Vitrification of mammalian oocytes is an indispensable approach for assisted reproductive technologies. However, the underlying mechanisms responsible for low developmental competence of vitrified germinal vesicle (GV) oocytes remain unknown. We show here that vitrified oocytes had a significant increase of chromosomal misalignment and abnormal spindle either at Metaphase (Met) I or II. Using monastrol assay, vitrified GV oocytes showed an increased incidence of aneuploidy at Met II. The high incidence of aneuploidy suggests that Spindle Assembly Checkpoint (SAC) is perturbed in vitrified oocytes. To investigate our hypothesis, we conducted nocodazole (NOC) and DNA damage assays. In contrast to control oocytes, vitrified oocytes extruded first polar bodies (PBs) in the presence of NOC or etoposide which are known to induce SAC activation. We then examined the localization of Mad2, an essential regulator of SAC signaling. Under NOC-induced Met I arrest status, in contrast to Mad2 localization at kinetochores in control oocytes, vitrified oocytes showed diffused localization across chromosomal arms. These results indicate that SAC is no longer functional in vitrified oocytes. Importantly, we found that vitrification stimulated the activities of cathepsin B and caspase 3 which are the putative pathways of impaired SAC function. This hypothesis was confirmed by restoring Mad2 localization and SAC function in vitrified oocytes treated with E-64, a cathepsin B inhibitor. These results provide the first demonstration that vitrification perturbs faithful chromosome segregation by impairing SAC function through activation of cathepsin B in mouse oocytes.