP ($P < 0.01$). Frequency of ovarian cyst occurrence had significant effect ($P < 0.001$) on PR (62.77% in case of 1 times occurrence vs. 33.82%). [3] detected, that older cows showed an increased risk of retained placenta or ovarian cysts. Healthy cows achieved better results of fertility compared to cows with some reproduction disorders (difference of interval 8.82 days, of SP 42.75 days and index 0.44) in our study.

Table 4 describes relationships between the sperm motility during the survival test in cervical mucus (30, 60, and 90 minutes) and evaluated reproduction parameters. The best PR was assumed in case of the best motility during all survival test (50.79% and 53.71% and 52.55%), but with no statistical significance.

One of the major contributors to poor fertility of a dairy herd is ineffective detection of estrus [24]. [10] indicated that in high-yielding dairy herds, the difficulty associated with successfully estrus detection is a problem that limits the reproductive performance of the herd. Crystallization pattern could be used to detect oestrus. [19] indicated the relationships between the ultrastructural and rheological properties of bovine cervical mucus and sperm motion. [12] and [11] performed tertiary branching of the fern pattern and sperm migration tests with thawed mucus. Table 5 represents relationships between cervical mucus crystallization (club moss-like patterns, club moss-fern patterns, fern-like patterns, fern-like patterns decomposition, none or dotted structures) and reproduction results in evaluated herd of Holstein cows. Oestrus and ovulation were associated with "fern-like" patterns of air-dried cervical mucus from cows [8]. The cervical mucus crystallization affected PR of observed cows ($P < 0.05 – P < 0.001$), the best result of conception (62.74%) was assumed in case of ferny-like crystallization of mucus sample collected in time of insemination, i.e. in the best stage of estrus. The differences of PR in the case of the other type of mucus crystallization were from 22.67 to 49.15%. The same trend was detected in length of SP, the lowest SP (189.12 days) had cows with ferny-like crystallization of its mucus, but with no statistical significance. According to [14] the type of crystallization and protein concentration in cervical mucus had a significant effect on the rate of sperm penetration and motility.

We monitored 3-year period in herd of Holstein cows and we can demonstrate the significant differences of sperm motility in survival test and arborisation test of cervical mucus and their relationships to reproductive indicators within this years. [22] found an individual effect among bulls on sperm penetration and pregnancy rate. Results of [5] were confirmed by sperm penetration into cow cervical mucus. In our study, the dependence of pregnancy rate on results of sperms motility in cervical mucus survival test was assumed. The pregnancy rate was the highest, 50.79%, 51.38%, and 52.55%, in the case of higher motility of sperm (40%, respectively 30%) in the time of test (30, 60, and 90 minutes).

[25] tested the hypothesis that the composition of cervical mucus can be used as an indicator of reproductive

Table 5: Relations of cervical mucus crystallization patterns to fertility indicators
Tabulka 5: Vliv krystalizace cervikálního hlenu na ukazatele plodnosti

	n	interval			SP			index			PR		
		μ+α	SE	P	μ+α	SE	P	μ+α	SE	P	μ+α	SE	P
crystallization	1.	30	91.36	5.07	196.59	17.57		2.96	0.34	1,2,3-5*	20.24	8.93	1-2*1-3***,2-
	2.	84	84.18	3.57	203.89	10.65		3.21	0.20		40.07	6.28	3**,2-4*,2-
	3.	78	84.98	3.71	189.12	10.60		3.10	0.20		62.74	6.51	5***,3-4,5***
	4.	29	85.57	5.53	198.25	16.96		3.54	0.32		15.02	9.42	
	5.	64	91.47	3.98	222.45	12.18	3-5*	3.78	0.23		13.59	7.00	

Legend of crystallization:
1 - club moss -like patterns
2 - club moss -fern patterns
3 - fern-like patterns
4 - fern-like patterns decomposition
5 - none or dotted structures

efficiency in the cow; and their results suggest that the composition of cervical mucus may be a useful indication of potential fertility in cattle. The dependence of sperms motility during survival test in cervical mucus on cervical mucus crystallization patterns is described in Table 6. The higher motility of sperms during the survival test time with club moss-ferny and ferny-like patters of crystallization was detected. The lowest motility of sperms at all times (30, 60, and 90 minutes) was in mucus with nothing or dotted crystallization with statistically significance ($P < 0.05$, respectively $P < 0.01$).

Presumption of relevance time of estrus, ovulation prediction, consecutive best conception rate in cows with fern-like patterns crystallization of their cervical mucus and higher motility or longevity of sperms in test in their heat mucus was confirmed.

ACKNOWLEDGEMENTS

This research was funded by the Ministry of Agriculture of the Czech Republic (Projects No. 1G46086) and by the Ministry of Education, Youth, and Sports of the Czech Republic (Project No. MSM 6046070901).

REFERENCES

[1] Collier, R.J., Dahl, G.E., VanBaale, J., Major Advances Associated with Environmental Effects on Dairy Cattle, J. Dairy Sci., 2006; 89: 1244-1253.

[2] Crane, M.B., Melendez, P., Bartolome, J., de Vries, A., Risco, C., Archbald, L.F., Association between milk production and treatment response of ovarian cysts in lactating dairy cows using the Ovsynch protocol, Theriogenology, 2006; 66: 1243–1248.

[3] De Rensis, F., Scaramuzzi, R.J.: Heat stress and seasonal effects on reproduction in the dairy cow - a review, Theriogenology, 2003; 60, 1139-1151.

[4] De Vries, A., Steenholdt, C., Risco, C.A., Pregnancy Rates and Milk Production in Natural Service and Artificially Inseminated Dairy Herds in Florida and Georgia, J. Dairy Sci., 2005; 88: 948-956.

[5] Elgaafary, M. N., Graves, C.N., Goncalves, P.B., Re-activated bull spermatozoa motility, acrosome status and ability to penetrace cervical mucus and zona-free hamster oocytes, Anim. Repris., 1993; 32 (3-4): 163-172.

[6] Firk R., Stamer, E., Junge W. and Krieter J., Automation of oestrus detection in dairy cows: a review, Livest.Prod. Sci. 2002; 75, pp. 219–232.

[7] Friggens, N.C., Body lipid reserves and the reproductive cycle: towards a better understanding,

Table 6: Relations of cervical mucus crystallization patterns to sperm motility during survival test
Tabulka 6: Vliv krystalizace cervikálního hlenu na aktivitu spermií během testu přežitelnosti.

		n	test30			test60			test90		
			μ+α	SE	P	μ+α	SE	P	μ+α	SE	P
crystallization	1.	30	20.09	2.45		12.51	2.39		6.51	2.10	2,3-5*
	2.	84	23.38	1.77		14.80	1.72		7.96	1.51	
	3.	78	22.73	1.82		13.82	1.77		8.47	1.58	
	4.	29	24.62	2.64		12.56	2.57	2-5**,	6.99	2.26	
	5.	64	18.69	2.03	2,4-5*	8.19	1.98	3-5*	4.21	1.74	

Livest. Prod. Sci. 2003; 83, pp. 209–226.

[8] Hafez, B., Hafez, E.S.E., Reproduction in Farm Animals. Maryland, USA, 2000, pp.495

[9] Chebel, R.C., Santos, J. E. P., Reynolds, J.P., Cerri, R.L.A., Juchem, S.O., Overton, M., Factors affecting conception rate after artificial insemination and pregnancy loss in lactating dairy cows, Anim. Reprod. Sci., 2004; 84(3-4): 239-255.

[10] Kerbrat, S., Disenhaus, C., A proposition for an updated behavioural characterisation of the estrus period in dairy cows, Applied Animal Behaviour Science, 2004; 87 (3-4): 223-238

[11] Kumar, R.A., Devanathan, T.G., Effect of spermatozoal quality on sperm progression speed in cervical mucus, Indian-Veterinary-Journal. 1996; 73(6): 645-648

[12] Kumar, R.A., Devanathan, T.G., Pattabiraman, S.R., Effect of storage of bovine cervical mucus on sperm migration in-vitro, Indian Journal of Animal Reproduction. 1996; 17(1): 65-66

[13] Lucy, M.C., Reproductive loss in high-producing dairy cattle: where will it end?, J Dairy Sci 2001; 84, pp. 1277–1293

[14] Matoušek, J., Říha, J., Sršeň, V., Veselský, L. and Louda, F., Penetration of cervical mucus and other body fluids by bull sperm in capillary tube, Anim. Reprod. Sci. 1989;18 (1-3): 161-166

[15] Peters, A.R., Ball, P.J.H., Reproduction in cattle, Blackwell Science, Oxford, UK, 1995.

[16] Pryce, J.C., Nielsen, B.L., Veerkamp, R.F. and Simm, G., Genotype and feeding system effects and interactions for health and fertility traits in dairy cattle, Livest. Prod. Sci. 1999; 57, pp. 193–202.

[17] Pryce, J.E., Coffey, M.P., Brotherstone, S., Woolliams, J.A., Genetic relationships between calving interval and body condition score conditional on milk yield, J. Dairy Sci. 2002; 85, pp. 1590–1595.

[18] Royal, M.D., Darwash, A.O., Flint, A.P.F., Webb, R., Woolliams, J.A., Lamming, G.E., Declining fertility in dairy cattle: changes in traditional and endocrine parameters of fertility, Anim. Sci. 2000; 70, pp. 487–501.

[19] Rutllant, J., Lopez Bejar, M., Lopez Gatius. F., Ultrastructural and rheological properties of bovine vaginal fluid and its relation to sperm motility and fertilization: a review, Reproduction in Domestic Animals. 2005; 40(2): 79-86

[20] Santos, J.E.P., Cerri, R.L.A., Ballou, M.A., Higginbotham, G.E., Kirk, J.H., Effect of timing of first clinical mastitis occurrence on lactation and reproductive performance of Holstein dairy cows, Anim. Reprod. Sci., 2004; 80:31–45.

[21] Stott, A.W., Veerkamp, R.F., Wassell T.R., The economics of fertility in the dairy herd, Anim. Sci. 1999; 68, pp. 49–58.

[22] Taş, M., Bacinoglu, S., Cirit, Ü., Özdaş, Ö.B., Ak. K.: Relationship between bovine fertility and the number of spermatozoa penetrating the cervical mucus within straws, Anim. Reprod. Sci., 2006; 10.1016/j.anire prosci.2006.08.020, in press

[23] Tekerli, M., Gündoğan, M., Effect of Certain Factors on Productive and Reproductive Efficiency Traits and Phenotypic Relationships Among These Traits and Repeatabilities in West Anatolian Holsteins, Turk. J. Vet. Anim. Sci., 2005; 29: 17-22.

[24] Van Eerdenburg, F.J.C.M., Karthaus, D., Taverne, M.A.M., Merics, I., Szenci, O., The relationship between estrous behavioral score and time of ovulation in dairy cattle, J. Dairy Sci. 2002; 85 (5): 1150-1156

[25] Zaaijer, D., Counotte, G. H. M., Sol, J., Smidt, W. J., Broadbent, P. J, Changes in the composition of cervical mucus of the cow during the estrus cycle as parameters for predicting potential fertility, Theriogenology, 1999; 39 (3): 569-580