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A Competitive *In Vitro* Assay of Human Sperm Fertilizing Ability Utilizing Contrasting Fluorescent Sperm Markers

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Fluorescein isothiocyanate (FITC) and tetramethyl rhodamine isothiocyanate (TRITC) were evaluated for use as contrasting fluorescent labels on living human spermatozoa. Unlabeled (control), FITC-labeled (green fluorescence), TRITC-labeled (red fluorescence), and mixed FITC-TRITC-labeled sperm suspensions were incubated with non-fertilizable human oocytes for assessing sperm penetration of the zona pellucida and with zona-free hamster eggs for assessing sperm incorporation into the ooplasm. Fluorochrome-labeled spermatozoa were as efficient as unlabeled cells from the same donors in penetrating the human zona pellucida and in entering the hamster ooplasm. The middle and principal pieces of spermatozoa undergoing nuclear decondensation within the hamster ooplasm retained their fluorescent label, allowing visual differentiation between FITC- and TRITC-labeled spermatozoa. Videomicrographic analyses of the movement characteristics (percentage of motile cells and mean swimming speeds) of labeled and unlabeled spermatozoa before and after incubation with ova revealed no detrimental effect on the motility of labeled cells. We conclude that the fluorescent dyes FITC and TRITC do not impair the function of human spermatozoa as assessed by motility characteristics and by their ability to penetrate ova *in vitro*. The contrasting colors of the two fluorochromes make them particularly useful in the competitive assessment of the *in vitro* fertilizing potential of human spermatozoa from different donors or after different experimental treatments.

Key words: Spermatozoa, fertilization, sperm motility

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