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### Identification of Calcium Flux in Single Preimplantation Mouse Embryos with the Calcium-Sensitive Vibrating Probe

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Although aging affects nearly every aspect of female reproduction, oocytes are believed to be major targets. Oocytes are long-lived, post-mitotic cells and donation of these cells by young females to older ones clearly ameliorates the effects of aging on reproductive success (1). Elucidation of the mechanisms underlying reproductive aging is of basic importance because the senescence of oocytes provides a model of aging of other long-lived, post-mitotic cells. Moreover, many women now delay marriage and childbearing until their late thirties, when the effects of aging on fertility can become clinically significant (2). A non-invasive technique that could be used to assay the developmental potential of an embryo before implantation would both facilitate the diagnosis of reproductive senescence and help middle-aged women decide whether to depend on their own oocytes and pursue costly reproductive therapies, or to pursue alternatives such as adoption or oocyte donation.

Disruption of intracellular calcium  $[Ca]_i$  regulation is an important mechanism underlying senescence in many long-lived cells (3). Normally, the plasma membrane has at least two systems that contribute to maintaining low  $[Ca]_i$ : a Na/Ca exchanger powered by the Na-K ATPase, and a Ca-ATPase (4). Both of these systems have been implicated in cell injury and senescence (3, 4). Inhibition of the Na-K ATPase by ouabain alters *in vitro* development of mouse preimplantation embryos (7).

In this study we employed the calcium-selective vibrating probe to test directly the hypothesis that mouse preimplantation embryos exhibit steady-state calcium currents. Moreover, to develop the vibrating probe as a non-invasive assay of the developmental potential of such embryos, we have begun to map steady state calcium flux in mouse embryos with differing developmental potential.

After hybrid matings (B6C3F1  $\times$  B6D2F1), mouse embryos were removed surgically at the two cell stage and either studied at this stage or cultured to the four- or eight-cell stages in M2 medium supplemented with 0.4% BSA at 37°C in 5% CO<sub>2</sub>. The two-cell stage embryos were washed at least twice in a modified M2 medium containing only 50  $\mu$ M calcium and then transferred in the same medium to petri dishes coated with high-molecular weight polylysine to which the embryos adhered. Embryos were then examined for a calcium flux at room temperature ( $\approx$ 22°C). Measurement of the voltages associated with steady-state transmembrane calcium flux were done as previously described (8). The calcium-sensitive electrodes, after calibration to determine their Nernstian characteristics, were positioned within about 1  $\mu$ m of an embryo's zona pellucida. The distribution of the efflux was mapped by moving the probe to at least four quadrants of the embryo's circumference. Images of the embryos were recorded with a video printer so that morphology could be correlated with steady-state calcium flux. After experimentation

embryos were routinely returned to normal M2 medium and kept at 37°C in 5% CO<sub>2</sub>, where cell division was monitored.

Morphology combined with growth allowed us to divide the two cell stage embryos into two classes:

1. Morphologically normal or capable of further cleavage.
2. Morphologically fragmented or incapable of further cleavage.

In the case of the first class of embryos, there was a strong calcium efflux signal measured in all cases. In 10 preparations this signal had an amplitude of  $-21.22 \mu$ V  $\pm$  5.7 (mean  $\pm$  standard deviation). Initial observations from the four quadrants did not exhibit any differences in the microvolts recorded. In class 2 embryos there was no measurable calcium efflux signal. Of 5 embryos examined, the signal at the plasma membrane was  $-1.75 \mu$ V  $\pm$  5.28. Background was  $2.07 \mu$ V  $\pm$  2.24.

We conclude that mouse preimplantation embryos, which retain their developmental potential (Class 1), exhibit a steady state transmembrane calcium efflux as measured by the non-invasive, vibrating calcium selective electrode. The efflux mapped in a symmetrical pattern about the embryo, with no polarity observed. Embryos with impaired developmental potential, as measured either by a fragmented morphology or subsequent failure to divide (Class 2), failed to exhibit a steady state calcium efflux equivalent to that observed in Class 1 embryos. The absence of an equivalent efflux in those embryos, which subsequently failed to grow further, suggests that the steady state calcium efflux may be a viable assay of the health of the embryo.

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