BRIEF COMMUNICATION

EXPERIMENTS IN SEDIMENTATION AND
CENTRIFUGATION OF BULL SPERMATOZOA AND
THE SEX RATIO OF BORN CALVES*

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Man's control of the sex ratio of progeny remains one of the most interesting unsolved problems in biology. Many experiments have been done to separate the male-determining Y-spermatozoa and the female-determining X-spermatozoa. Up to the present time, no method to influence sex ratio in mammals has been successfully developed. After the war, the theory of size-differences between X- and Y-spermatozoa was again tested. Lindahl (1958) carried out experiments with counter-streaming centrifugation of bull spermatozoa. Inseminations with heavy spermatozoa produced more female calves (57%), with light spermatozoa more male calves (59%).

In order to separate X- and Y-sperm-cells by their different size and weight, Bhattacharya (1958, 1962) made use of the spermatozoa's own gravity dropping through a medium of high viscosity. He took advantage of an instrumental principle developed by Kampschmidt, Mayer, Hermann & Dickerson (1951) for measurement of the sedimentation-rate in bull spermatozoa. In this method bull or rabbit spermatozoa were layered on the top of a burette, filled with 20 ml of a medium consisting of a mixture of egg yolk and glycine. The sedimentation of spermatozoa was carried out at 0° C. After 12 hr sedimentation was stopped and the sperm-cells could be found distributed into 10 to 12 ml fractions. The frequency of numbers of spermatozoa within the different fractions was said to follow curves with two peaks. Spermatozoa from the lower and upper fractions were used to inseminate rabbit does. The fertility of treated semen was very low; no more than forty-one of 176 inseminated does (i.e. 23.3%) became pregnant and the litter size averaged only three. However, there was a deviation in sex ratio in the expected direction. Inseminations with lighter spermatozoa from the two upper fractions resulted in twenty-four males and seven females, while those from the two lower fractions (i.e. heavy spermatozoa) produced twenty-seven females and eleven males. During 1960, Bhattacharya inseminated cows of the dairy herd of the Max-Planck-Institute

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Mariensee with bull spermatozoa fractionated by the method as just mentioned but without success. Andersen & Rottensten (1962), using Bhattacharya's method, could not confirm his results. They inseminated rabbits with light and heavy spermatozoa. From both fractions more female rabbits were born.

In our experiments we at first tested the original method given by Bhattacharya. Our work, however, was limited to cattle, because in monotocous mammals the proportion of foetal mortality can be exactly determined. This is important to know, since Lindahl (1960) has shown that foetal mortality will influence the sex ratio. We also found that within 12 hr the sperm-cells had fallen to approximately half of the length of burette and were distributed into 10 to 12 ml fractions. More than 200 semen samples were fractionated and counted. In no case, however, were curves with two peaks found. Distribution of sperm-cells either followed a unimodal curve or there was an accumulation in the lower fractions. After 12 hr at 0° C, in that highly viscous medium, motility and survival of spermatozoa were adversely affected. Although we knew this treated semen was highly infertile, thirty-one cows were inseminated with spermatozoa from the lower fractions. As we expected, none of these animals became pregnant; again demonstrating the inadequacy of this medium for bull spermatozoa.

In our subsequent trials, we also used the very suitable equipment of Kampschmidt *et al.* (1951), but we were forced to develop a totally new medium. Our preliminary aim was to find a medium which preserved motility and survival of spermatozoa and guaranteed normal fertility. After much testing we found such a medium but it was very different from that of Bhattacharya. It consisted of other components (skim milk, variable concentrations of salt-solutions and egg-yolk) and it was nearly homogenous. The viscosity of our medium was very low, only 7 to 10 cP (which is 10 times lower than Bhattacharya's medium). The specific gravity was a little higher, ranging between 1,037 and 1,044. In this new medium, sedimentation of bull spermatozoa into 10 to 12 fractions was completed within 60 min, not after 12 hr. No further sedimentation could be observed after 60 min. In our opinion, this may be a true separation process depending on a certain specific gravity and viscosity of the medium and not an interruption of a lengthy sedimentation process. When counting sperm-cell numbers in different fractions, we only found curves with single peaks and never curves with two peaks. Motility and survival were hardly affected during the brief process of sedimentation—decreasing by only 10%. Dilutions of the fractions caused no harmful effects and the spermatozoa were able to swim freely in this medium.

Following the sedimentation process spermatozoa of the lowest fractions were centrifugated at 4000 rev/min for 15 min. By this method small doses (1 to 1.5 ml) with high number of sperm-cells (about 40 to 80 millions) were obtained. Centrifugation probably will cause a further selection of heavy spermatozoa, because in the supernatant fluid a small number of spermatozoa could always be found.

For 3 successive years we inseminated 139 cows with spermatozoa from the two lowest fractions, i.e. nearly 10 to 15% of the whole sperm number. Last year, we used the four lowest fractions, i.e. nearly 30% of all spermatozoa, in order to

test the efficiency of separation and to obtain more pregnancies. Of the inseminated cows 67% became pregnant after first insemination and came to term (some pregnant cows were sold by the owners before calving). These are nearly the same results as those obtained by normal artificial insemination. The sex ratio of newborn calves is given in the following table.

As can be seen, in all 3 years, there was a deviation in sex-ratio towards females. In the 3rd year, too, when using more fractions, there was a significant deviation from the normal 1:1 ratio ($P < 0.05$). Out of a total of eighty-six calvings there were 69.8% female calves born, i.e. forty-three males to 100 females. Normally in artificial insemination the sex-ratio is somewhat higher than 50% for male calves. In Germany, Baier & Haeger (1958) calculated in 17,000 calvings a ratio of 55.2% males: 44.8% females, i.e. 123 females to 100 males.

In the light of our results in sex-distribution we believe, that we have successfully isolated X- and Y-chromosome-bearing spermatozoa by sedimentation. Thus, the hypothesis, that X-bearing spermatozoa are larger and heavier than Y-bearing spermatozoa is once again confirmed. Future inseminations will be

TABLE 1
SEX-DISTRIBUTION OF CALVES FOLLOWING INSEMINATION WITH
HEAVY SPERMATOZOA

<i>Year</i>	<i>Females</i>	<i>Males</i>	<i>Females (%)</i>	<i>Males to 100 females</i>
1961 to 1962	6	1	85.7	17
1962 to 1963	15	5	75.0	33
1963 to 1964	39	20	66.1	43
Total	60	26	69.8	43

done with spermatozoa from upper fractions too, here we expect more male calves will be born.

In comparison with Bhattacharya's method it is of interest that our sedimentation curves had normal distribution in sperm numbers. As mentioned before, curves with two maxima never could be seen. More critical remarks to Bhattacharya's method have been published by Schilling (1966).

In spite of our promising results in sex distribution, this method of sperm separation is not yet suitable for practical use. Up to now, the technique of separation is very expensive and tedious, and requires much time. In addition many more calvings are necessary to demonstrate the accuracy of this method for sex-control. For our future work we also believe that attempts should first be made to discover more differences between X- and Y-spermatozoa in highly purified fractions with either heavy or light spermatozoa, in morphological, physical, physiological or biochemical sense as stated by Krajnc (1964). Only when we have an answer to all these questions, can we have a basis for effective sex-control.

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