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Frequency of Polyploid Spermatozoa in Man

DIPLOID spermatozoa have been found in mouse, rabbit, bull (reviewed by Beatty[1]) and man[2,3]. In the rabbit they form 0.03% of the total sperm and in the bull 0.1% to 0.17%, but the incidence in man is not known. Because such sperm could well be involved in the formation of triploid embryos, which are almost invariably aborted in man[4], knowledge of their frequency would be of interest.

Of 1,670 sperm measured in this study, sixteen were diploid, and one was tetraploid, giving 1.02% of polyploid sperm (Table 1). The mean optical density of the diploid sperm was 1.990 ± 0.070 times, and the mean area 1.578 ± 0.069 times, that of haploid sperm. These figures are very close to the expected values of 2.0 and 1.587 ($2^{2/3}$). The corresponding values for the single tetraploid sperm were 3.68 for density and 1.84 for area. The subjects studied were aged between 23 and 27 yr, and the proportions of polyploid sperm ranged from 0.74% to 1.53%, the highest proportion occurring in the youngest subject. Our data are, however, clearly inadequate to make any deductions about a possible effect of age.

If a diploid spermatozoon fertilizes a normal ovum, the resulting zygote would be triploid. Triploid embryos are almost invariably aborted, and form about 5% of spontaneous abortions[5]. Estimates of the spontaneous human abortion rate vary from about 15% to 27%, so that roughly 1% of all conceptions are triploid[5]. Boué *et al.*[6] and Schindler and Mikamo[7] considered the origin of human triploid embryos, and concluded that they result both from digyny, through suppression of the second polar body, and diandry. Penrose and Delhanty[8] showed that a triploid abortus had resulted from digyny, two

Table 1 Characteristics of the Sperm Samples used and Frequency of Polyploid Sperm

Subject	Age	Sperm count * $\times 10^{-6}$ ml.$^{-1}$	Sperm motility * (%)	Sperm morphology * % normal	Number of sperm measured	Number diploid	Number tetraploid	% Polyploid
1			Data not available		450	3	1	0.89
2	26	81	75	59	408	3	0	0.74
3	27	44	69	73	419	4	0	0.95
4	23	35	60	52	393	6	0	1.53
Total	—	—	—	—	1,670	16	1	1.02

* The following values are regarded as normal: sperm count, above 30×10^6 ml.$^{-1}$; sperm motility, above 50%; sperm morphology, above 55%.

Ejaculated sperm were obtained from men attending a subfertility clinic; all were chromosomally normal, and their sperm counts, percentage of normal sperm and sperm motility were within normal limits (Table 1). Except for subject 2, a drop of whose semen was smeared on a slide and fixed for 30 min in methanol–acetic acid (3 : 1), samples of semen were fixed in suspension in three changes of methanol–acetic acid, and a drop of the resulting suspension dropped onto a slide and allowed to spread. This technique gave more uniform results with Feulgen's method than smearing before fixation. To measure the DNA content of the sperm, Feulgen's method was used on the slides, hydrolysing in 5 M hydrochloric acid at room temperature for 1.5 h. Slides were subsequently mounted in DPX. A Vickers M85 microdensitometer was used to measure the integrated optical density and the area of the sperm; three measurements of each parameter were made for each sperm, and we used the means of these values in subsequent calculations. The slides were sampled at regular intervals (usually 2 mm in each direction) and all sperm within the field of the instrument were measured until approximately 400 had been measured from each subject; these occupied about half the slide in each case.

Because there was considerable variation in the integrated optical density and the area of the sperm, an objective criterion of diploidy was used. Sperm were regarded as diploid if their densities were more than 1.64 standard deviations (95% limit) above the mean value, and if half their density was within the normal range of all the sperm measured; a similar criterion was used for the areas, except that the area divided by $2^{2/3}$ was required to fall within the normal range (because area is a measure of only two of the three dimensions which should be increased in a larger sperm). In fact, almost all the polyploid sperm found by measurement were easily recognizable visually by their larger areas and greater densities.

of the three sets of chromosomes resembling the mother's. Schindler and Mikamo[7] regarded double fertilization (by two haploid sperm) as the only likely mechanism of diandry, and stated that there is no evidence that diploid sperm are capable of successful fertilization. Nevertheless, as there is a significant proportion of diploid sperm in human semen (between 6 and 30 times greater than that reported in other mammals[1]) it seems unwise to ignore them as a possible cause of triploid embryos. Even if they are capable of fertilization, however, there is clearly some selection against them, because their frequency is comparable with that of triploid embryos, many of which are formed by other mechanisms. It seems, therefore, that diploid sperm are almost as frequent as 24 *YY* sperm[3] and are similarly less efficient than normal haploid sperm in fertilization.

I thank Mr P. Edmond and Sister M. Isdale for sperm samples, Professor H. J. Evans and Miss K. Buckton for advice, and Mrs Sheila Christie and Mrs Lily Gowans for technical assistance.

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Received February 12, 1971.

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