BRIEF COMMUNICATION

THE EFFECT OF RESTING ROOSTERS FROM
EJACULATION ON THE QUALITY OF SPERMATOZOA
IN SEMEN

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During an investigation of the chemical composition of fowl semen it was felt necessary to study the effect of varying the length of rest periods between ejaculations on the quality of fowl spermatozoa. Such information could be useful for future work on the storage of semen *in vitro* for long periods, since there might be an optimum ejaculation rate for obtaining spermatozoa of the best quality and thus likely to survive best *in vitro*.

Twenty related males of the Breeding Line of Brown Leghorns at the Poultry Research Centre, Edinburgh (Blyth, 1954) were selected randomly from roosters trained for the artificial collection of semen. Semen had been collected and examined at least once weekly from all the males for a month before the start of the experiment. The males were subdivided randomly into five groups; one group was kept as a control and the other four were rested for 2, 4, 6 and 8 weeks respectively, between ejaculations.

Semen was collected consistently from the males in the control group throughout a 10-week experimental period at intervals of 2, 3 or 7 days; each time the morphology of spermatozoa in semen samples was examined in nigrosin–eosin smears (see below). Semen was taken from the four groups of experimental roosters at the end of their allotted rest periods and examined; thereafter semen was collected as frequently as in the controls and during the 9th and 10th weeks was examined once again.

A nigrosin–eosin stain technique was used to examine the quality of spermatozoa in smears of semen samples. Nigrosin (0.6 g) was added, with constant stirring at room temperature, to 10 ml isotonic glutamate solution (Lake, 1960) containing 0.16 g eosin. Aliquots of 0.50 ml of this stain mixture were put into small test-tubes kept in ice-cold water. Clean uncontaminated semen was collected and 0.02 ml added to the stain and mixed thoroughly. After 10 min one drop of the stain–semen mixture was placed on a clean slide and a smear made. The slide was dried quickly and thoroughly in warm air and the smear mounted with DPX (Gurr Ltd) mounting fluid and a coverslip. Duplicate

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smears were made and a total of 300 spermatozoa was counted on each slide using an oil immersion objective; duplicate counts were made of each specimen. The proportion of unstained, normally-shaped, intact spermatozoa (Lake, 1954) was determined in each semen sample and was considered to be an estimate of viable spermatozoa.

TABLE 1
THE NUMBER OF NORMAL (UNSTAINED) SPERMATOZOA IN NIGROSIN-EOSIN
SMEARS OF SEMEN TAKEN FROM BROWN LEGHORN ROOSTERS AFTER
VARIOUS PERIODS OF REST BETWEEN EJACULATIONS

Bird No.	Weeks after commencement of experiment						
	0	2	4	6	8	9†	10†
Control group							
1	277.0	281.5	282.0	283.0	284.2	285.0	285.5
7	270.0	285.0	271.0	269.0	278.2	272.0	283.5
8	277.0	282.5	276.0	277.0	270.0	281.0	274.0
20	272.3	275.0	276.0	268.5	278.7	276.0	273.5
Mean	274.1	281.0	276.2	275.4	277.8	278.5	279.1
2-weeks rest group							
2	275.6	273.0				283.0	282.0
5	234.6	243.5				212.6	249.0
12	277.3	212.0				279.5	284.5
18	278.6	271.0				261.0	269.5
Mean	266.5	249.9				259.1	271.2
4-weeks rest group*							
3	274.0		246.0			269.3	261.0
6	266.0		256.0			260.5	273.5
9	279.0		253.0			273.5	281.5
Mean	273.0		251.8			267.7	272.0
6-weeks rest group							
4	275.0			274.2		276.5	283.0
10	282.6			271.0		273.5	270.5
15	270.0			236.5		276.0	283.0
19	270.0			147.0		253.6	233.5
Mean	274.4			232.2		269.9	267.5
8-weeks rest group							
11	258.0				184.0	232.0	248.5
14	276.0				272.6	279.5	280.5
16	279.0				184.0	272.0	286.0
17	285.0				195.5	258.0	267.0
Mean	274.5				209.0	260.4	270.5

* One rooster in this group died during the experiment for reasons unconnected with the experiment.

† The experimental males were milked at weekly intervals after their selected rest period and test samples were taken twice from all the males during weeks 9 and 10.

The results in Table 1 showed that there was a tendency for the number of degenerating spermatozoa in an ejaculate to rise after roosters had been rested between ejaculations for more than 2 weeks. There were individual variations in the degree of deterioration of ejaculates and some roosters appeared not to be appreciably affected. However, analysis of variance revealed that over the 8-week experimental period there was a trend to an increase in the proportion of degenerating spermatozoa in semen significant at the 5% level.

When experimental males were milked frequently after a rest period the percentage of normal spermatozoa in the semen samples returned towards the pre-rest value.

The observations made in this experiment showed that the number of degenerating spermatozoa in semen samples was liable to increase in some roosters when rest periods between ejaculations were extended for more than 2 weeks. It seems desirable, therefore, that for work on the chemical composition and storage of semen *in vitro*, roosters should have semen collected from them regularly to ensure uniform samples.

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