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Detection of Aneuploidy in Human Sperm

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Adequate methods for monitoring any type of gametic mutation directly in man are virtually nonexistent. A method is presented by which one can monitor Y chromosomal nondisjunction directly in the male gamete by quantifying the number of spermatozoa with two fluorescent bodies (YFF) in 1000 sperm counted. Dried semen slides are stained with quinacrine dihydrochloride and examined under a fluorescent microscope with dark field illumination. This method eliminates the biopsy required for other meiotic studies and further eliminates bias in gametogenic selection by evaluating ejaculated mature spermatozoa.

Since chromosomal numerical errors are found in 0.4% of term births and 35% of miscarriages, it is evident that chromosomal aneuploidy constitutes the major mutagenic load in man. In view of the increases observed in the incidence of YFF sperm in patients receiving antineoplastic therapy and in the DBCP-exposed workers, it may be prudent for men who have a history of exposure to mutagens and who are contemplating reproduction to be evaluated by this method prior to attempting conception.

Further, this procedure could also be applied to the clinical phase of new drug testing to evaluate the effects of that agent with respect to aneuploidy since the increases in Y chromosomal nondisjunction may well act as a barometer for increases in overall autosomal nondisjunction.

Introduction

Mutagenic agents can impede the flow of genetic information by various mechanisms. If these agents effect the gametic cells, it is possible that those gametes which are not selected against meiotically will persist in a viable form. Should fertilization occur the outcome can end only in abortion or abnormal offspring.

The most clinically relevant stage of human mutation is gametically transferred, since it becomes incorporated into the gene pool. Gametes are subject to several types of mutation. Point mutations can be either base-pair substitutions or frameshift type mutations. In the case of base-pair substitutions, the purines convert to pyrimidines or vice versa. If this occurs in the first or second position of the triplet code, there is a high probability that there will be an insertion of an incorrect amino acid in that protein sequence. In frameshift type mutations, bases are added or deleted, resulting in a translation of the triplet code which is "out of frame" from that point on.

Chromosomal mutations can be either structural or numerical. In the case of structural aberrations, pieces of chromosomes are broken and either added to another chromosome or deleted. The second type of chromosomal mutation is numerical. Numerical errors are termed aneuploidy or nondisjunction and can occur during meoisis or in the early stages of mitosis in the zygote and represent a failure of homologous chromosomes to separate during cell division. The net result is cells which possess one too few chromosomes (monosomy) or those which possess one too many chromosomes (trisomy).

Aneuploidy has a high background rate in man — approximately 0.4% of term births (*1*) and over 35% of early spontaneous abortions (*2*). One in every 700 term births displays the aneuploidic condition Down's syndrome (*3*), whereas phenotypically expressed recessive point mutations such as Pompe's disease are found in about only 1 in 400,000 term births (*4*). Hence, Down's syndrome is about 570 times as prevalent as Pompe's disease.

Aneuploidy clearly burdens society and the human gene pool and contributes to mutation on the worst biological level, since these individuals display many biochemical and structural deficiencies which are usually accompanied by severe mental retardation. Coupled with the biological problems is the economic drain on society and the family involved since many of these individuals must be maintained in long-term health care facilities.

Based upon these data, it was decided that one of the most clinically relevant signs of germinal mutation in man is that of chromosomal aneuploidy.

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Ideally, one should examine gametic tissue from humans for the presence of aneuploidy.

Therefore, human semen was collected and analyzed for the presence of Y chromosomal aneuploidy. Zech (*5*) and Vosa (*6*) noted that the distal portion of the long arm of the Y chromosome fluoresces brightly when stained with quinacrine dihydrochloride. The same phenomenon can be observed in the interphase Y chromosome of human spermatozoa as described by Barlow and Vosa (*7*).

Since the XYY male is also an aneuploidy condition with an incidence of 1 in 1000 male term births (*8*), it is believed that Y chromosomal aneuploidy is a relevant marker for human gametic nondisjunction.

Materials and Methods

Ejaculates were collected into a sterile siliconized glass bottle and were immediately brought to the laboratory. Slides were prepared by placing a drop of semen upon a slide and then pressing another slide firmly against the first. The two slides were pulled apart to produce a uniform film. The slides were dried horizontally for about 24 hr. The dried semen was fixed in absolute methanol for 15 min and subsequently stained for 40 min in a solution made up from 50 mg of quinacrine dihydrochloride (Sigma Chemical Co., St. Louis), 50 ml of tap water, and 5 ml of ethanol. The slides were then drained and rinsed for 30 sec in standing tap water. The slides were mounted in McIlvaine's buffer solution (pH 5.5) and left undisturbed until the time of evaluation, approximately 3 hr later (*9*).

All slides were coded and examined on a Nikon Model FL-S set up for evaluation of fluorescent material with dark field illumination (100x objective and HBO 200 high-pressure mercury vapor lamp with BG 12 exciter filter and No. 53 barrier filter).

Only normal spermatozoa were scored, i.e., those unremarkable in size and shape, intact with complete membranes, and possessing an attached tail. Further, each fluorescent body was required to fall within the intact membranes and it had to form a distinct point of light. Ideally, 1000 sperm were examined from each specimen; however, this was not possible in some cases. A minimum of 400 sperm per sample was examined where slides were difficult to read.

Results

As seen in Table 1, evaluation of 30 semen specimens from a single individual (case 1) with no known mutagenic exposure demonstrated an average YF frequency of 39.3% (range: 32.8-44.1%) and an average YFF frequency of 1.3% (range: 0.7-2.2%). The sampling period for case 1 was approximately 400 days. Figure 1 displays the incidence of YFF sperm as a function of time. There are sporadic changes in these YFF values; therefore, serial sampling is necessary to establish a baseline. The overall background frequency for YFF sperm is 1.3% as determined from analysis of 262 semen samples (*10*).

Case 6 (Fig. 2) was a 22 year-old patient with metastatic osteogenic sarcoma. He had previously undergone a lobectomy and combined radiation-chemotherapy treatment. Two semen samples were collected prior to a standard three-month course of doxorubicin hydrochloride (Adriamycin). The YFF values for this patient showed a three to four-fold increase approximately one month after starting therapy. YFF values generally remained elevated after the next five samples; however, the patient's condition deteriorated, and further cooperation became impossible.

Case 7 (Fig. 3) was a 28 year-old physician who began fluoroscopy residency at the point indicated by the arrow. Three to five weeks after exposure there is a sharp increase in the frequency of YFF sperm observed.

Table 1. YF and YFF frequencies in serial semen samples from a nonexposed donor (case 1).

Sample No.	YF, %	YFF, %
1	36.2	1.6
2	37.8	1.4
3	34.4	1.2
4	39.1	0.7
5	41.0	0.8
6	44.1	1.1
7	38.6	1.1
8	36.9	1.1
9	37.2	1.3
10	34.1	1.4
11	32.8	1.6
12	38.1	1.8
13	39.9	1.8
14	43.0	1.9
15	41.4	1.2
16	41.3	1.1
17	44.0	1.0
18	40.4	0.8
19	38.6	0.8
20	37.7	0.9
21	38.2	1.1
22	39.9	1.2
23	41.1	1.1
24	43.2	1.9
25	42.0	2.2
26	38.9	2.1
27	38.8	1.0
28	36.4	1.2
29	40.8	1.4
30	43.1	1.1
Mean	39.3	1.3

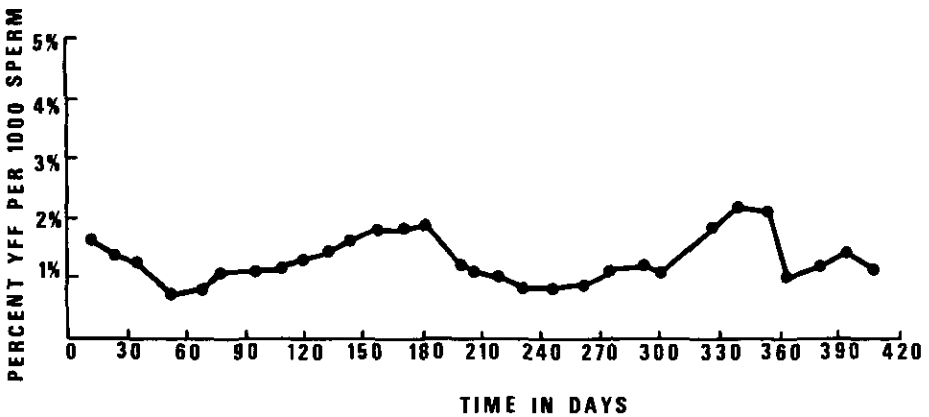

FIGURE 1. YFF frequency of 30 serial semen samples taken from a donor (case 1).

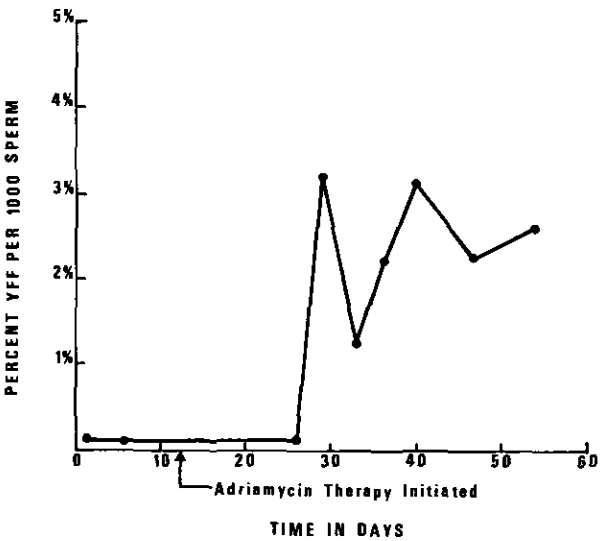

FIGURE 2. YFF frequency of nine serial semen samples taken from an individual being treated with Adriamycin for osteogenic sarcoma (case 6).

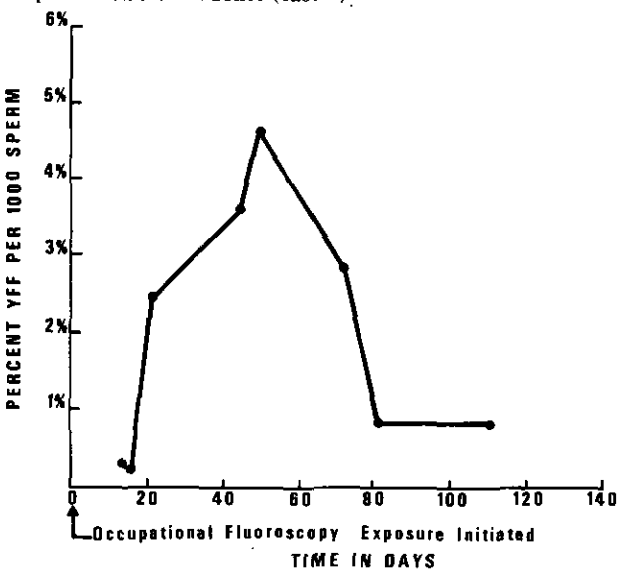

FIGURE 3. YFF frequency of eight serial semen samples taken from a physician on fluoroscopy residency (case 7).

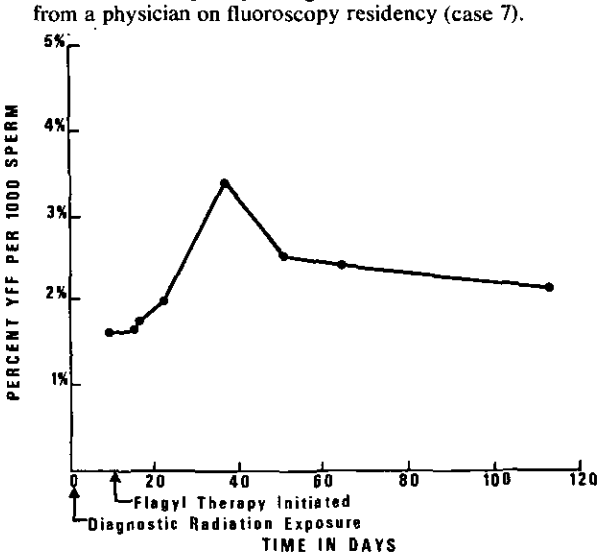

FIGURE 4. YFF frequency of eight serial semen samples taken from a research scientist exposed to x-ray radiation and Flagyl (case 8).

Case 8 (Fig. 4) was a 32-year old research scientist with intestinal amebiasis who underwent diagnostic radiation 9 days before starting on a 10-day regimen of Flagyl. YFF values for this individual revealed a doubling five weeks post-radiation exposure. Personal communications with A. Wyrobek of Lawrence Livermore Laboratories, Livermore, California, indicate that the sperm morphology abnormalities on these same semen specimens also show increases at the same intervals. Further investigations are currently underway.

Figure 5 shows the incidence of YFF sperm plotted against time in a 28 year-old individual (case 14) with a seminoma (testicular tumor). The initial sample was collected prior to serial x-ray therapy. Note that three to four weeks after initiation of treatment there is a five to six-fold increase in the incidence of YFF sperm.

Table 2 shows data from a group of 18 men who

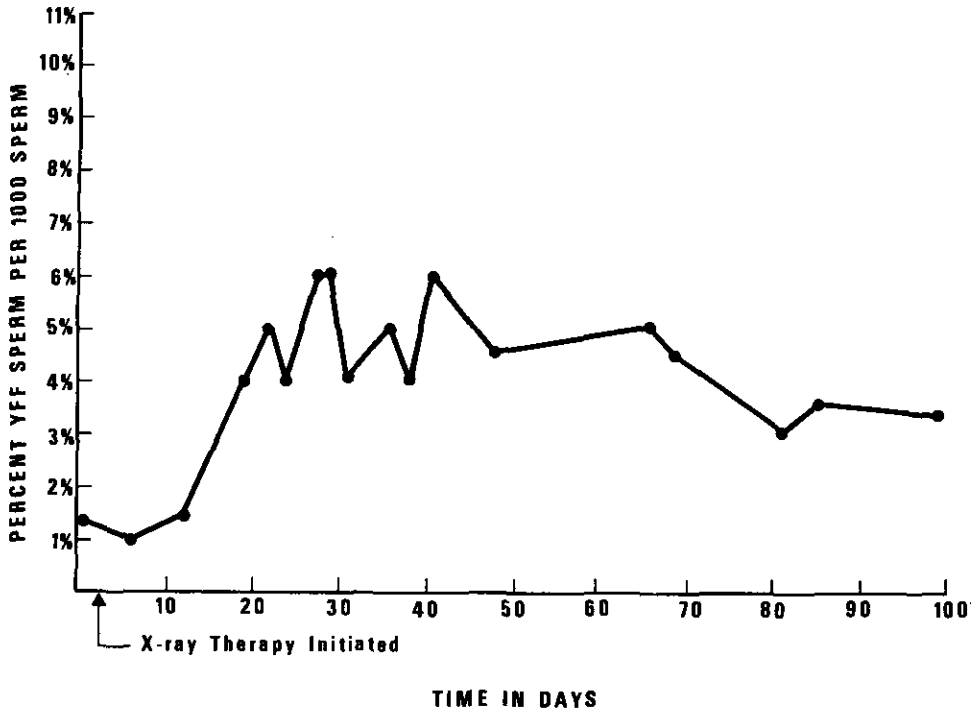

FIGURE 5. YFF frequency of 18 serial semen samples taken from an individual being treated with x-ray therapy for seminoma (case 14).

Table 2. YF and YFF frequencies in semen samples from DBCP-exposed workmen.

Case	YF, %	YFF, %
16	44.5	3.3
17	41.3	2.8
18	42.3	5.0
19	40.0	4.5
20	41.5	3.8
21	46.3	3.8
22	42.5	2.0
23	40.3	2.8
24	37.3	4.0
25	41.3	4.3
26	36.3	2.0
27	40.5	5.0
28	43.3	5.3
29	39.0	3.5
30	43.0	4.0
31	46.0	4.0
32	39.5	4.5
33	44.8	4.0
Mean	41.8	3.8

have experienced occupational exposure to dibromochloropropane (DBCP). There were originally 30 semen samples collected; however, 12 of the samples were aspermic and analysis was not possible. The 18 analyzable semen samples (cases 16-33) from DBCP-exposed workers revealed an average YF frequency of 41.8% (range: 36.3-46.3%) which is similar to that of the nonexposed individuals (Table 1). The average incidence of YFF sperm in the exposed workers was 3.8% (range: 2.0-5.3%). Table 3 presents the distribution of individuals with normal (less than 2%) (*11*, *12*) and abnormal (greater than 2%) (*13*) incidence of Y chromosomal nondisjunction. As can be seen, 43 of 45 nonexposed individuals fell within the normal range, while 16 of 18 DBCP-exposed workers fell outside the normal range. These differences between exposed and nonexposed control individuals are statistically significant ($p < 0.001$) as determined by X^2-analysis with one degree of freedom.

Table 3. Distribution of nonexposed and DBCP-exposed individuals as a function of the percentage of YFF sperm.

Group	Number of samples with YFF sperm[a]	
	0-2%	> 2%
Nonexposed individuals	43	2
DBCP-exposed individuals	2	16

[a]$X_1^2 = 40.88, p < 0.001$.

Discussion

The 24, YY gamete represents a disjunctional error in spermatogenesis in which the replicated male chromosomes have failed to separate. It is well established that chromosomal numerical errors constitute a significant portion of mutation in man since 0.4% of all term births display aneuploidy (*1*). The fact that the 24, YY sperm exists and constitutes a viable gamete is evident, since the XYY individual exists in a frequency of about 1 in 1000 male term births (*8*).

Since autosomal aneuploidy has been shown to increase with age (*14*) and since paternal age has been suggested to be a factor in the incidence of aneuploidy (*15*), it is possible that an increase in the frequency of 24, YY sperm could infer a potential increase in autosomal aneuploidy as well. Thus, this is the rationale in utilizing this unique gametic marker to screen at risk populations (*10*).

The increase in YFF sperm appears to be correlated to the exposure of the individual to radiation (cases 7 and 14), to Adriamycin (case 6) and radiation and Flagyl (case 8). It is possible that the exposure of the workers to DBCP as shown in Table 2 increased the incidence of YFF sperm. Clearly, as a group, the DBCP workers displayed a significantly elevated incidence of YFF sperm when compared to the YFF sperm in unexposed males (*9*).

It is believed that YFF monitoring of sperm could be used to detect gametic nondisjunction in the human male. In view of the changes observed in the patients receiving antineoplastic therapy and in the DBCP-exposed workers, it may be prudent for men who have a history of exposure to mutagens and who are contemplating reproduction to be evaluated for evidence of Y chromosomal aneuploidy before attempting conception. Genetic counseling coupled with chromosomal analyses by aminiocentesis should be made available to the wives of these men in the event of pregnancy.

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