EFFECT OF HOT SEASON ON LIVE SPERMATOZOA WITH INTACT ACROSOME IN HOLSTEIN BULLS

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Summary

Semen characteristics were examined to find the deterioration of the percentage of live spermatozoa with intact acrosome during hot season using 5 Holstein bulls located in Shimizu-cho Hokkaido Japan. Spermatozoal viability and acrosomal status were observed simultaneously with triple-stain technique for each spermatozoon. Spermatozoa were divided in four categories (live spermatozoa with intact acrosome, live spermatozoa without intact acrosome, dead spermatozoa with intact acrosome and dead spermatozoa without intact acrosome). Bull and collection month had significant effects on semen characteristics ($p < 0.01$). The percentage of live spermatozoa with intact acrosome and the percentage of live spermatozoa had the lowest least squares mean by collection month in August (72.7% and 76.7%). These two characteristics indicated the obvious deterioration during hot season. But the fluctuation of these two characteristics were not parallel and the differences between the two characteristics were largest during July to September. The present results indicate the necessity for the simultaneous determination of viability and acrosomal status of each Holstein bull's spermatozoa in order to keep fertility above an acceptable minimum level during hot season.
(**Key Words**: Intact Acrosome, Holstein Semen, Hot Season)

Introduction

The relationships between fertility and some semen characteristics of bulls have been studied. Wood et al. (1986) reported that fertility of a bull could be predicted from semen characteristics. These characteristics include some assessments of motility, categories of abnormal spermatozoa, acrosomal changes and the release of hyaluronidase. Also, Smith et al. (1981) indicated that fertility could be estimated by combining several characteristics of semen. Fukui et al. (1983) estimated the close negative relationship between fertility and morphologically abnormal ratio of spermatozoa in bull's semen.

The seasonal fluctuation in the semen quality of bulls has been studied (Stalhammar et al., 1989; Taylor et al., 1985; Chandler et al., 1985; Sekoni et al., 1988). The adverse effects of elevated temperatures on spermatogenesis have been documented for the bull and consist of impaired efficiency of spermatogenesis, as reflected by increased spermatozoal abnormalities and reduced spermatozoal output and viability. Vogler et al. (1991) reported the adverse effect of elevated testicular temperatures on epididymal spermatozoa as noted by their decreased viability to maintain motility and acrosomal integrity following cryo-preservation.

The acrosome contains some enzymes which play significant roles on fertilization (Austin and Short, 1982). Therefore, it is important that spermatozoa just after ejaculation should have intact acrosome. The live spermatozoa with intact acrosome at ejaculation can contribute to fertilization. So, in the systems of semen production for artificial insemination (A.I.), the percentage of live acrosome-intact spermatozoa is important criterion of semen quality.

In the reports on seasonal fluctuation of semen quality and the relationships between fertility and semen quality, different samples of spermatozoa have been used for assessing the status of acrosome and spermatozoal viability, respectively. Triple-stain technique (Talbot and Chacon, 1981; Didion and Graves, 1986) enables us to examine the status of acrosome and viability of each spermatozoon simultaneously. There are no reports on

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seasonal fluctuation of semen quality with the triple-stain technique. The percentage of live spermatozoa with intact acrosome may decrease severely in comparison with the deterioration of acrosomal status and spermatozoal viability, assessed independently.

The purposes of the present study are to examine the fluctuation of the percentage of live spermatozoa with intact acrosome and to clarify the difference among the degree of deterioration of the percentage of live spermatozoa with intact acrosome, the status of acrosome and the viability of spermatozoa from Holstein bulls during hot season.

Materials and Methods

Semen samples used in this study were collected from 5 Holstein bulls located in Shimizu-cho (42° 55′N, 142° 42′E) Hokkaido, Japan during June 1991 to November 1991. Monthly mean temperatures during June to Novemer were 17.3, 17.0, 18.7, 15.7, 9.9 and 3.3℃. June, July and August is the hottest month throughout the year. Mean and standard deviation for age of these bulls were 7.7 and 1.52 year of age at the beginning of this study. These bulls have been ejaculated twice per each collection day and in 2 collection days per week. The two ejaculates of each collection day were pooled and used for examining the status of acrosome and the viability of spermatozoa. The fresh semen samples were stained using the triple-stain method described by Didion and Graves (1986) every other week.

Four hundred spermatozoa in each sample were observed by a phase contrast microscopy at 1000 x magnification and divided into four categories: (1) live spermatozoa with intact acrosome, (2) live spermatozoa without intact acrosome, (3) dead spermatozoa with intact acrosome and (4) dead spermatozoa without intact acrosome. The percentage of live spermatozoa, the percentage of live spermatozoa with intact acrosome and the percentage of spermatozoa with intact acrosome were estimated from the counts of the four categories. These 3 original percentage data have been transformed to angles (arcsin $\sqrt{\%}$), and then fluctuation of these 3 characteristics with season was evaluated by General Linear Model Procedure of the Statistical Analysis System (SAS) (SAS, 1990).

Results

Means, standard deviations and coefficients of variation for three semen characteristics are shown in table 1. The means of the percentage of live spermatozoa and the percentage of live spermatozoa with intact acrosome were 82.4% and 79.9%, respectively. The difference between these two means was 2.5% and revealed the percentage of live spermatozoa without intact acrosome. The percentage of spermatozoa with intact acrosome had the largest means and the smallest standard deviation and coefficient of variation. These figures indicate that the percentage of spermatozoa with intact acrosome is a stable trait in comparison with other two characterisitics.

Table 2 shows the results of the analysis of variance for the three characteristics. The effects of bull and collection month on the three characteristics were statistically significant ($p < 0.01$).

TABLE 1. MEANS, STANDARD DEVIATIONS (S.D.) AND COEFFICIENTS OF VARIATION (C.V.) FOR THREE CHARACTERISTICS

Characteristics	No. of sample	Mean (%)	S.D.	C.V.
Percentage of live spermatozoa with intact acrosome	60	79.87	8.59	10.80
Percentage of spermatozoa with intact acrosome	60	96.32	4.07	4.23
Percentage of live spermatozoa	60	82.38	7.15	8.68

TABLE 2. ANALYSIS OF VARIANCE FOR THREE CHARACTERISTICS

Source	d.f.	Percentage of live spermatozoa with intact acrosome	Percentage of spermatozoa with intact acrosome	Percentage of live spermatozoa
		M.S.($\times 10^3$)	M.S.($\times 10^3$)	M.S.($\times 10^3$)
Bull	4	75.7**	27.6**	48.9**
Collection month	5	34.7**	23.7**	18.4**
Error	50	6.4	1.4	3.8

** Statistically significant ($p < 0.01$).

Least squares means by collection month (transformed to original percentage) for the three characteristics are

shown in table 3. The percentage of live spermatozoa with intact acrosome had the lowest least squares mean in August (72.7%). The least squares mean for the percentage of live spermatozoa with intact acrosome in August was significantly lower than another collection month except July and September ($p < 0.01$). the percentage of spermatozoa with intact acrosome had the lowest least squares mean in July (93.9%). The least squares mean for the percentage of spermatozoa with intact acrosome in July was significantly lower than in October and November. The least squares means for the percentage of live spermatozoa in August was lowest and had statistically significant difference from other collection months except July. Generally, these three characteristics indicated lower least squares means by collection month during July to September. Especially, the percentage of live spermatozoa with intact acrosome and the percentage of live spermatozoa were remarkably low during hot season. On the other hand, the fluctuation of the percentage of spermatozoa with intact acrosome was obscure in the collection months.

TABLE 3. LEAST SQUARES MEANS BY COLLECTION MONTH FOR THREE CHARACTERISTICS

Collection month	Percentage of live spermatozoa with intact acrosome	Percentage of spermatozoa with intact acrosome	Percentage of live spermatozoa
June	83.5[a]	96.6[ab]	85.8[a]
July	75.1[bc]	93.9[b]	79.2[bc]
August	72.7[c]	95.0[ab]	76.7[b]
September	79.7[abc]	94.6[ab]	83.8[ac]
October	83.4[a]	98.6[a]	84.2[ac]
November	82.5[ab]	98.8[a]	83.1[ac]

Least squares means with same superscripts within columns do not differ significantly ($p = 0.01$).

Discussion

A.I. studs have striven to improve the efficiency of production of semen straws by searching the feasible minimum number of spermatozoa per straw and developing new methods for semen treatment. On the other hand, A.I. stud must keep fertility being above a certain level. The concentration of spermatozoa and the spermatozoal motility have been observed for each ejaculated semen in routine. The number of spermatozoa per straw and the dilution rate of ejaculated semen are determined mainly by the two semen characteristics. Usually, the status of acrosome has not been examined in routine.

The results of the present study have indicated that the percentage of live spermatozoa with intact acrosome and the percentage of live spermatozoa decreased during hot season especially in August. Moreover, the differences between these two characteristics were 0.6% to 4.1%, particularly the differences were increased during July to September (4.1, 4.0, 4.1%). This fact has indicated that when the minimum number of spermatozoa per straw was determined on the routine examination in the cool season, it is possible that fertility may reduce to below a certain level during hot season especially July to September. Vogler et al. (1991) reported that the deterioration of spermatozoal motility and status of acrosome by scrotal insulation were observed earlier in frozen-thawed semen than on fresh semen. This fact indicates the necessity of the simultaneous determination of viability and acrosomal status in frozen-thawed semen for a longer period.

Terawaki et al. (1991) reported that the concentration of spermatozoa and the total number of spermatozoa in Holstein bull's semen in the same location were deteriorated in the same way as the results of the present study, but the deterioration of the characteristics dealt with in the present study was observed about 1 or 2 months earlier. It has been known that spermatogenesis takes about 45 days (Hafez, 1974) and 8 to 11 days required for epididymal passage (Koefoed-Johnsen, 1960) in a bull. Spermatocytogenesis (spermatogonium to spermatid) and spermiogenesis (spermatid to sperm) require about 30 and 15 days, respectively. If spermatozoa at the stage of spermiogenesis are exposed by heat stress, the percentage of morphologically abnormal spermatozoa may increase and the viability of spermatozoa may decrease. Also, the influences of heat stress at the stage of spermatocytogenesis may result in the decrease of the total number of spermatozoa and the concentration of spermatozoa. Therefore, the influences of heat stress on the morphology of spermatozoa could be observed about 30 to 45 days earlier than on the total number of spermatozoa and the concentration of spermatozoa. The differences between the present and the previous results have coincided with the above consideration.

In conclusion, the percentage of live spermatozoa with intact acrosome decreased during July to September in comparison with the percentage of live spermatozoa. Especially, acrosomes of live spermatozoa were more sensitive on heat stress than one of dead spermatozoa. In order to keep fertility above an acceptable minimum level during hot season, it is recommended that the simultaneous determination of viability and acrosomal status for A.I.

Holstein bull's spermatozoa should be required several times in a year, throughout the seasonal changes.

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