One of the first major milestones in early mammalian development is blastocyst formation and yet, less than half of pre-implantation embryos from most mammals will reach this stage following \textit{in vitro} fertilization (IVF). A leading cause of IVF failure and embryo loss in humans is the presence of whole chromosomal imbalances, or aneuploidy. Although more likely to arrest at the cleavage-stage, aneuploid embryos may still form blastocysts and often morphologically indistinguishable from chromosomally normal (euploid) embryos. Chromosomal mis-segregation in oocytes during meiosis is considered the primary reason for aneuploidy in cases of advanced maternal age. However, mosaic aneuploidies, which are mitotically derived, occur just as frequently and irrespective of maternal age. The potential cause(s) of mitotic aneuploidy, whether it can be non-invasively detected, and if pre-implantation embryos from other mammalian species are also chromosomally unstable was the focus of this study. Using a combination of time-lapse imaging to monitor embryo development, immunofluorescent analysis of nuclear structure, and next generation RNA-Sequencing (RNA-Seq), we assessed mitotic divisions in rhesus macaque (N=54), equine (N=11), bovine (N=48), and mouse embryos (N=45) up to the blastocyst stage. While similar mitotic timing was observed between rhesus, equine and bovine embryos, mouse embryos exhibited a particularly long second division of 19.8±3.2 hours (p<0.0001), which is likely due to species-specific differences in the onset of embryonic genome activation. Upon immunostaining with the nuclear envelope marker, LAMIN-B1, only intact primary nuclei were detected in cleavage-stage mouse embryos, whereas chromosome-containing micronuclei were observed in rhesus, equine, and bovine embryos in order of decreasing frequency. Besides persisting up to the blastocyst stage, these micronuclei were often positive for gamma-H2AX, a marker of DNA breaks and chromosome fragility. RNA-Seq analysis of rhesus, equine, and bovine blastocysts showed high expression of ribosomal proteins, cytochrome oxidases, and housekeeping genes as somewhat expected. Interestingly, exceedingly high expression levels (RPKM≥2,400) of \textit{ANXINN A2} (ANXA2), a gene known to be involved in diverse cellular processes, was detected in blastocysts of all three species. Given recent findings that ANXA2 participates in cytokinesis and is associated with chromosomal instability in other cell systems, its abundant and conserved expression may be important for maintaining embryo ploidy status during pre-implantation development across higher-order mammalian species.

\textbf{Keywords:} Aneuploidy, imaging, micronuclei, mitosis, sequencing