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## JOURNAL ARTICLE

# The use of two fluorescent dyes to identify sperm in a competitive binding assay to oocytes

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The relationship of most sperm laboratory assays to male fertility is inconsistent. Assays that measure traits required to fertilize oocytes are expected to have the most predictive value. A new assay that measures the competitive ability of two sperm samples to bind to oocytes was developed. Two populations of sperm were labeled using a pair of lipophilic dyes. A concentration of 75  $\mu\text{M}$  of the two dyes, DiQ (4-[4-(dihexadecylamino)styryl]-N-methylquinolinium iodide; an orange-red dye) and DiOC16 (3,3'-dihexadecyl oxacarbocyanine perchlorate; a yellow-green dye), intensely stained 66 and 73% of sperm, respectively, without affecting sperm motility or oocyte-binding ability. Because sperm could be stained with fluorescent dyes, sperm from two semen samples were mixed together in a droplet, and oocytes were added to allow sperm to bind oocytes competitively. Oocyte-bound sperm from each sample were counted. Binding was specific; nonspecific sperm binding was estimated by sperm bound to two-cell mouse embryos and averaged one to three sperm per embryo. Staining with DiQ or DiOC16 did not affect oocyte-binding ability since more than 80% of the sperm bound were stained with either dye. Furthermore, if different ratios of DiQ- or DiOC16-stained sperm from the same ejaculate were prepared in droplets and oocytes were added, the percentage of sperm bound to the oocytes reflected the percentage of sperm in the droplet; there was no differential effect of either dye. This assay used fixed oocytes because sperm bound equally to fixed and fresh bovine oocytes. This competitive oocyte-binding assay allows one to make a series of pairwise comparisons between a group of males or to include an internal control sample in sperm-oocyte binding assays. This assay may allow more accurate prediction of the oocyte-binding ability of sperm.

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