23 Nuclear and cytoskeletal analysis of southern white rhinoceros (Ceratotherium simum simum) arrested presumptive zygotes following intracytoplasmic sperm injection

E. Ruggeri A , C. Young A , N. Ravida A and B. Durrant A
+ Author Affiliations

Reproduction, Fertility and Development 35(2) 137-137 https://doi.org/10.1071/RDv35n2Ab23
Published: 5 December 2022

Oocyte chromosome segregation and embryo development are guided by intensive actin-microtubule interactions and remodelling. Analysis of spatial and temporal configurations of nuclear and cytoskeletal structures provide a method to assess zygote development. With a conservation urgency to develop successful reproductive technologies in the southern white rhinoceros (SWR) as a model for the nearly extinct northern white rhinoceros (NWR), it is imperative to craft a proper in vitro culture system to produce SWR embryos. If fertilisation fails, it is necessary to understand the causes of arrest. Our goal was to investigate the causes of developmental arrest in presumptive zygotes generated by intracytoplasmic sperm injection (ICSI) in the SWR. Because cytoskeletal and nuclear modifications postfertilisation have not been described, and limited information is available on timing of events post-ICSI in this species, the aims of our study were: (1) to develop an immunostaining protocol to visualise α/β-tubulin and F-Actin cytoskeleton in this species, and (2) to describe abnormal cytoskeletal and nuclear configurations associated with failure of fertilisation after ICSI. Oocytes were retrieved via ovum pickup and matured in vitro in M199 or DMEMF12 media for 36–38 h. ICSI was performed using frozen-thawed sperm from a proven bull. At 72 h after ICSI, injected SWR oocytes that arrested were fixed in MTSB-XF and washed with a solution including 1% bovine serum albumin (BSA) and 0.1% Triton X-100. The oocytes were stained with monoclonal mouse anti-α/β-tubulin, followed by incubation with a secondary antibody Alexa 488, phalloidin, and Hoechst 33258. Finally, the oocytes were mounted for imaging. Eight arrested presumptive zygotes were analysed. Images were acquired with a Keyence BZ-X Series All-in-One fluorescence microscope (Keyence Corporation). We collected measurements and data using ImageJ software (National Institutes of Health). The results are expressed as average ± standard deviation as a.u. (arbitrary units). Several categories of abnormal morphologies were evaluated and associated with each of the arrested zygotes. Of these categories, the most recurrent were condensed sperm head and intact tail in 4/8 (50%); large maternal chromosome mass in prometaphase in 3/8 (37%); karyomeres in 3/8 (37%); tubulin filament network in 2/8 (25%); and maternal chromatin undergoing decondensation in 2/8 (25%). Presumptive zygote actin filament total intensity averaged 440,772 ± 193,464 a.u. and actin vesicles were observed in 2/8 (25%) of the arrested zygotes. Premature sperm chromosome condensation was only observed in 1/8 (12%), and spindle chromatin-induced PB extrusion was present in 1/8 (12%) arrested zygotes. Overall, the majority of the presumptive zygotes arrested due to sperm decondensation failure, therefore, sperm activation will be evaluated in the future. Maternal chromatin appeared to be arrested at prometaphase or failing proper chromosome migration, leading to incomplete female pronuclear reconstitution. This is the first report to investigate causes of zygote arrest in the SWR.