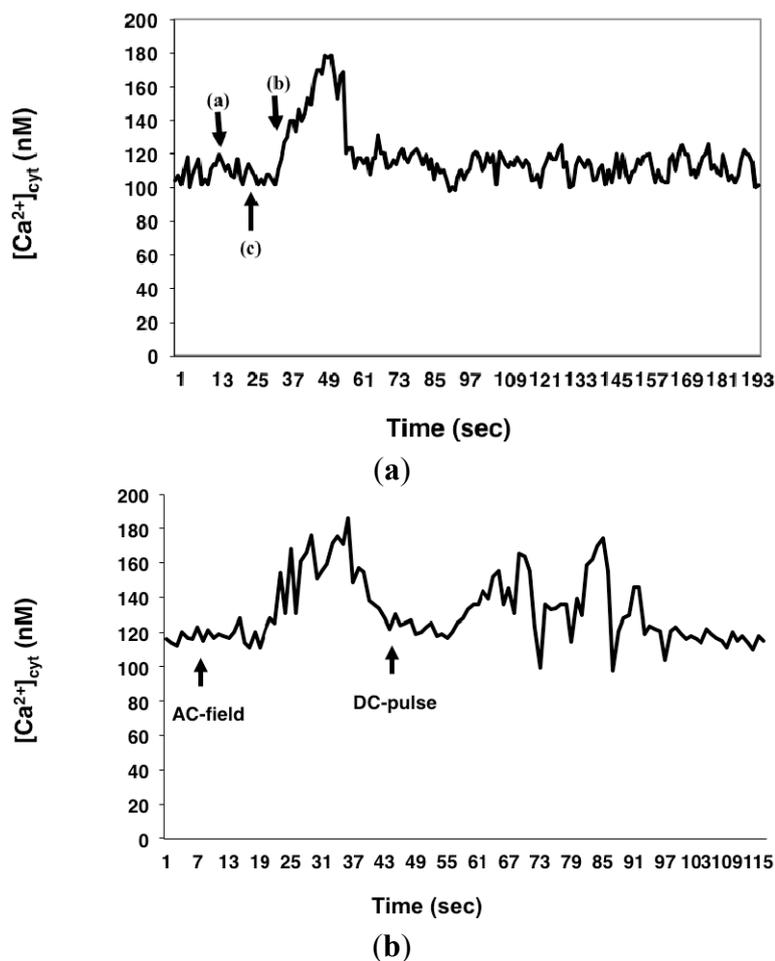


Supplementary Information

In accordance with the criterion of insuring that the observed $[Ca^{2+}]_{cyt}$ changes possess biological relevance indeed, a series of control experiments was performed including injection of the injection buffer into unfertilised wheat eggs cells ($n = 18$) injected previously with fura-2 dextran, during which $[Ca^{2+}]_{cyt}$ was monitored at each phase of the injection such as: impaling the cell membrane by the microcapillary, injection and withdrawing the capillary (Figure S1a).

Figure S1. Time-course of the $[Ca^{2+}]_{cyt}$ transient produced by the microinjection- and electro-fusion procedures. **(a)** Representative $[Ca^{2+}]_{cyt}$ measurement of a microinjected wheat egg cell incubated in Ca^{2+} -free IVF medium. Arrows “a”, “b” and “c” indicate the time of microneedle impalement, injection and needle retraction, respectively; **(b)** Control experiment of the electrofusion procedure. Egg cells transferred into droplets of calcium-containing IVF medium (following their injection with fura-2 dextran in calcium-free IVF medium), were exposed to A.C. field and D.C. pulses with the same parameters used during sperm-egg fusion.

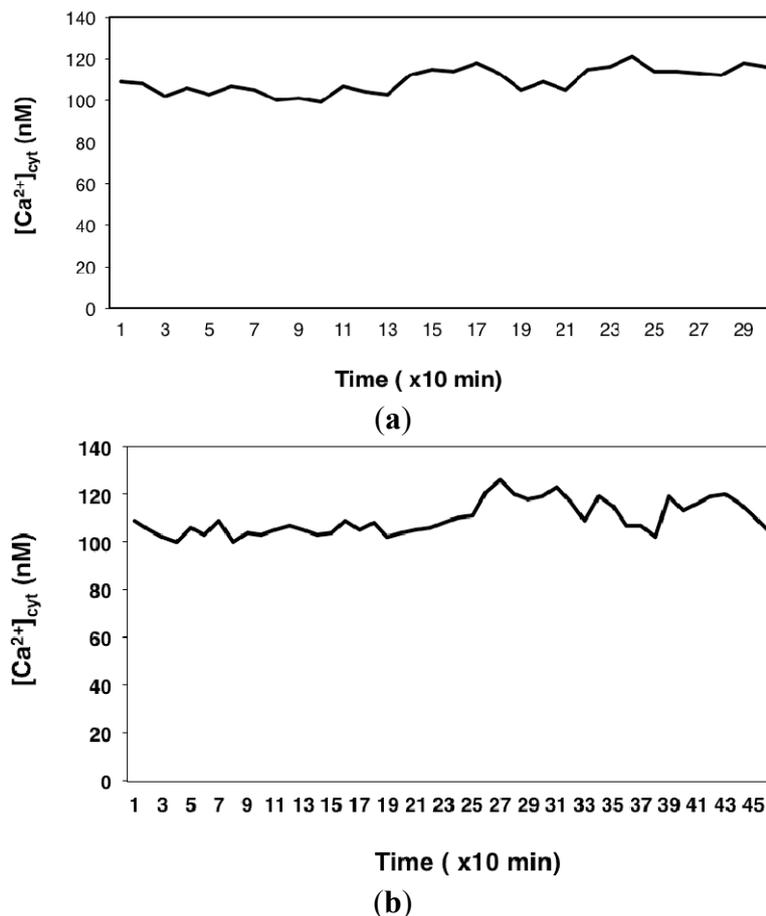


As a control experiment for the electrofusion procedure, unfertilised egg cells were exposed to the same A.C.-(alternating current) field strength, which was routinely applied during electrofusion (for cell alignment and cell-cell membrane contract), and to the D.C.-(direct current) pulse used to trigger cell fusion, and $[Ca^{2+}]_{cyt}$ was monitored. As Figure S1b demonstrates, neither the A.C. nor the D.C. pulse caused $[Ca^{2+}]_{cyt}$ changes differing significantly from the basal level. Change in the $[Ca^{2+}]_{cyt}$

level did not exceed 186 ± 18 nM in any of the cells examined ($n = 15$). Figure S1a,b illustrate that neither the microinjection procedure performed in egg cells nor electrofusion caused elevation in $[Ca^{2+}]_{cyt}$ resembling to those observed during sperm-egg fusion. Thus, it could be concluded that the establishment of plasmogamy and the subsequent incorporation of the male gamete is the trigger for $[Ca^{2+}]_{cyt}$ elevation in the egg cytoplasm.

As Figure S2a,b depict, the characteristic pattern of $[Ca^{2+}]_{cyt}$ changes observed in egg cells fused with the sperm was never seen when $[Ca^{2+}]_{cyt}$ was measured in the course of incubating unfertilised egg cells in IVF medium with or without extracellular calcium. Throughout the recording period no spontaneous increase in $[Ca^{2+}]_{cyt}$ was found to occur in any of the egg cells incubated in IVF medium with or without calcium prior to sperm-egg cell fusion (number of cells analysed was 55 and 49, respectively) (Figure S2a,b).

Figure S2. Representative $[Ca^{2+}]_{cyt}$ measurement with fura-2 dextran in wheat egg cells incubated in IVF medium without (a) and with (b) $CaCl_2$.



Addition of calcium to Ca^{2+} -free IVF medium in which the female gametoplasts were incubated, caused only a slight (132 ± 19 nM, $n = 17$) elevation in the level of $[Ca^{2+}]_{cyt}$ (Figure S3). As Ca^{2+} indicators such as fura-2 are thought to bind to cellular proteins, which can markedly alter the indicator's response to Ca^{2+} , we used the dextran conjugate of the ion indicator as an additional control. The spreading of dextran-conjugate in the cell's cytoplasm was examined by injecting fluorescein-dextran into wheat egg cells using identical set of parameters for the injection procedure

that was employed for introducing fura-2 dextran into the cells to be image-analysed. As Figure S4 demonstrates, after 1 h following injection a homogenous diffusion of the dye was seen throughout the whole cytoplasm and no visible compartmentalisation of fluorescein-dextran into organelles or vesicles occurred. Fluorescein diacetate (FDA)-test according to Heslop-Harrison and Heslop-Harrison [40] was performed to check the viability of the fura-2 dextran-injected, *in vitro* fertilised egg protoplasts. Figure S5a shows that following microinjection and electrofusion the microinjected cells to be subsequently ratio-imaged were viable as demonstrated by the positive membrane esterase activity. Further, the potential impact on the continuation of zygote development of buffering free Ca^{2+} by fura-2 dextran was studied. During these experiments, egg cells injected with fura-2 dextran prior to sperm cell fusion were individually cultured and monitored following IVF and their development was compared with that of non-injected control cells. As Figure S5a–c demonstrate the Ca^{2+} -buffering effect of fura-2 did not cause “disturbance” in intracellular calcium homeostasis or in the cellular characteristics of the female gametes to the extent that would impair the ability of the fertilised egg cells to continue further development. The cells retained their capability of cell wall regeneration, the first sign of which could be detected 40 min following sperm-egg fusion (Figure S5b,c). Further, the potentially cytotoxic effect of the high energy UV light employed for exciting the cells during $[\text{Ca}^{2+}]_{\text{cyt}}$ measurements on zygote development was studied by checking if the fertilised (and imaged) egg cells could maintain normal development, the sign of which were deemed to be cell cleavage and nuclear division of the fusion products (Figure S6a,b). As it is shown in Figures S5–S7, the *in vitro* fertilised and $[\text{Ca}^{2+}]_{\text{cyt}}$ -imaged wheat egg cells recovered from the experimental intrusion and were able to follow their developmental fate the apparent signs of which were their capability of cell division and further development under *in vitro* culture conditions (Figure S7a–c).

Figure S3. The effect of addition of extracellular calcium on the resting level of $[\text{Ca}^{2+}]_{\text{cyt}}$ measured in egg cells incubated in calcium-free IVF medium. Arrow shows when Ca^{2+} was added to the microdroplet containing the egg cell.

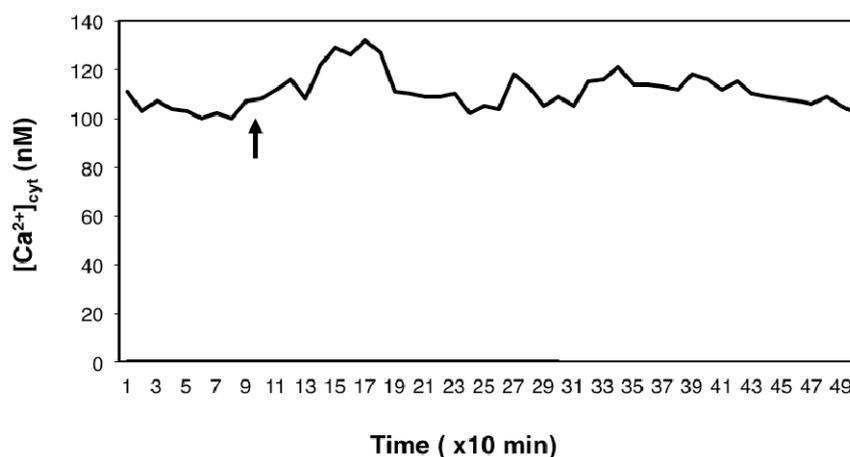


Figure S4. Distribution pattern of fluorescein-dextran injected into a wheat egg cell. Fluorescent image taken of an egg cell injected with fluorescein-dextran and incubated in IVF medium for 1 h following injection. Scale bar: 25 μm .

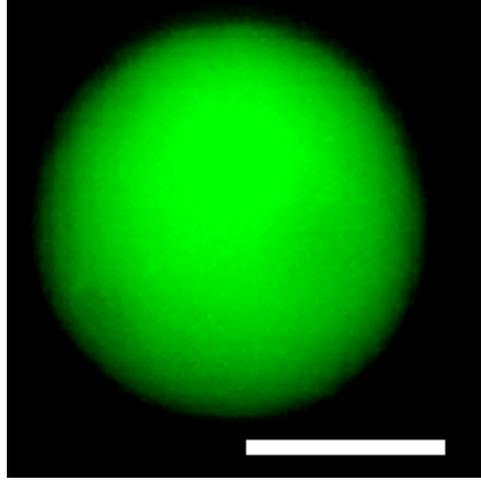


Figure S5. Demonstration of the capability of *in vitro* fused egg cells injected with fura-2 dextran to continue further development. (a) Viability test performed on a wheat egg cell. Following microinjection and *in vitro* fusion, the egg cell was incubated in 0.4% FDA dye solution for 30 min. Fluorescence was measured at 490–515 nm. Scale bar: 25 μm ; (b) Overlay image gained from cell wall staining in viable egg cells used for sperm-egg fusion and $[\text{Ca}^{2+}]_{\text{cyt}}$ imaging. The blue patch on the cell surface in the merged image demonstrates the commencement of cell wall synthesis as revealed by the fluorescent dye, calcofluor white. Green fluorescence derives from FDA. The image was taken 40 min after completion of sperm-egg fusion. Scale bar: 20 μm ; (c) Completion of cell wall synthesis in *in vitro* fertilised egg cells. Blue fluorescence reveals the cell wall around the entire egg cell used for IVF and imaging $[\text{Ca}^{2+}]_{\text{cyt}}$. Micrograph was taken 2 h after sperm incorporation into the egg cytoplasm. Scale bar: 15 μm . Note that cell wall staining revealed a strongly-staining patch on the cell surface, which is shown by the arrow.

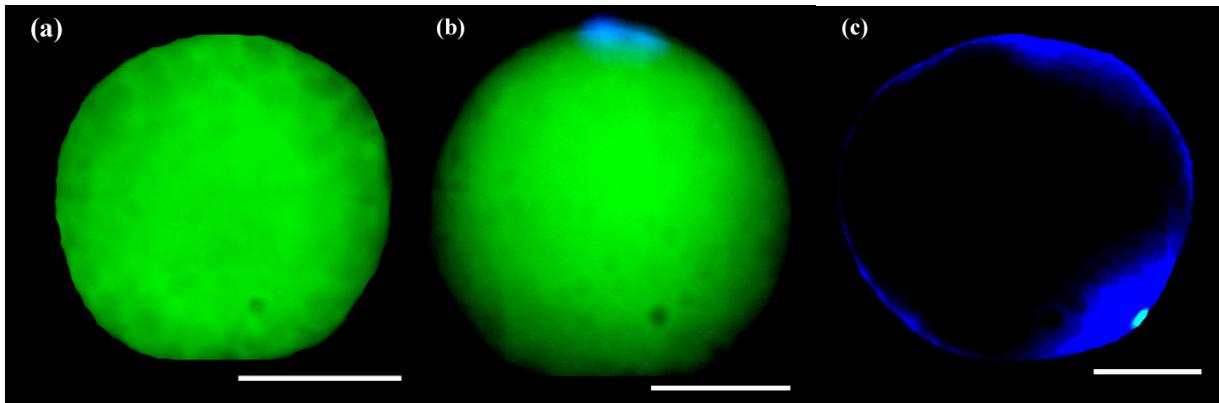


Figure S6. Fusion product completing cellular and nuclear division. (a) Light microscope image taken of an egg cell deriving from a batch of female gametes used for IVF and $[Ca^{2+}]_{cyt}$ imaging. The image was taken after 28 h *in vitro* culture following IVF. Scale bar: 40 μm ; (b) Fluorescence micrograph showing the nuclei of the daughter cells stained with SYTO (16)-Green nucleic acid stain. Scale bar: 15 μm .

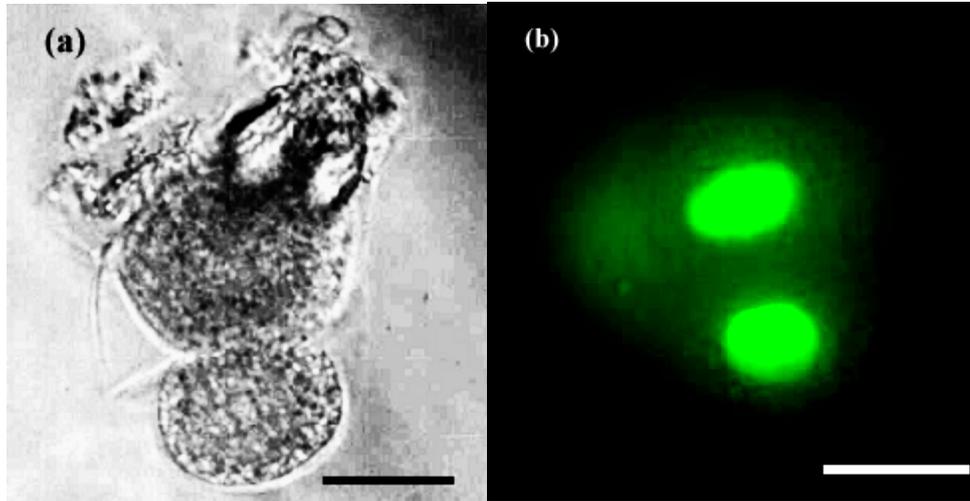
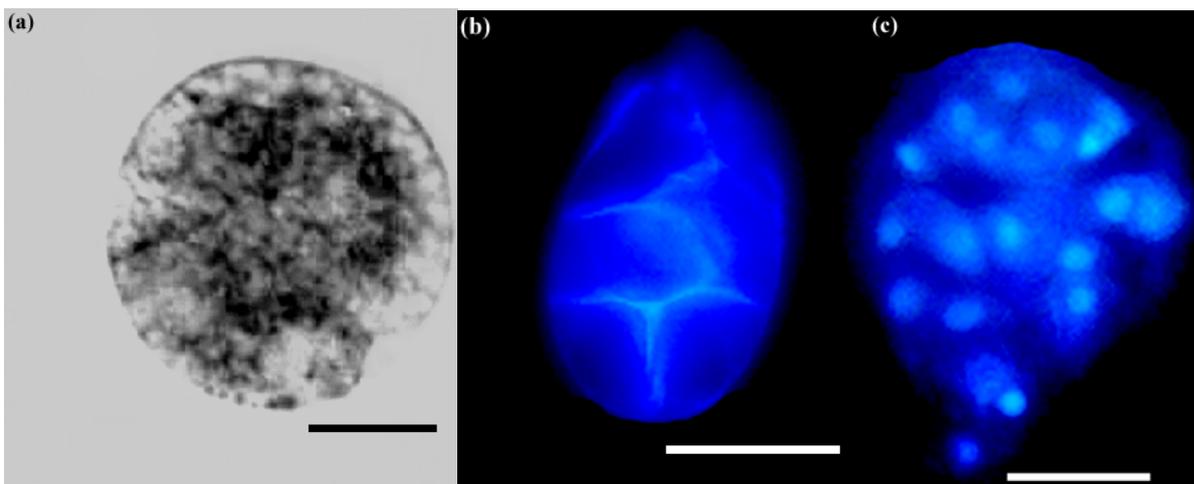


Figure S7. Developing structure deriving from an *in vitro* fertilised and calcium-imaged egg cell. (a) *In vitro* produced zygote photographed after 72 h in culture. Scale bar: 30 μm ; (b) Cell wall staining as revealed by the CW (Calcofluor White) dye in a multicellular structure developed from an egg cell used for $[Ca^{2+}]_{cyt}$ imaging after it had been fused *in vitro* with a sperm cell and immobilised on thin alginate layer. Image was taken on the 7th day of *in vitro* culture. Scale bar: 50 μm ; (c) Epifluorescent image showing the nuclei of an embryo-like structure developed from an *in vitro* fertilised egg cell used for $[Ca^{2+}]_{cyt}$ measurement and stained with DAPI after 12 days of *in vitro* culture. Scale bar: 80 μm .



Experimental Section

1. Nuclear-Staining with SYTO Green/DAPI

In order to verify that nuclear division occurred in the fusion products, they were stained with the fluorescent DNA stain Syto 16 (Molecular Probes, Eugene, OR, USA) added to the fusion medium at a final concentration of 1 μM [45] or with 100 $\mu\text{g}/\text{mL}$ DAPI-solution purchased from Sigma–Aldrich (St. Louis, MO, USA) prepared in 600 mosmol/kg mannitol-Triton X-100 (0.001% v/v) solution pH 4.0 for 5 or 30 min, respectively, following washing them in the same solution without DAPI (and Triton X-100) 2 times for 10 min each.

2. FDA Test

Fluorescein-dextran ($M_r = 10,000$) was purchased from Molecular Probes (Eugene, OR, USA) and injected under the same injection conditions and parameters that were applied when fura-2 dextran injections were performed.

FDA-test was performed according to Heslop-Harrison and Heslop-Harrison [40] to check the viability of the egg cells following injection and *in vitro* fusion. Essentially, 2% acetone solution of FDA was added at the incubation (IVF) medium to reach 0.4% final concentration of the dye. The staining was carried out at room temperature for 30 min. The fluorescence was measured at 490–515 nm.

3. Visualization of Thapsigargin-Sensitive Ca^{2+} Pumps in the Wheat Egg

BODIPY FL[®] thapsigargin was obtained from Molecular Probes (Eugene, OR, USA). For localising thapsigargin-sensitive Ca^{2+} pumps, egg protoplasts were incubated for 5 min with BODIPY FL thapsigargin diluted from a stock (1 mg/mL) solution dissolved in DMSO to reach the concentration of 1 μM in the fusion medium. The cells were washed before images were acquired through a 40 \times lens and FITC filter.