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FACTORS AFFECTING SPERM SURVIVAL IN CERVICAL MUCUS AND PREGNANCY RATES IN SYNCHRONIZED HOLSTEIN COWSs

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Abstract:

The objective of this study was to determine the relationship between calving year and season, parity, number of AI, days in milk, milk production in the 1st 100 lactation days, disease occurrence (retained placenta, endometritis, or cysts) and their influence on sperm motility (SM) during 30, 60 and 90 minutes of the cervical mucus survival test (n=284) or pregnancy rates (PR) in Ovsynch-treated Holstein cows (n=238). Significant differences of SM in the 30th minute and PR ($P < 0.05$) were determined in relation to the calving year. Cows calved from July to September had the lowest SM in the 30th minute ($P < 0.05$) and also the lowest PR. The best SM was defined in the 1st lactation after 60 and 90 minutes ($P < 0.05$ to $P < 0.01$). No statistical significance was detected for the effect of the number of inseminations. Cows inseminated before the 72nd lactation day showed lower SM and the lowest PR ($P < 0.05$ to $P < 0.01$). The lowest milk production during the first 100 lactation days significantly correlates with the lowest SM during the survival test ($P < 0.05$ to $P < 0.01$). The lowest PR was detected in cows with milk production over 3831 kg ($\geq \bar{x} + 0.25\ s_d$). Health traits affected SM and PR significantly ($P < 0.05$ to 0.01). The best SM at the beginning of the test signified the best results during the entire test ($P < 0.05$ to 0.001) and also higher PR, but with no statistical significance.

Materials and methods

Data and samples were collected at the university dairy farm with 423 purebred Holstein cows. Cervical mucus samples were collected and analysed from March 2002 to December 2005, (n=284). Cows were treated with OVSYNCH heat synchronization with timed AI. A sperm survival test in the cervical mucus for assessment of cows´ ability to conceive was performed. The motility values were detected after 30, 60, and 90 minutes of the test duration in a water bath at a temperature of $38 \pm 1^{o}C$. Sperm motility in the cervical mucus survival test was estimated on the following scale: 0%-without, 1%-sporadic, 10%, 20%, 30%, 40%, and 50%.

Data were analyzed by the statistical program SAS STAT 8.0 - GLM, the general linear model being:

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

$Y_{ijklmno}$ … observed value of the trait as a dependent variable (cervical mucus sperm motility in %, AI result – pregnant/non-pregnant),

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 (j = 1st , 2nd , 3rd and other lactations)

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

F_m … effect of m-interval of lactation days 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

Evaluation of the effect of health disorders, or cervical mucus sperm motility was based on the same general linear model supplemented with

H_o … fixed 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 cyst occurrence) or fixed effect of o-cervical mucus sperm motility in % after 30,60, and 90 minutes (o = 1 - ≤ 1%, 2 – 10%, 3 – 20 %, 4 – 30% after 30, 60, and 90 minutes, and also 5 – 40% ≥ after 30 minutes).

The numbers of animals in the individual classes within the framework of constant effecsts by AI number/order were : 1^{st} = 127, 2^{nd} = 69, 3^{rd} = 88.

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

Results

Sperm motility was determined in 284 cervical mucus samples from 238 Holstein cows after 30, 60 and 90 minutes of a survival test. Cows were calved from March 2002 to December 2005 from the first to the eighth lactation, with the average parity 2.28. The average daily milk production in the first month of lactation was 33.82 kg with a protein content of 3.27%, 3612.1 kg of milk with a protein content of 3.19% in the first 100 days of lactation, and 9855.1 kg of milk with a protein content of 3.30% in 305 days of lactation. Cows with or without health disorders before insemination were treated with OVSYNCH for estrus synchronization and bred by timed AI. The average values of reproduction parametres were 3.00 AI services per conception, 106.4 days from calving to the first AI, and 208.6 open days. Average sperm motility in the cervical mucus samples were 19.78%, 12.37%, and 7.69% at times of 30, 60 and 90 minutes during the survival test. Health disorders were noted in 85.2% of cows of this trial group. Retained placenta was reported in 7.04%, endometritis in 10.92%, and ovarian cysts in 83.8% of cases, with 48.32% of multiple incidence.

The results are expressed in relation to calving year and season, lactation number, and number of AI services, and groups which were established in accordance with lactation days and milk production during 100 days of lactation in Table 1.

Calving year also had a significant effect on sperm motility ($P < 0.05$) and the pregnancy rate ($P < 0.05$).

The best sperm motility was found in cows during the 1st lactation. This trend was also significant ($P < 0.05$ to $P < 0.01$) after 60 and 90 minutes of the test. Differences in sperm motility increased from 3.17% in the 30th minute to 7.17% in the 90th minute. Decline in primiparous cows´ sperm motility from the 30th minute to the 60th minute constituted 31.48%, and 59.90% to the 90th minute, while in cows in their 2nd and later lactations sperm motility decreased from 47.93% to 49.03% to the 60th minute and from 75.26% to 78.22% to the 90th minute of the test. In primiparous cows, higher sperm survival ability was not confirmed by better conception.

No significant differences in sperm motility during the survival test or in the pregnancy rate in relation to the number of inseminations were detected.

Significant differences related to the calving season were determined only in the 30th-minute sperm survival test ($P < 0.05$). In the case of cows calved from July to

September and from October to December lower sperm motility was detected than in cows calved in the first half of the year, from 3.28% to 5.45%. Cows calved in the last calving season demonstrated lowest conception, only 27.12%, with differences of 7.19%-14.77%, but without statistical significance.

Cows in the 1st group, i.e., with insemination before the 72nd lactation day, showed lower sperm motility in cervical mucus and the worst pregnancy rate, 6.49% only ($P < 0.05 – P < 0.01$). Differences in conception ranged from 10.08% to 41.36%. Decline of motility during the test was highest in cows inseminated between 152-196 lactation days.

The lowest milk production during the first 100 lactation days significantly relates to the lowest sperm motility during the survival test ($P < 0.05 – P < 0.01$). Differences amounted to 3.34%-5.38% after 30, 4.49%-8.94% after 60, and 4.72%-7.75% after 90 minutes of the test. The group of cows giving less than 2742 kg of milk ($< \bar{x} - s_d$) had the greatest decline in sperm motility, i.e., 58.81% to the 60th minute and 86.01% to the 90th minute. However, the lowest pregnancy rate was detected in cows with milk production during the first 100 days of more than 3831 kg of milk ($\geq \bar{x} + 0.25\ s_d$), with differences from 7.57% to 10.32%.

Table 2 illustrates the effect of the cows' health. Significant differences were observed, when evaluation was made, depending on the type of disorder. Cows with retained placenta after calving had lower sperm motility by 6.06% in the 30th minute ($P < 0.05$), 5.87% in the 60th minute, and 5.73% in the 90th minute, and worse pregnancy result by 16.82%. In endometritis-affected conception after monitored inseminations, the difference was 24.5% in favour of cows without endometritis disorder ($P < 0.05$). A trend of higher sperm motility in cervical mucus of healthy cows was determined, but differences were not significant. Cyst occurrence had no significant influence differences. While multiple cyst frequency during lactation influenced decreasing pregnancy by 23.57% in observed inseminations ($P < 0.01$), on the other hand, no differences were detected in sperm motility during the test.

Table 3 describes relationships between sperm motility in the survival test and observed insemination results. The lowest sperm motility at the beginning of the test meant the worst results during the entire test time. The best sperm motility after 30 minutes signified the highest motility in the 60th or 90th minute as well ($P < 0.05 – P < 0.001$). Pearson´s correlation coefficients confirm this significant relationship.

Conclusion

Our results documented significant effects of the calving year and season, parity, the number of inseminations, days in milk, milk production in the 1st 100 days of lactation, retained placenta, and endometritis or cyst frequency during lactation on sperm motility during 30, 60 and 90 minutes of the survival test or pregnancy rates in Ovsynch-treated Holstein cows. Definition of this relationship offers the possibility of their use for a detailed study and determination of cows´ biological ability to conceive, which is the one of the components of cow reproduction parameters. Understanding the essentials of all factors which affect the cows´ reproduction abilities will faciliate the choice of traits for breeding value prediction and objective cow selection focusing on conception ability. Follow-up research is necessary to determine the basis for objective selection of traits and prediction of breeding values.

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Table 1: Sperm motility after 30 (SM30), 60 (SM60), and 90 (SM90) minutes of survival test and pregnancy rate (PR) in relation to calving year (CY) and season (CS), number of lactation (NL), days in milk (DIM), and milk production in the 1st 100 days of lactation (M100)

		n	SM30			SM60			SM90			PR		
			μ+α	SE	P	μ+α	SE	P	μ+α	SE	P	μ+α	SE	P
CY	1.(2002)	48	19.98	2.10	1-2*, 2-3*	12.23	2.06		7.03	1.80		23.49	7.76	1-2*
	2. (2004)	49	26.59	2.73		14.26	2.68		6.81	2.34		49.50	10.62	
	3. (2005)	187	19.23	1.22		11.65	1.20		7.37	1.04		32.15	4.72	
CS	1.(January-March)	65	23.41	2.29	2-3,4*	12.93	2.25		7.09	1.96		36.88	8.72	
	2.(April-June)	46	24.82	2.37		14.79	2.33		7.65	2.03		41.89	9.08	
	3.(July-September)	97	20.13	1.64		11.09	1.61		6.28	1.40		34.31	6.23	
	4.(October-December)	76	19.37	1.59		12.03	1.55		7.27	1.39		27.12	6.14	
NL	1st lactation	107	24.84	2.07		17.02	2.03	1-2,3*	11.70	1.77	1-2,3**	35.54	8.02	
	2nd lactation	70	20.17	2.06		10.28	2.02		4.99	1.76		37.22	7.72	
	3rd and later lactation	107	20.80	1.70		10.83	1.67		4.53	1.45		32.38	6.57	
DIM	1. (≤ 72 days)	16	21.20	4.51	2-5*, 2-7**, 3,4-7*	12.25	4.45	2-5,6,7*	6.28	3.88	2-5,6,7*	6.49	17.64	1-3**, 1-4,5*, 1-6,7*, 2-3*
	2. (73-96 days)	40	28.77	3.42		20.57	3.37		13.36	2.94		16.57	12.59	
	3. (97-120 days)	27	24.53	3.28		14.38	3.24		9.79	2.82		47.11	12.56	
	4. (121-144 days)	58	22.49	2.50		14.41	2.47		8.33	2.15		34.82	9.68	
	5. (145-168 days)	34	19.08	2.38		10.85	2.35		5.87	2.05		42.40	9.19	
	6. (169-216 days)	31	20.75	2.60		9.95	2.57		3.52	2.24		39.81	9.90	
	7. (217 days ≥)	78	15.56	2.08		9.65	2.05		5.41	1.79		47.85	7.93	
M100	1. (≤ 2742 kg)	46	18.45	2.43	1-2*	7.60	2.38	1-2,5**	2.58	2.08	1-2*, 1-5**	38.94	9.44	
	2. (2743-3395 kg)	80	23.48	2.04		14.03	2.01		7.64	1.75		39.87	7.76	
	3. (3396-3830 kg)	53	22.12	2.34		13.30	2.30		7.30	2.00		37.22	8.77	
	4. (3831-4483 kg)	55	21.79	2.13		12.09	2.09		7.52	1.82		29.55	8.28	
	5. (4484 kg ≥)	50	23.83	2.20		16.54	2.16		10.33	1.88		29.65	8.46	

Table 2: Sperm motility after 30 (SM30), 60 (SM60), and 90 (SM90) minutes of survival test and pregnancy rate (PR) in relation to retained placenta (REPL), endometritis (END), ovarian cyst (OC), and multiple ovarian cyst occurrence (MOC)

		n	SM30			SM60			SM90			PR		
			μ+α	SE	P	μ+α	SE	P	μ+α	SE	P	μ+α	SE	P
REPL	1. (no)	264	22.35	1.30	1-2*	13.11	1.28	1-2*	7.47	1.11	1-2*	36.30	4.92	
	2. (yes)	20	16.29	3.08		7.24	3.02		1.74	2.63		19.48	11.81	
END	1. (no)	253	21.97	1.32		12.79	1.29		7.18	1.12		36.65	4.89	1-2*
	2. (yes)	31	20.53	2.86		10.74	2.80		4.08	2.43		12.12	10.95	
OC	1. (no)	46	21.86	2.07		11.79	2.03		5.99	1.76		36.61	7.79	
	2. (yes)	238	21.89	1.51		13.06	1.47		7.44	1.28		34.46	5.70	
MOC	1. (1x)	121	20.45	1.82		11.86	1.82		6.84	1.59		45.69	6.91	1-2**
	2. (2x ≥)	115	21.51	1.89		13.13	1.90		7.87	1.66		22.15	7.34	

Table 3: Relationships between sperm motility after 30 (SM30), 60 (SM60), 90 (SM90) minutes of survival test, and also pregnancy rate (PR)

			SM60				SM90				PR			
		n	μ+α	SE	P	r (P)	μ+α	SE	P	r (P)	μ+α	SE	P	r (P)
SM30	1. (≤1%)	48	-0.67	1.21	1-2*,1-3,4,5***, 2-3,4,5***, 3-4,5***,4-5***	0.78799 (<.0001)	-0.91	1.36	1-3*,1-4,5***, 2-3*,2-4,5***, 3-4,5***, 4-5***	0.61207 (<.0001)	34.13	8.02		0.11205 (0.0628)
	2. (10%)	56	3.29	1.19			0.74	1.34			42.20	7.90		
	3. (20%)	68	8.10	1.10			3.98	1.24			43.39	7.29		
	4. (30%)	85	20.00	0.97			11.69	1.09			49.89	6.44		
	5. (40 %≥)	27	31.21	1.55			21.34	1.74			50.79	10.26		
SM60	1. (≤ 1%)	118					-0.06	0.72	1-2*,1-3,4***, 2-3,4***, 3-4***	0.83233 (<.0001)	43.80	5.68		0.05953 (0.3174)
	2. (10%)	59					2.61	0.93			39.40	7.34		
	3. (20%)	47					13.24	1.08			40.67	8.51		
	4. (30% ≥)	60					22.56	0.95			53.71	7.52		
SM90	1. (≤ 1%)	176									42.55	4.96		0.04153 (0.4857)
	2. (10%)	40									51.38	8.65		
	3. (20%)	37									48.00	9.26		
	4. (30% ≥)	31									52.55	10.03		