Sperm enzyme activity analysis in individual sperm for detection of heritable mutations in mammals

B. B. Panda [1], G. Ficsor [2] and L. C. Ginsberg

Department of Biology and Biomedical Sciences, Western Michigan University, Kalamazoo (Michigan 49008, USA)
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Summary. Male mice were injected i.p. with 2.5 mg/kg mitomycin C, 100 mg/kg ethyl nitrosourea or saline and mated with untreated virgin females five weeks later. Sperm from 64 of the F_1 male progeny were analyzed histochemically for acrosin, succinic dehydrogenase and alpha-glycerophosphate dehydrogenase activity. The frequency of F_1 males with sub-normal sperm enzyme activity was significantly higher among progeny from treated males than in controls. These results show that analysis of sperm enzyme activity in F_1 males is a practical method for detection of transmitted mutations induced in a treated parent.
Key words. Mice; heritable mutations; sperm enzyme activity.

Mammalian germ cell tests are important for human genetic risk assessment [3, 4]. We report a germ cell test in which enzyme activity is evaluated in individual sperm of F_1 male mice from treated or control sires. We found that the mutagenicity of ethyl nitrosourea (ENU) or mitomycin C (MC) is detected by this method. HA (ICR) F_0 males were injected once i.p. with 2.5 mg/kg MC, 100 mg/kg ENU or saline vehicle. Five weeks later each male was caged with two virgin HA (ICR) females for one week. After mating, the males were discarded and the females individually caged. The F_1 males were either killed by cervical dislocation or were semicastrated under ether anesthesia to obtain sperm samples.

The sperm from each F_1 male was used for histochemical detection of acrosin, succinic dehydrogenase (SDH) and alpha glycerophosphate dehydrogenase (AGPDH) activity. At least two microscope slides were made from each F_1 male and for each assay. The slides were randomized and blindly scored. 250 sperms were examined at × 400 magnification from each F_1 mouse and for each assay. A sperm was scored as positive for acrosin activity if its head was surrounded by a yellow halo in a dark blue background [5, 6]. Sperm without acrosin activity lacked a halo. Both SDH and AGPD are located in the mid-piece region of the sperm. When active, both enzymes produced the reduced violet precipitate of paraiodonitrotetrazolium violet localized in the mid-piece region [6, 7]. Sperm without SDH or AGPD activity lacked the violet color [8, 9].

Two successive statistical analyses were performed: First, the number of enzyme inactive sperm in each F_1 male was compared to similar numbers in F_1 males from control sires. If enzyme inactivity in F_1 males exceeded the 99.9% confidence interval of the control mean such male was classified as a variant male [10] (fig., open shapes). Second, the statistical significance of differences between the number of variant F_1 males from treated or control sires was determined using homogeneity chi-square test on a 2 × 2 contingency table [11].

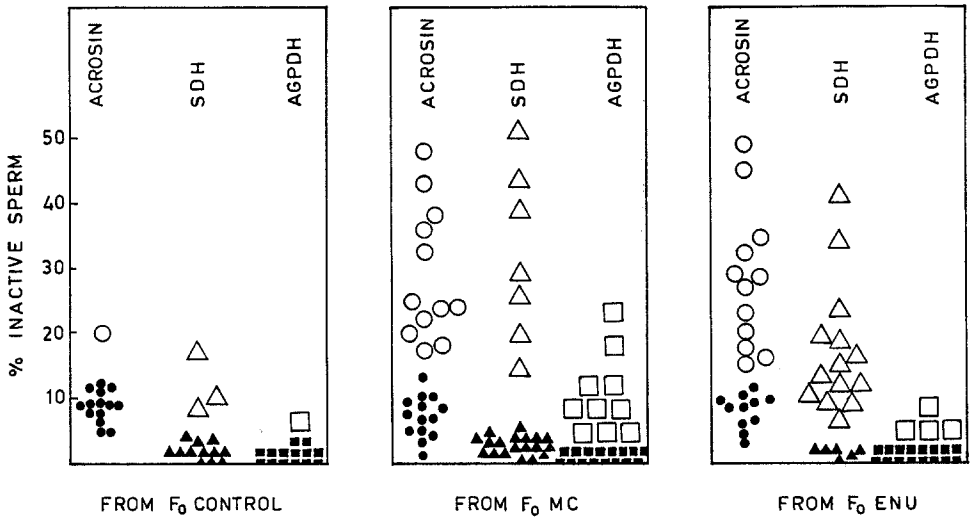
The figure shows the number of F_1 mice with percentage of inactive sperm for acrosin, SDH and AGPDH in individual F_1 males from treated or control sires. Generally more variant males were present among F_1 males from treated sires than in controls. The statistically significant effect of treatments is presented in the table.

Judging from the high frequency of F_1 variant males from treated sires, we conclude that mutation of many different genes may result in loss of enzyme activity. A large genetic target, as might be the case with this test, means that fewer F_1 animals are required (only 64 in this study) to detect a

Sperm enzyme inactive variant F_1 males from treated or control sires [a]

Agents tested in F_0 males	Dosages (mg/kg)	Variant F_1 Acrosin	Males/total F_1 SDH	Males [b] AGPDH
Control	0	1/15	3/15	1/15
MC	2.5	12/26*	7/26	10/26
ENU	100	12/23*	14/20**	4/20

[a] 24 F_1 males from ENU-treated sires reached sexual maturity. Only 23 of these had enough sperm for statistical evaluation for acrosin activity and 20 each for SDH and AGPD activity. [b] Significantly different from controls at * $p \leq 0.05$ and ** $p < 0.01$.



Percentage of sperm without acrosin, succinic dehydrogenase or alpha-glycerophosphate dehydrogenase activity in F_1 sons sired by control, ethyl nitrosourea or mitomycin C treated F_0 male mice. The enlarged open shapes represent mice with inactive sperm that exceed 99.9% confidence interval of the control mean.

positive genetic response than for tests relying on smaller genetic targets, e. g. mouse specific locus test[12,13].

Since most phenotypes in sperm are under diploid control, we assume that at least some of the enzyme inactivity resulted from dominant mutations[14]. However, since several sperm proteins are translated from mRNA first transcribed in haploid cells[15], it is possible that at least some of the enzyme activity loss in individual sperm was caused by recessive mutations.

A practical strength of the heritable sperm enzyme test (HSET) is that it can be performed simultaneously with other mammalian transmission genetic tests that keep F_1 progeny alive until sexual maturity.

The HSET allows progeny testing of F_1 males either by mating before killing for sperm examination or after examination of sperm obtained by semi-vasectomy or semi-castration. With the latter approach only variant males need to be progeny tested. Sperm from F_1 or later progeny could be obtained by ejaculation in some species of mammals.

It remains to be seen whether or not the HSET in conjuction with the heritable sperm morphology assay[10] and other sperm tests[16–18] will prove to be practical tests for detection of mutations in humans. A possible application of these tests may be with sperm of males contemplating parenthood and who may be concerned with receiving mutations from their parents who were exposed to known or suspected mutagens. Undoubtedly more developmental and validating work needs to be done in animals with progeny sperm tests before testing human sperm for parentally induced mutations could be recommended.

1 Present address: Department of Botany, Berhampur University, Berhampur, India. We gratefully acknowledge USPHS, NIEHS grant 1 RO1 ES02607-02 and technical assistance by G. M. Oldford.

2 To whom correspondence and reprint requests should be addressed.
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Sex chromatin analysis of unstained mouse amniotic cells using brightfield microscopy

T. Watanabe, K. Ohwada and A. Endo*

Department of Hygiene and Preventive Medicine, Yamagata University School of Medicine, Yamagata 990–23 (Japan)
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Summary. Sex chromatin of unstained mouse amniotic cells was identifiable using ordinary brightfield microscopy with optimal setting of the illumination.
Key words. Sex chromatin; mouse amniotic cells; unstained examination.

Our laboratory is at present involved in improving the techniques for sex chromatin analysis of rodent embryos at midgestation. During the study, we found that mouse sex chromatin is easily and quickly identifiable with unstained slides under ordinary light (brightfield) microscopy if we optimize the illumination setting. This was unexpected, since several reports have described the difficulty of identifying mouse sex chromatin even with stained preparations[1,2]. Therefore we describe the method here.

The extra-embryonic membranes were removed from the mouse conceptuses. This is possible as early as the 9th day of gestation. The amniotic membrane was separated and immediately placed in a fixative (60% glacial acetic acid) at room temperature for 30 min (20 min at least). Then, the specimens were gently touched on the surface of the slide using sharpened forceps, and dissociated amniotic cells were attached to the surface in a small spot (5 mm in diameter). About 10–15 spots (enough for the embryos from a single litter) can be taken on a single standard size (75 × 25 mm) glass slide.

After air-drying, the unmounted slides were examined under medium power (dry × 40 objective and × 10 ocular) with a brightfield microscope (Olympus BH-2) while finely focusing up and down with low illumination. For optimal viewing, it is essential that, as with a conventional analysis of unstained urinary sediments, illumination intensity be low enough. For this, the condenser was placed almost in its lowest position, the size of the diaphragm aperture was diminished, and also the voltage of illumination lamp was set at about one half of the maximum (12 V). With this setting, scattering refraction of the light within the nucleoplasm may produce phase contrast-like effect to reveal the structural contours of the nuclei more distinctly. As shown in figure a, the sex chromatin mass is easily identifiable as a dark mass adjacent to the inner nuclear membrane in amniotic cells from female embryos. Such a mass was observed very seldom in male amniotic cells (fig. b). In female cells, more than 50% of the cells had a sex chromatin mass, whereas such a mass could be seen in less than 2–3% of the male cells. Analysis of aged slides (more than two months) produced the same results. In more than 30 embryos at near term, sex diagnosis by this method coincided with morphological diagnosis by internal sex organs without a single exception. It was also found that carbol fuchsin staining of the slides after un-