Supplementary figures and movies.

Fig. S1. Immunofluorescent staining for NOS of primary cultures (endothelial cells) derived from explants of human oviduct (ampullary region). A, C, E and G show phase images, B, D, F and H show corresponding immunofluorescence images. Cultures were approximately 24 hours old and retained beating cilia. Constitutive (eNOS, nNOS) and inducible (iNOS) isoforms were clearly present in all cells. No staining was detectable in control incubations in which the primary antibody was omitted. Fluorescence pictures were all taken with the same settings. Scale bars show 50 μm.

Fig. S2. Immunofluorescent staining for NOS of COV434 (human granulosa) cells. A, C, E and G show phase images, B, D, F and H show corresponding immunofluorescence images. Constitutive NOS isoforms (eNOS, nNOS) were clearly present in all cells but inducible NOS (iNOS), though detectable, appeared to be present at lower levels. No staining was detectable in control incubations in which the primary antibody was omitted. Fluorescence pictures were all taken with the same settings. Scale bars show 50 μm.

Fig. S3. A; Human cumulus stained with DAF (green fluorescence), demonstrating NO synthesis by all or nearly all cells. B; Phase image of mouse oocyte-cumulus, overlaid with fluorescent image from DAF staining, showing production of NOS by a proportion (≈10%) of cells. C: Phase image of oviductal explant (ampullary region), overlaid with fluorescent image from DAF staining. NO production is occurring throughout the explant. Arrows show beating cilia which are visible where they extend beyond the edge of the explant.

Fig. S4. Dose-dependence of the action of NO on $[Ca^{2+}]_i$ in human spermatozoa. Traces show the responses of 7 separate cells to sequential addition of 1 μM, 10 μM and 100 μM spermine NONOate (shown by bars above the traces. Only one cell (yellow trace) responds to 1 μM NONOate. At 10 μM many cells show a slow increase in $[Ca^{2+}]_i$ and at 100 μM the response is increased and occurs in many cells as irregular transients or oscillations. In these experiments less than 20% of cells showed no response (blue trace).

Fig. S5. Simple model to illustrate the relationship between increase in fluorescence induced by NO [Δ fluorescence (NONOate)] and fall in fluorescence caused by subsequent application of DTT [Δ fluorescence (DTT)]. Panel A shows the saturating relationship of fluorescence to $[Ca^{2+}]$, modelled using the equation y=1-(1/exp(x)) (where x represents $[Ca^{2+}]$ and y represents fluorescence). Panels B and C show two alternatives for the relationship between the rise in Ca^{2+} caused by NO (ΔCa^{2+} NONOate) and the fall in Ca^{2+} caused by DTT (ΔCa^{2+} DTT). In B the amplitude of the response to DTT is determined by the preceding action of NONOate (85% reversal of NONOate effect ± randomly generated 'noise'). In C the effect of DTT is to reduce $[Ca^{2+}]_i$ by a constant increment (± randomly generated 'noise'). Panels D and E show the changes in fluorescence that would be recorded (based upon the

relationship shown in A) in scenarios B and C respectively. The results in fig 5C (and 4 other experiments) resemble panel D.

Supplementary movie 1**.** Stimulation of flagellar activity by progesterone in the presence of NO•. Movie shows 4 cells imaged in the presence of 100 μM spermine NONOate (added approximately 8 minutes previously). Cells were imaged at 1 Hz and total length of recording is 5.1 minutes. Yellow marker (top left) shows point at which progesterone (3 μM) was added (approximately 1 minute into recording). After progesterone application the excursion of the flagellum increases, resulting in noticeable movement of the head in some of the cells. This increase in flagellar activity is prolonged (see fig 6 D), consistent with the prolonged/oscillatory effect of progesterone on $[Ca^{2+}]_i$ under these conditions (fig 6A, B).

Supplementary movie 2**.** Stimulation of flagellar activity by progesterone without pre-treatment with NO. Movie shows 5 cells, imaged at 1 Hz and total length of recording is 8.33 minutes. Yellow marker (top left) shows point at which progesterone (3 μM) was added. After progesterone application the excursion of the flagellum increases in some transiently in some cells, but the amplitude and (more particularly) the duration of this effect are brief compared to that seen in the presence of 100 μM NONOate.

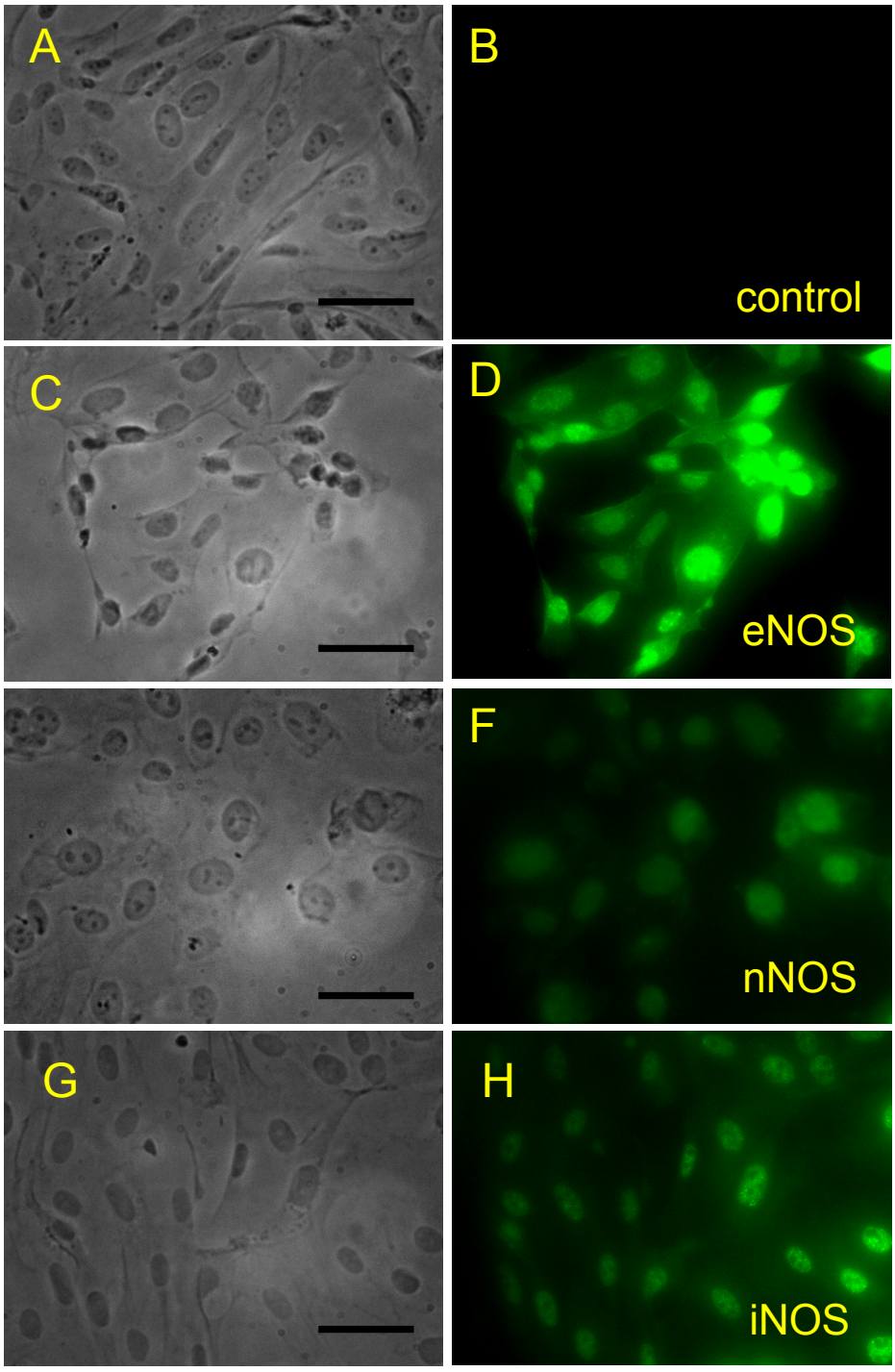

Fig S1

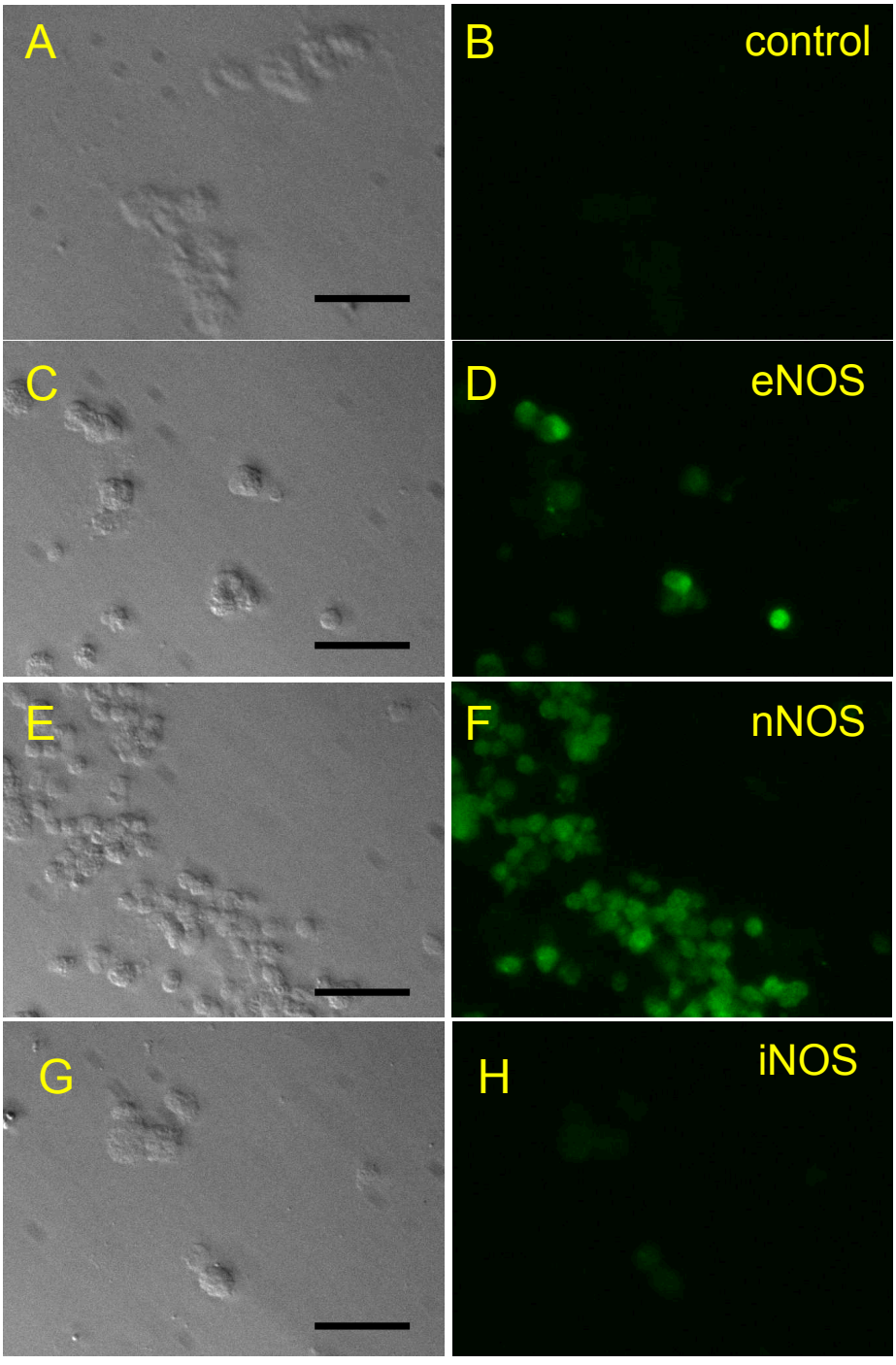

Fig S2

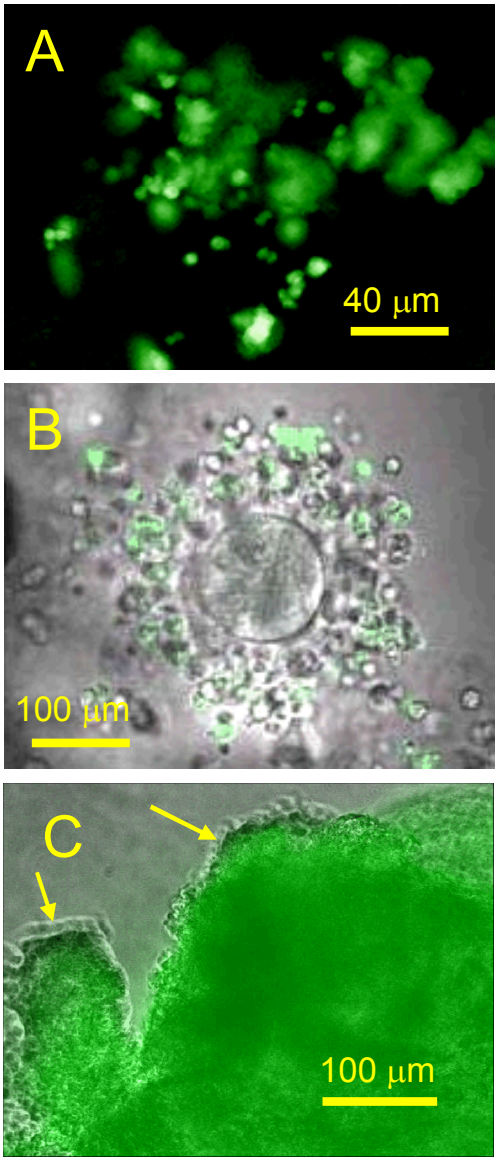

Fig S3

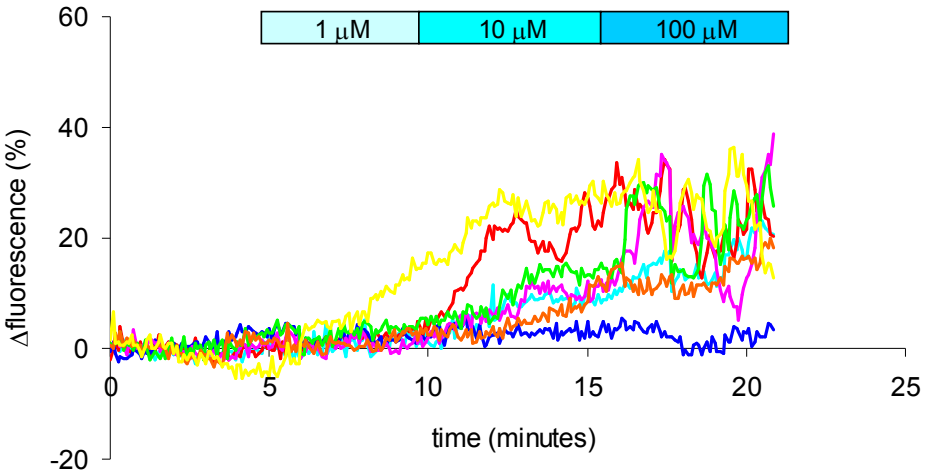
1 μM
10 μM
100 μM
60
40
20
0
-20
Δfluorescence (%)
0
5
10
15
20
25
time (minutes)

Fig S4

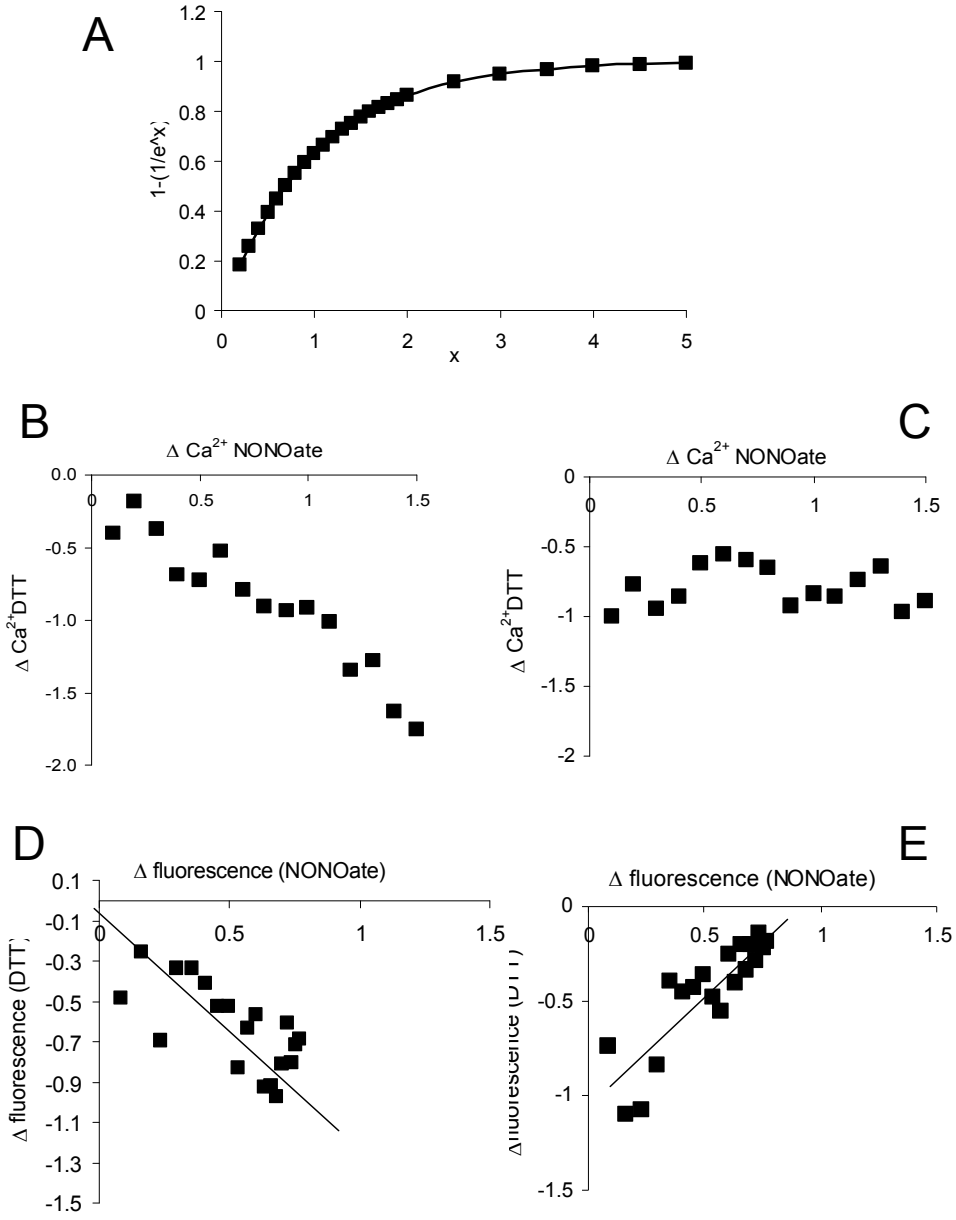
A
1-(1/e^x)
x
B
Δ Ca2+ NONOate
Δ Ca2+DTT
C
Δ Ca2+ NONOate
Δ Ca2+DTT
D
Δ fluorescence (NONOate)
Δ fluorescence (DTT)
E
Δ fluorescence (NONOate)

Fig S5