SEASONAL VARIATION OF THE PROPERTIES AND CHEMICAL COMPOSITION OF BOVINE SEMEN

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Abstract : Since the present experiments have been conducted in the warm climate of South-Western Japan (i.e. Kochi Prefecture), it was prerequisite to study the seasonal variation of bovine semen in order to obtain as stable materials as possible for further studies.

1) Seasonal variations of semen volume, sperm concentration and sperm number showed a fluctuation among the bulls but they showed a tendency to decrease in September and October.

Mounting desire decrease in and around July, August and September and decremental effects of high temperatures during summer appeared after a lag of time.

Fructose concentration gradually decreased preceding the decrease in sexual desire. The minimum concentration was observed in August, when temperature was the highest and a gradual increase was shown with the fall of temperature.

Citrate concentration showed the maximum in June and the minimum in October. Its decrease was observed later than the decrease in fructose concentration.

2) Bulls having stronger mounting desire had a tendency to show greater semen volume and higher citrate concentration. Fructose concentration and semen concentration showed a minus correlation.

3) Spermatozoa of the Holsteins showed greater semen volume, greater sperm number and higher motility than those of the Wagyus, while the latter showed higher frctose concentration than the former.

Mounting desire of the Holsteins decreased in July and August, while that of the Wagyus decreased in September and October.

4) Sperm concentration, sperm number and protein concentration of the first ejaculates showed higher figures than those of the second.

In making experiments on the spermatozoa of farm animals, it is necessary to study beforehand the seasonal variation of their properties and chemical composition in order to avoid the seasons when the materials are more deteriolated and less stable.

On the seasonal variation of spermatozoa, Mizuho & Niwa[1], Yoshioka & Sakai[2], Fulka & Hofrajterova *et al*[3] reported that their properties deteriorate and conception rate lowers as the temperature rises in summer. On the seasonal variation of their chemical composition Mann[4] clarified that the quantity of citrate contained in horse spermatozoa increases in

breeding season and decreases in non-breeding season. According to Mann[4], Hiroe & Masaki[5][6] *et al* similar seasonal variation of the quantity of fructose was observed in the semen of horses, sheep and goats but no marked variation was recoginized in the case of bulls and swine. Nishikawa & Horie[7], Iritani & Nishikawa[8] also reported on the similar kind of seasonal variation. Further, Hiroe et al[8] reported that the quantity of ascorbic acid did not show such a marked seasonal variation as that of fructose.

As has been mentioned above spermatozoa of horses, sheep and goats have been known to show seasonal variation of their properties and chemical composition and this fact must always be taken into consideration in studying the semen of other farm animals. Since the present experiments have been conducted in the warm climate of South-Western Japan(i.e., Kochi Prefecture), it was prerequisite to study the seasonal variation of bovine semen in order to obtain as stable materials as possible for further studies.

Experimental Procedure

Three Holstein bulls, A (2.6 years old), B (5.9 years old), C (4.6 years old) and three Wagyu bulls, D (2.3 years old), E (1.3 years old) and F (4.6 years old) were selected for the present experiments. From each of them two consecutive ejaculates were collected every two weeks with an artificial vagina and their properties and chemical composition were studied immediately after the collection.

Strength of sexual desire was measured by the time necessitated for ejaculation. As to the properties of semen such factors as volume, number, pH, and motility were studied. As to chemical composition, the quantities of fructose[9], citrate[10] and protein which are vitally related with the motility of the sperm, were measured.

The preparation for the analysis of the above-mentioned chemical components was made as follows.

Semen sample 0.3 ml + 15 % TCA 15 ml
Centrifuged for
15 minutes at 2,000 r.p.m.
Precipitate … (1) Supernatant … (2)

(1) was used for the analysis of protein and (2) for that of citrate

Semen sample 0.3 ml + 10g/dl $ZnSO_4$ 5 ml + 0.5N NaOH 5 ml
Precipitate Supernatant … (3)

(3) was used for the analysis of fructose.

Because of high temperatures during the summer, some of the bulls lost their mounting desire and no ejaculates could be collected as a result. Following collections were made during the 9 months of experimental period. A−36, B−34, C−29, D−27, E−29, F−12, total 167.

Result and Discussion

A) Seasonal Variation of Mounting Desire

Those bulls that reached ejaculation in a shorter time were considered to have a storonger sexual desire and vice varsa. Seasonal variation of the ejaculation time is shown in Table 1.

Table 1 Seasonal Variation of the Ejaculation Time (min.)

MONTH		APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
BULLS	WAGYU HOLS. A	0.10	0.30	0.25	0.17	2.28	1.15	0.45	1.03	1.14
	B	2.34	1.47	3.03	6.33	5.03	6.15	5.39	4.55	3.50
	C	0.12	0.17	0.19	4.39	0.30	0.39	1.09	0.23	1.17
	D			0.37	1.13	1.03	4.10	2.35	2.36	6.58
	E	2.15	2.57	1.16	3.16	0.42	4.31	3.20	3.54	4.32
	F	1.03	2.43	1.13	0.07		1.06	6.33	6.30	
BREED	HOLSTEIN	1.08	0.48	1.03	3.48	2.34	2.29	2.44	2.21	2.18
	WAGYU	1.25	2.13	0.44	1.54	0.57	4.11	3.16	3.39	5.45
EJACU-LATION	1st EJAC.	1.05	1.08	1.44	3.20	2.08	4.07	3.30	4.08	3.35
	2nd EJAC.	1.24	2.06	0.57	2.44	1.40	3.00	2.22	1.58	4.05

Generally speaking, sexual desire decreases at high temperatures. Bull A required the longest time in August, B, C in July and D, E in September. F showed no desire at all in August and no ejaculates could be collected. It is interesting to note that Holsteins A, B, and C required the longest ejuculation time in July and August, while Wagyu bulls D, E, and F spent a longer time gradually from summer on requiring the longest time in October, November and December.

It follows that these two species have different sensitivity to high temperatures. That is Holstein, which have more liability to high temperatures, show the decline of sexual desire with the rise of temperature, while Wagyu bulls show the decline a few months later. On the other hand, Wagyu bulls are rather nervous, showing a liability to the change of various conditions at the time of collection.

In the season of higher temperature they required a longer time before mounting for the first ejaculation. For the second ejaculation, however, they tended to mount in a relatively shorter time and the ejaculation time was shortened in consequence.

In the case of the Holsteins the first ejaculation required a longer time than the second, while the Wagyu bulls showed a reverse tendency. Bull F had very weak mountind desire and collection of the second ejuculate was almost impossible after August.

The mean of ejaculation time for the total 167 ejaculations was 2 minutes 28 seconds.

B) Seasonal Variation of the Semen Properties

a) Semen Volume

Seasonal variation of semen volume of each bull is shown in Table 2, Fig. 2.

The rate of seasonal variation varies with each bull, but all the bulls except D showed a decrease in volume in September and October. Bull A, which had the strongest mounting

Table 2 Seasonal Variation of the Properties and Chemical Composition of Bovine Semen

Heads		Treatment	Semen Vol. (ml.)	Sperm Conc. ($\times 10^8$/ml.)	Total Sperm No.	Motility	pH	Fructose	Citrate (mg/dl)	Protein
Holstein Bulls	A	36	7.5	7.4	56.4	82.3	6.47	339.7	896.8	7165.9
	B	36	4.5	11.3	50.7	81.3	6.41	297.4	628.5	5185.3
	C	32	4.6	10.1	51.5	62.1	6.51	303.8	778.4	7535.1
Holstein Av.		104	5.5	9.6	52.9	76.2	6.46	313.6	767.9	6628.8
Wagyu Bulls	D	28	4.4	11.6	49.4	84.6	6.57	527.2	847.2	6029.7
	E	28	2.3	7.0	18.4	56.6	6.35	534.8	565.9	6738.1
	F	13	5.2	13.1	43.3	79.6	6.35	260.2	859.6	6914.0
Wagyu Av.		69	4.0	10.7	37.0	73.6	6.42	440.7	758.9	6560.6
Total Av.		173	4.8	10.2	45.0	74.9	6.44	377.2	763.4	6594.7
Ejaculation Hols.	1st Ejac.	52	5.2	10.8	55.4	74.2	6.44	304.4	756.5	6725.7
	2nd Ejac.	50	6.2	8.4	43.4	76.4	6.47	298.1	812.4	6690.1
Wagyu	1st Ejac.	40	4.1	11.4	40.7	75.6	6.39	362.2	764.4	6928.1
	2nd Ejac.	30	3.6	9.3	38.6	68.8	6.48	370.0	771.7	6107.8
	1st Ejac.	92	4.7	11.1	48.1	74.9	6.41	333.3	760.5	6826.9
	2nd Ejac.	80	4.9	8.9	41.0	72.6	6.47	334.1	792.0	6399.0

desire, ejaculated the greatest amount of semen all through the period of experiments and it ejaculated the maximum of 9.1 ml (mean of 4 ejaculates) in August, when the tempera-

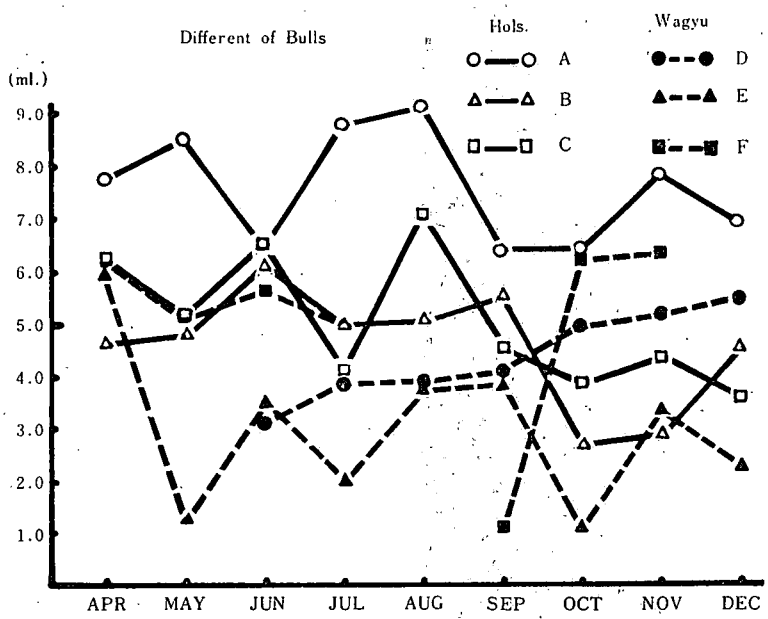

Fig. 1 Seasonal Variation of the Semen Volume

ture was very high. Bull B ejaculated the maximum amount of 6.08 ml in June, showing no marked variation from April through September and ejaculated the minimum amount of 2.65 ml in October. Bull C ejaculated 7.13 ml (maximum) in August and 3.53 ml (minimum) in December. The Holsteins showed a temporary decrease in their semen volume in June and July when humidity was very high. Bulls A and C, however, ejaculated the maximum amount in August, showing no decrease in high temperatures, while their semen volume decreased in Autumn (September, October and November). It is not clear whether the effects of the high temperatures in August presented themselves in October after a lag of time necessary for the reproduction of semen or the great humidity due to heavy rain fall in September and October decreased their sexual desire. It may be suggested, however, that in case of the Holsteins, which are heavier than Wagyus, their health conditions and the stresses caused by humidity might have influenced their semen volumes. Wagyu D, whose semen was collected in June for the first time, ejaculated only 3.13 ml in the month due to the lack of trainig but the semen volume increased month by month, showing the maximum amount of 5.45 ml in December. The youngest bull E, perhaps because of the lighest body weight ejaculated very small amount of semen, except 6.0 ml in April, showing the mean of 2.3 ml for 28 ejaculates (Table 2). This one also showed a marked decrease in May and October (1.10. ml). Wagyu F ejaculated comparatively great amount of semen at a time but, because of its weak mounting desire, it was almost impossible to collect the second ejaculates after August.

The mean of the semen volumes given in the table, therefore, is that of the first ejaculates, thus showing a relatively higher figure than that of the others'. This one did not mount at all in August and consequently no semen was collected.

It ejaculated only 1.06 ml in September.

Differences in the semen volumes of the Holsteins and Wagyus are shown in Fig. 2.

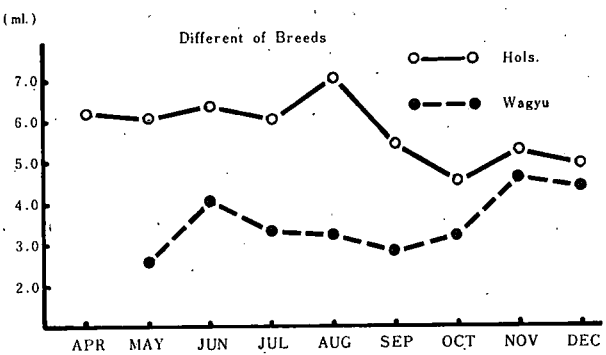

Fig. 2 Seasonal Variation of the Semen Volume

Throughout the period of experiments the Holsteins ejaculated greater volumes than the Wagyus. It was especially interesting to note that the former showed the maximum volume in August, when the latter showed a decrease in semen volume.

This may indicate that the sensitivity to high temperatures varies with the species. After October, however, difference in semen volumes of the two species was more or less levelled.

The average semen volumes of the whole period were 7.5 ml. in the case of the Holsteins

Seasonal Variation of Mean Temperature of Each Month

Month	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
Temp. (°C)	14.3	19.4	21.0	25.5	26.4	21.9	18.9	12.1	6.3

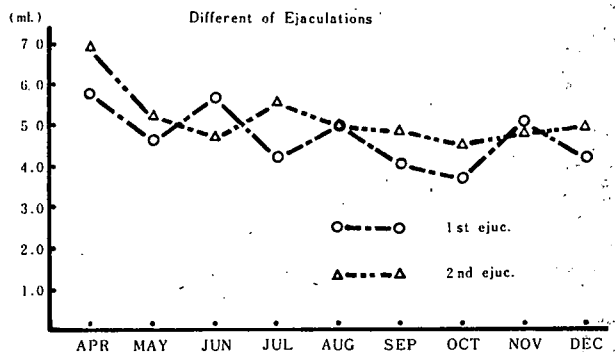

Fig. 3 Seasonal Variation of the Semen Volume

and 4.0 ml in the Wagyus and the mean of the tow species was 4.8 ml, which is very close to the mean reported by Kato & Nishikawa et al.[11], i.e., 5.0 ml.

Semen volumes of the first and second ejaculates of the two species are shown in Fig. 3.

No marked difference was observed between the two series of ejaculates. Generally speaking, however, the Holsteins ejaculated a greater volume (average 6.2 ml) in the second ejaculations than the first (average 5.2 ml). They showed stronger mounting desire and shorter ejaculation time in the second ejaculations. Those that showed stronger desire and shorter ejaculation time, had a tendency to give greater volume. In the case of the Wagyus, no significant difference was recognized between the first and second ejaculates (i,e., the average of the first ejaculates was 4.1 ml and that of the second 3.6 ml.). Mean of the two species also showed a very approximate figures- 4.7 ml for the first ejaculates and 4.9 ml for the second. According to Mackenzie & Berliner[12] et al, semen volumes showed a gradual decrease in successive ejaculations. In the present experiments, however, no appreciable decrease was observed so far as the second ejaculations are concerned. Only some of the bulls showed a slight decrease in the second ejaculates. When the third ejaculates were tentatively collected from some of the bulls, they showed more substantial decrease than the former two collections.

b) Sperm Concentration

Seasonal variation of sperm concentration also differed from bull to bull throughout the period of experiments. It, however, showed more or less same tendency with the variation of semen volume, reaching the minimum concentration in September in all the bulls, as is shown in Fig. 4.

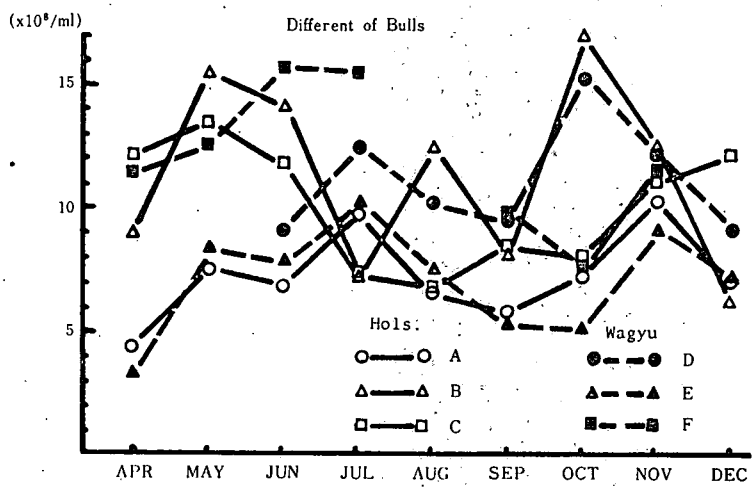

Fig. 4 Seasonal Variation of the Sperm Concentration

Bull A, which ejaculated the greatest semen volume, showed the lowest concentration. It showed the minimum concentration of 4.40×10^8/ml in April and the maximum 10.25×10^8/ml in November. Bull B, which ejaculated relatively smaller volume of semen, showed the highest concentration of all the bulls in October, reaching 17.0×10^8/ml It showed, however, a periodical decrease in concentration in April, July, September and December. Bull C showed the maximum concentration of 13.5×10^8/ml in May, then showed a decrease from July through Octobor, and restored a high concentration in November. In the case of the Holsteins, those that gave a greater volume of semen showed a lower concentration. The number of semen per ejaculation consequently showed no substantial difference for the three Holsteins. All the Wagyus, however, showed a contrary tendency of showing a higher concentration for a greater volume. Bull E, which ejaculated the smallest volume of semen, showed the lowest concentration, perhaps because of its younger age. Of all six bulls, Wagyu F showed the highest concentration of 13.1×10^8/ml, while Wagyu E showed the lowest 7.0×10^8/ml.

No appreciable difference was observed between the concentration of the two species: average concentration of the Holsteins was 9.6×10^8/ml and that of the Wagyus was 10.2×10^8/ml. Both of the species showed a seasonal decrease in concentration in August and September (Fig. 5).

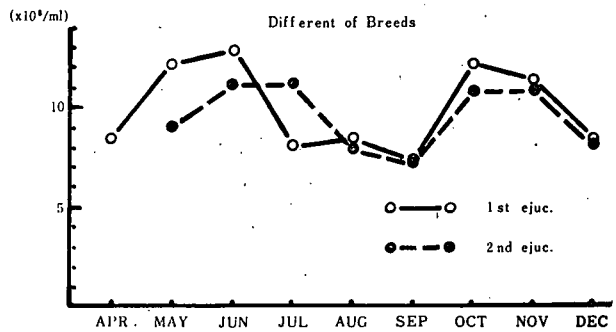

Fig. 5 Seasonal Variation of the Sperm Concentration

The average concentration of the first ejaculates (Holsteins: 10.8×10^8/ml, Wagyus: 11.4×10^8/ml, mean of the two species: 11.1×10^8/ml) showed a high figure than that of the second (Holsteins: 8.4×10^8/ml, Wagyus 9.3×10^8/ml, mean of the two species; 8.9×10^8/ml), as is shown in Fig. 6. The average concentration of all the ejaculates collected was 10.2×10^8/ml, showing an approximate result to the 10.0×10^8/ml given in the above-mentioned report[11].

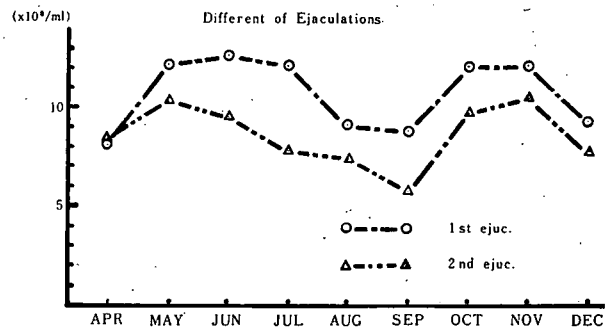

Fig. 6 Seasonal Variation of the Sperm Concentration

c) Total Sperm Number

Seasonal variation of sperm number is shown in Fig. 7.

Sperm number also showed a considerable fluctuation. Being influenced by sperm concentration, it showed the lowed the lowest figure in September and October in all the bulls. The maximum number of 9.2×10^8/ml was shown by Wagyu F in June. This bull, however, showed weaker sexual desires in the rest of the months and almost no second collections

could be made after August. Its average sperm nubmer, therefore, shows relatively higher figure than of the other bulls', being caluculated on the basis of the first ejaculates. The minimum sperm number of 5.8×10^8/ml was shown by Wagyu E in October, When its semen volume was the smallest.

Difference of sperm number between the two species is compared in Fig. 8.

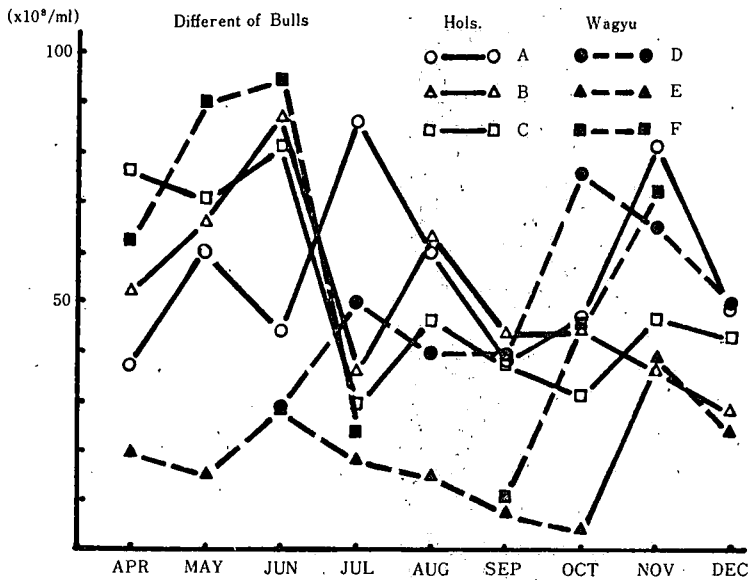

Fig. 7 Seasonal Variation of the Total Sperm Number

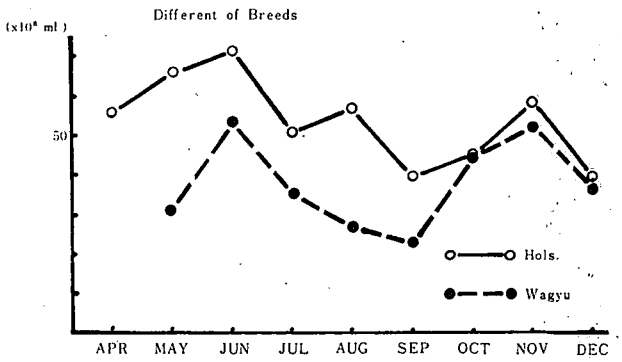

Fig. 8 Seasonal Variation of the Total Sperm Number

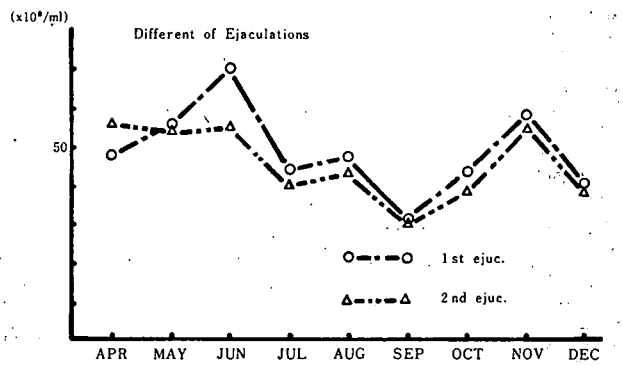

Fig. 9 Seasonal Variation of the Total Sperm Number

The Holsteins, which gave a greater semen volume, showed a greater sperm number, reaching considerably higher figures from May through September. The average number of all the Holsteins was 52.9×10^8/ml, while that of the Wagyu was 37.0×10^8/ml.

The first ejaculates, which had higher sperm concentrations in both the species, showed a slightly greater sperm number than the second ejaculates (Fig. 9).

The average sperm number of all the ejaculates was 45.0×10^8/ml. The fact that semen volume, sperm concentration, and semen number began to decrease in September may be explained by a decrease in secretory function and spermatogenesis of the reproductive organs

resulting from the aftereffects of higher temperatures in July and August.

d) Motility of Sperm

Fig. 10 shows the seasonal variation of motility of sperm caluculated in terms of viability index.

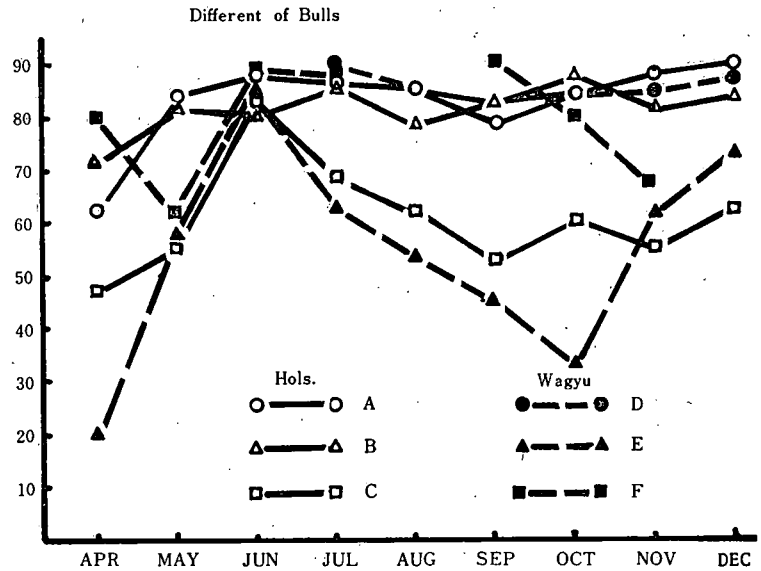

Fig. 10 Seasonal Variation of the Sperm Motility

Bulls A, B, C and F showed no marked variation, their viability indices being 75–95 throughout the seasons.

Holstein C and Wagyu E, however, began to show a decrease in motility in July and reached the minimun viability index of 53.8 (September) and 32.5 (October) respectively. They were, therefore, excluded from the experiments thereafter. Generally speaking, those that ejaculated highly motile semen showed higher mounting desires and gave greater semen volumes.

As is shown in Fig. 11, the Holsteins showed higher viability than Wagyus all through the period. The average motility index of the Holsteins was 76.2. while that of the Wagyus was 73.6. The average motility of all the ejaculates was 74.9.

No significant difference was observed between the first and second ejaculates of the two species (Fig. 12).

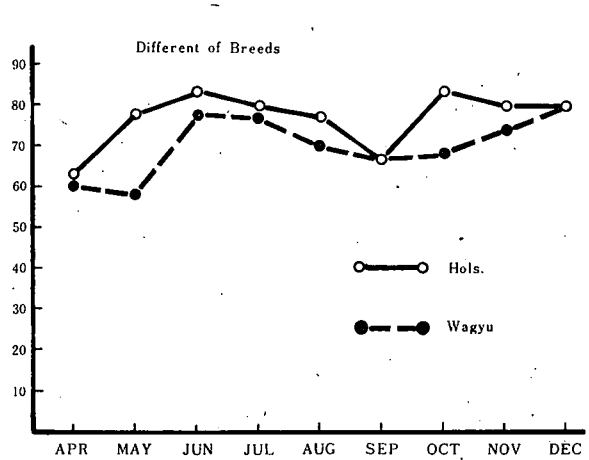

Fig. 11 Seasonal Variation of the Sperm Motility

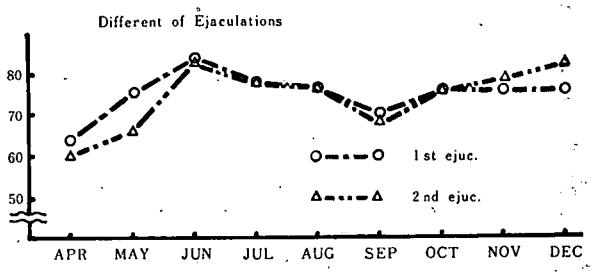

Fig. 12 Seasonal Variation of the Sperm Motility

e) pH

pH of spermatozoa, which is closely related with their survival, is mainly governed by accessory reproductive gland.

It is reported that, within a certain range, the concentration and viability of semen increase in proportion to the decrease of pH[13].

In the present experiments pH ranged from 6.2 to 7.0 for each bull and the average pH for the whole period was 6.44. No marked seasonal variation was observed (Fig. 13).

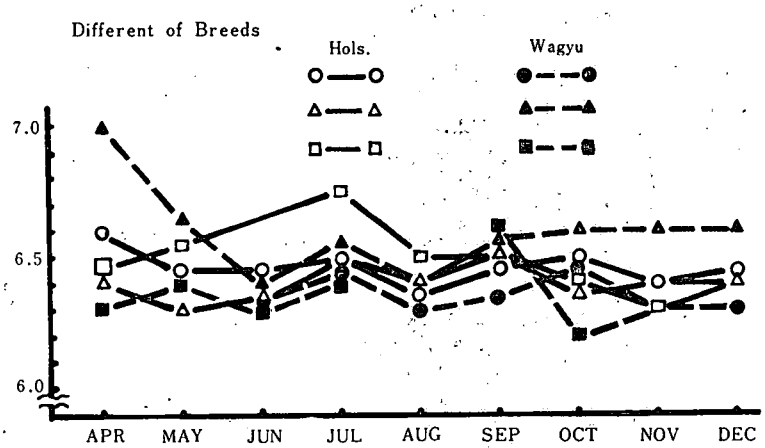

Fig. 13 Seasonal Variation of the pH of Semen

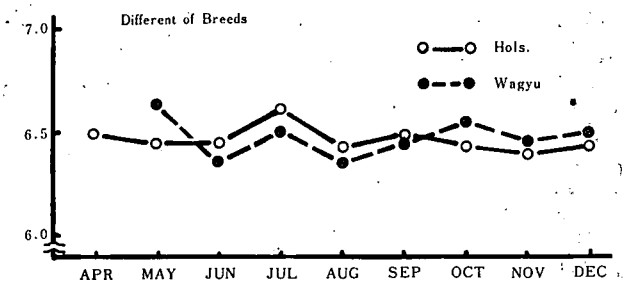

Fig. 14 Seasonal Variation of the pH of Semen

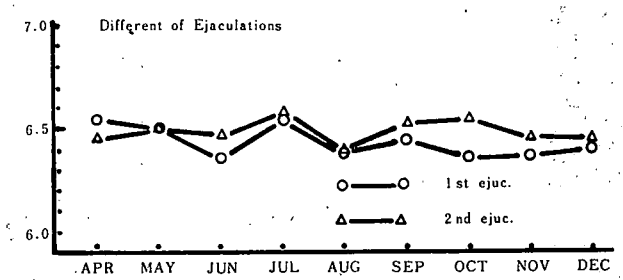

Fig. 15 Seasonal Variation of the pH of Semen

Bull E, showed the highest pH of 7.0 in April when its semen number was relatively small for its semen volume. Thus relatively greater volume of the fluid produced by accessory gland caused a rise in pH. On the whole, semen with higher concentration had lower pH and good motility, showing the same result as had been reported by Mann et al[14].

Fig. 14 and 15 respectively show the difference of pH between the two species and tow series of ejaculations. It was observed that pH showed a reverse tendency to the strength of sexual desire and motility.

C) Seasonal Variation of the Chemical Composition of spermatozoa

a) Fructose Concentration

It has been proved that considerable amount of sugars is contained in the seminal plasma and that the sugar is fructose in most of farm animals[15,16]. Fructose, which is produced mainly by seminal vesicle, has a close relation with survival and motility of spermatozoa, being utilized as the source of their energy and its metabolic pathway has been clarified. Seasonal variation of fructose concentration is shown in Fig. 16.

Fructose concentration of Holstein A began to decrease since the rainy season in May with its higher temperature and humidity and reached the minimum of 82.7 mg/dl in August, with temperature was the highest. Then it began to increase in September, when temperature began to fall, and showed the maximum of 871.6 mg/dl in November. Semen volume, semen number, and motility also showed a sudden rise in the same month.

Fructose concentration of the other bulls also decreased gradually in May or June, reached the minimum in August, and showed a sudden rise thereafter, reaching the maximum in October and November.

Fructose concentration showed a sensitive reaction to the effect of high temperatures and decreased rapidly preceding the decreases in mounting desire and semen properties. According to Mann et al[14], fructose and semen concentration have a significant minus correlation and the presnt experiments showed a similar pattern to their result. Mann[4] and Hiroe

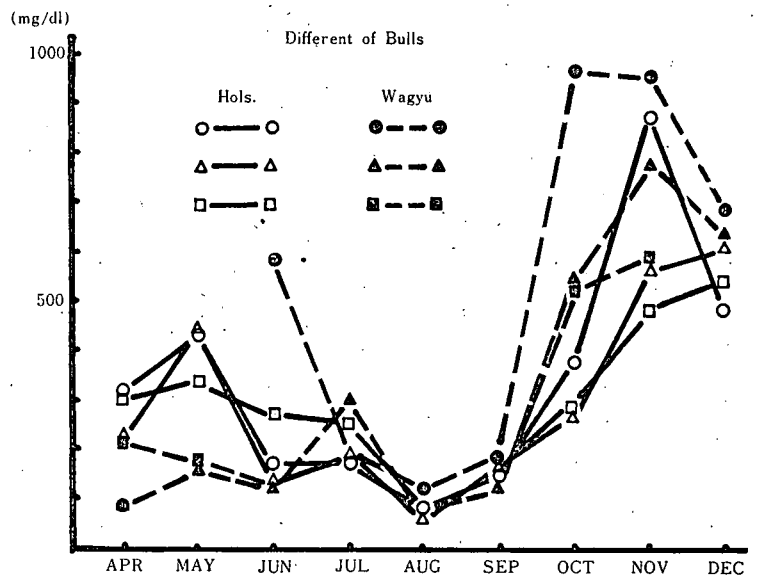

Fig. 16 Seasonal Variation of the Concentration of Fructose

et al[6] reported that no marked variation was observed in the fructose concentration of bulls and swine. The present experiments, however, being conducted in a warmer climate than theirs, where temperatures show a remarkable rise in August, concentration showed a marked seasonal decrease in summer.

Fructose concentration of Wagyu F (260 mg/dl), which showed the weakest sexual desire, was about half of that of the other Wagyus (c. 530 mg/dl). Such a marked individual

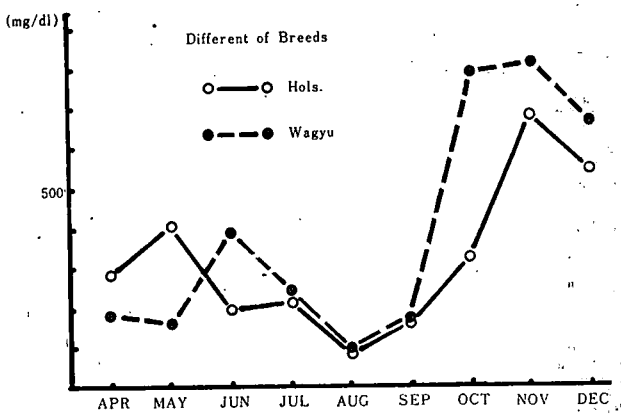

Fig. 17 Seasonal Variation of the Concentration of Fructose

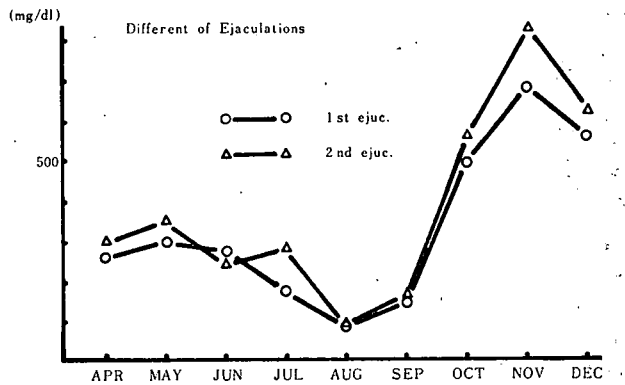

Fig. 18 Seasonal Variation of the Concentration of Fructose

difference is explained by Mann et al[14] that fructose, being produced by seminal vesicle and not by testicle, its quantity varies with individuals because of the difference in the size and secretory function of their seminal vesicles.

Generally speaking, the Wagyus showed higher fructose concentration than the Holsteins and they also showed a marked increase in it in October and November (Fig. 17).

The average fructose concentration of all the Holstein ejaculates was 313.6 mg/dl, while that of the Wagyus was 440.7 mg/dl, their mean being 377.2 mg/dl, which is a little lower than 500 mg/dl reported by white[17].

Fig 18 shows the difference of fructose concentration between the first and second ejaculates.

No significant difference was recognized between the two series of ejaculates. The average of the first ejaculates was 333.3 mg/dl, while that of the second 334.1 md/dl.

b) Citrate Concentration

Various reports have been made on the physiological significance of citrate. It has been proved that it is, as is the case with fructose, produced by seminal vesicle and that it has a close relation with the activity of activity of Androgen. It is also rported by Humphrey, Mann[18] that it gradually disappears when the animal is castrated and reappears with the transplantation or injection of Androgen. It has further been clarified that though it has no direct relation with the metabolism of spermatozoa, it inhibits their coagulation and liquefaction, protects them from detremental influence in combination with Ca contained in seminal plasma, and adjusts and maintains the equilibrium of their osmotic pressure in combination with K and Na. Seasonal variation of citrate concentration is shown in Fig. 19.

Citrate concentration of Holstein A reached the maximum of 1486.8 mg/dl in June and, after a gradual decrease, reached the minimum of 459.5 mg/dl in October. It then gradually increased after November. Those of the other bulls also showed a seasonal variations, reaching the maximum in June and July and the minimum in October. It was also observed

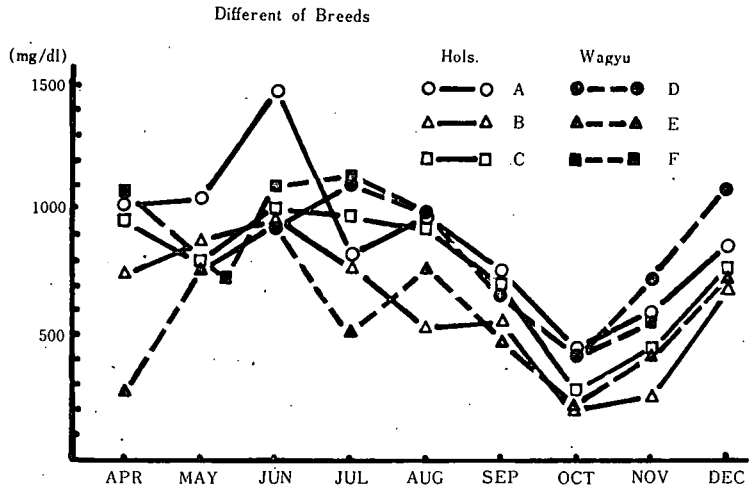

Fig. 19 Seasonal Variation of the Concentration of Citrate

that those that had stronger sexual desire and greater semen volume and mumber showed higher citrate concentration.

According to Kato & Nishikawa et al[12], citrate, like fructose is sensitive to Androgen but its reaction always appears later than that of fructose. A similar result was obtained from the present experiments. That is, citrate concentration reached its minimum in October, while fructose concentration in August, thus showing a time lag of two months.

No marked difference was observed between the citrate concentrations of the two species. (Fig. 20).

The average of the citrate concentrations of the Holstein was 767.9 mg/dl, while that of the Wagyus 758.9 mg/dl. That of all the ejaculates was 763.4 mg/dl, which is approximate to 720.0mg/dl reported by White[17].

The first (760.5 mg/dl) and second ejaculates (792.0 mg/dl) showed almost no difference in citrate concentration (Fig .21).

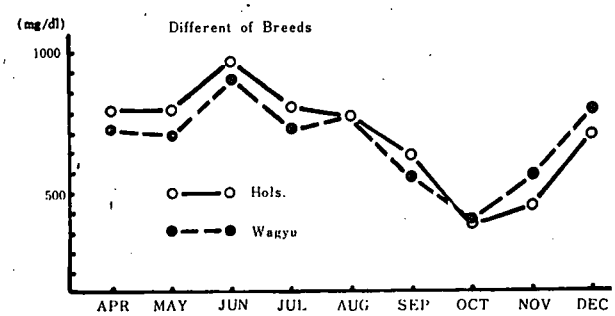

Fig. 20 Seasonal Variation of the Concentration of Citrate

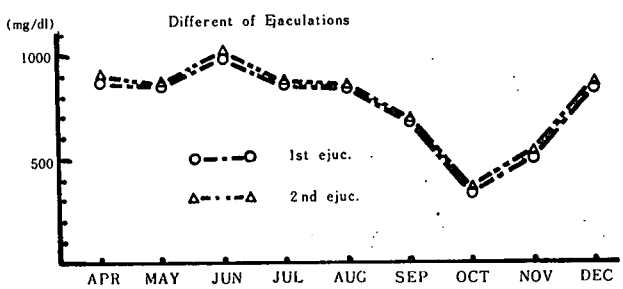

Fig. 21 Seasonal Variation of the Concentration of Citrate

c) Protein Concentration

Larson et al[19] reported that 90% of the protein in seminal plasma is non-dialytic and that its composition is peculiar to seminal plasma, containing eleven kinds or components but no sugars and fats. Its physiological significance is considered to

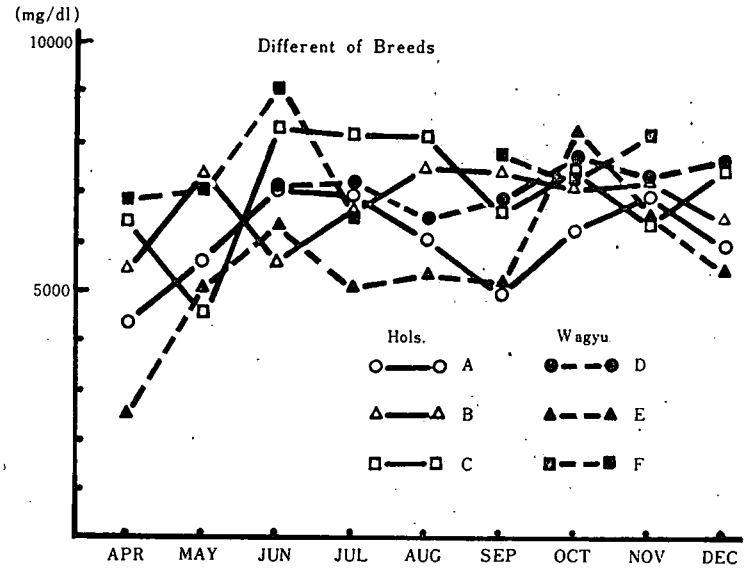

Fig. 22 Seasonal Variation of the Concentration of Protein

lie in the inhibition of the coagulation of spermatozoa in combination with heavy metals and the protection of the loss of materials within them. Fig. 22 shows the seasonal variation of protein.

Protein, unlike fructose and citrate did not show any marked seasonal variation and appreciable individual differences. Protein concentration also showed no particular tendency in relation to other components and properties.

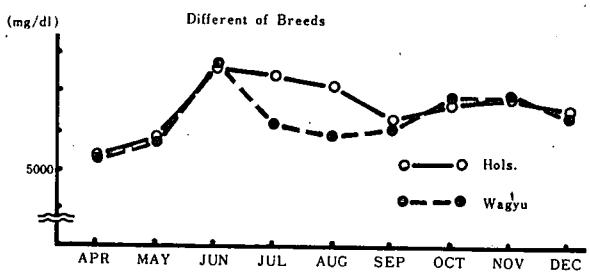

Fig. 23 Seasonal Variation of the Concentration of Protein

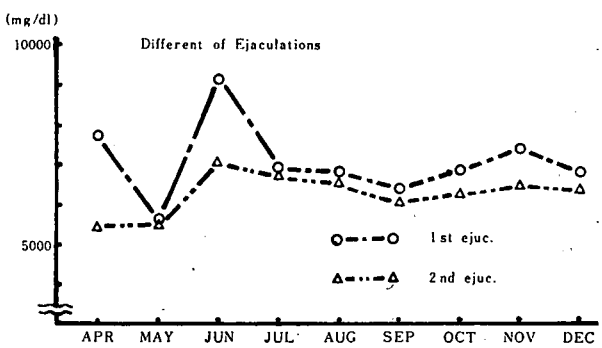

Fig. 24 Seasonal Variation of the Concentration of Protein

No notabl difference was found between the two species except that protein volume of the Wagyus decreased in July and August. (Fig. 23).

The average of the protein volume was 6628.8 mg/dl in the case of the Holsteins, while 6560.6 mg/dl for the Wagyus. The average of all the ejaculate was 6594.7 mg/dl, which is rather than 4718.8 mg/dl reported by White.

The first ejaculate (6826.9 mg/dl) showed a little higher protein concentration than the second ejaculates (6399.0 mg/dl). (Fig. 24)

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