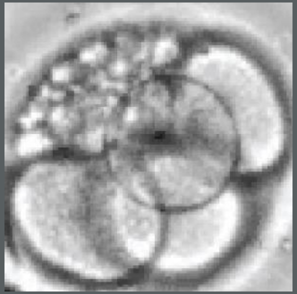
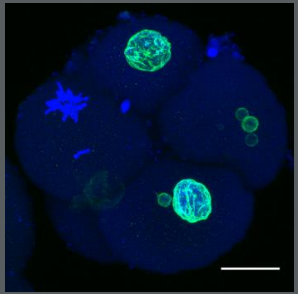
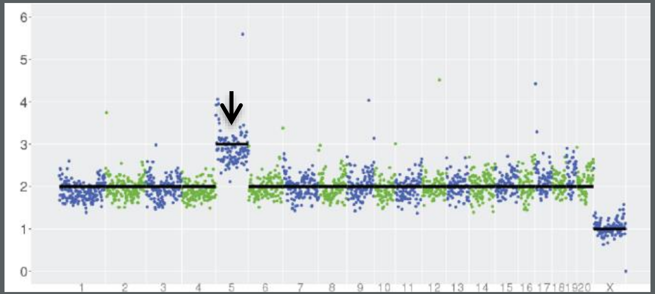
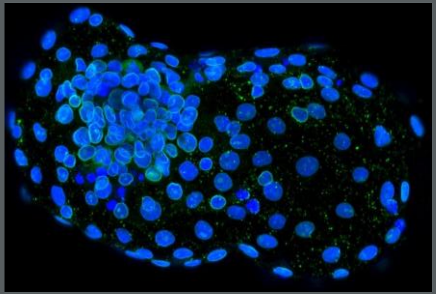
Session IV: Cross-Species Comparison of Preimplantation Chromosomal Instability









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Workshop on Mammalian Embryo Model Systems 1-17-20

The use of human IVF is increasing, but still only ~30-35% successful

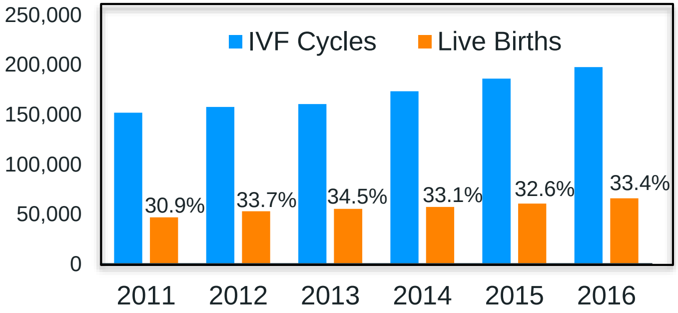
- Since the advent of human IVF **over 40 years ago**, IVF success as measured by live birth(s) has **not increased beyond 30-35%**.

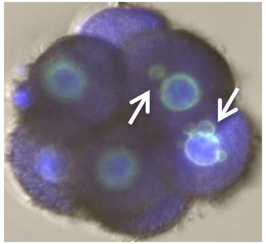
  (http://www.sart.org; cdc.gov/art)

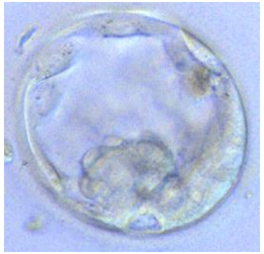
- A leading cause of IVF failure and embryo loss is the presence of **aneuploidy**, which effects **~50-80%** of cleavage-stage human embryos.

  (Vanneste *et al*., 2009; Johnson *et al*., 2010; Chavez *et al*., 2012; Chow *et al*., 2014; Huang *et al*., 2015; McCoy *et al*., 2015)

- While many will arrest (**~50% or more**), aneuploid embryos can still form blastocysts and may be **morphologically indistinguishable** from chromosomally normal (euploid) embryos.





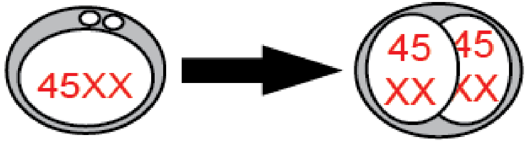


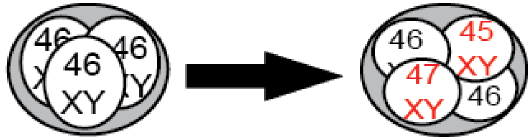
Both meiotic and mitotic errors contribute to aneuploidy

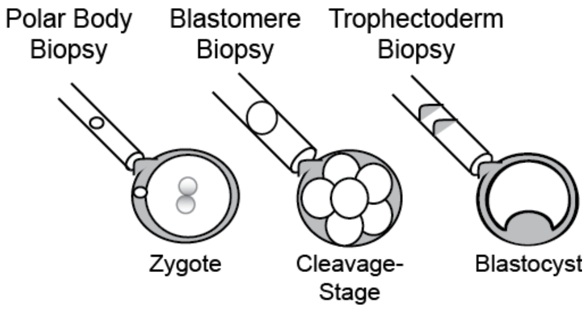
- Until relatively recently, chromosomal mis-segregation in oocytes during meiosis was considered the primary reason for aneuploidy, <u>especially in cases of advanced maternal age</u> **(~90% of meiotic errors)**.

- Chromosomal mis-segregation during mitosis occurs at an **equal or greater frequency** than meiotic errors and <u>irrespective of maternal age</u>.

- <u>Preimplantation genetic testing for aneuploidy (PGT-A)</u>: detects only **meiotic** errors, is **invasive**, suffers from **mosaicism**, and/or may require **extended culture**.



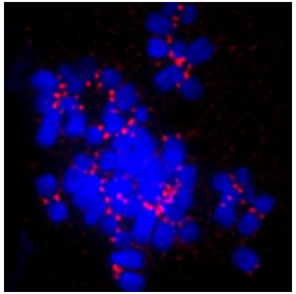


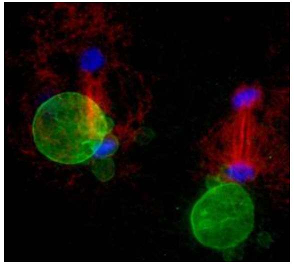


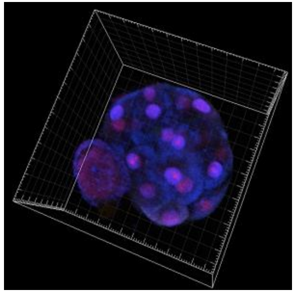
Aneuploidy frequency in <u>early</u> cleavage-stage embryos across mammals

Mammalian Species		Aneuploidy Frequency	Reference(s)
▪ Human		~50-80%	Vanneste *et al*., 2009; Johnson *et al*., 2010; Chavez *et al*., 2012; Chow *et al*., 2014; Huang *et al*., 2015; McCoy *et al*., 2015
▪ Rhesus		~74%	Daughtry *et al*., 2019
▪ Cow		~32-85%* (**in vitro* matured eggs)	Destouni *et al*., 2016; Hornak *et al*., 2016; Tsuiko *et al*., 2017, Brooks *et al*., submitted
▪ Horse		?%* (**in vitro* matured eggs)	
▪ Mouse		~1-4%	Lightfoot *et al*., 2006; Macaulay *et al*., 2015; Treff *et al*., 2016; Bolton *et al*., 2016

Remaining questions regarding aneuploidy in preimplantation embryos





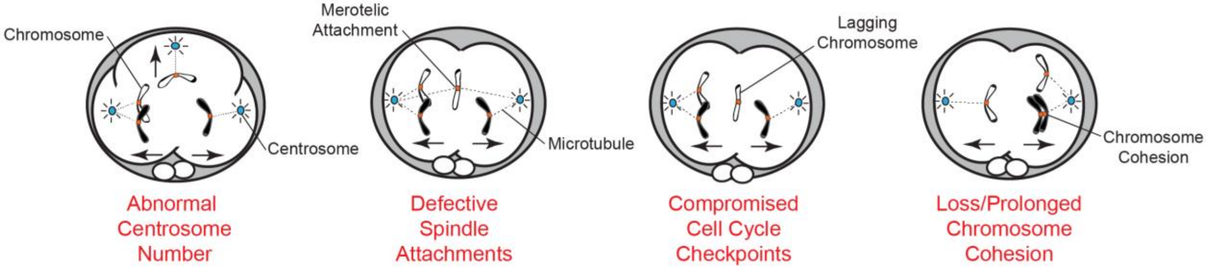


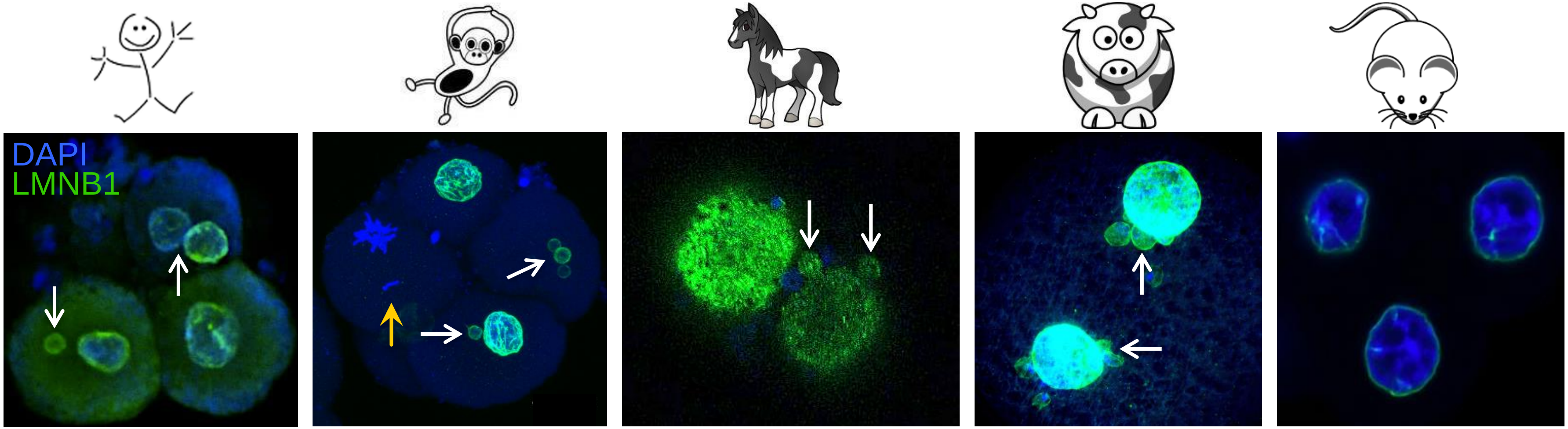
- Why is there such a high incidence of aneuploidy in preimplantation embryos?
  (underlying mechanisms)

- Can live-cell imaging with or without labeled markers be used to assess embryo developmental potential?
  (nuclear and cytoskeletal structure)

- Are there corrective means to overcome aneuploidy generation during preimplantation development?
  (naturally or therapeutically)

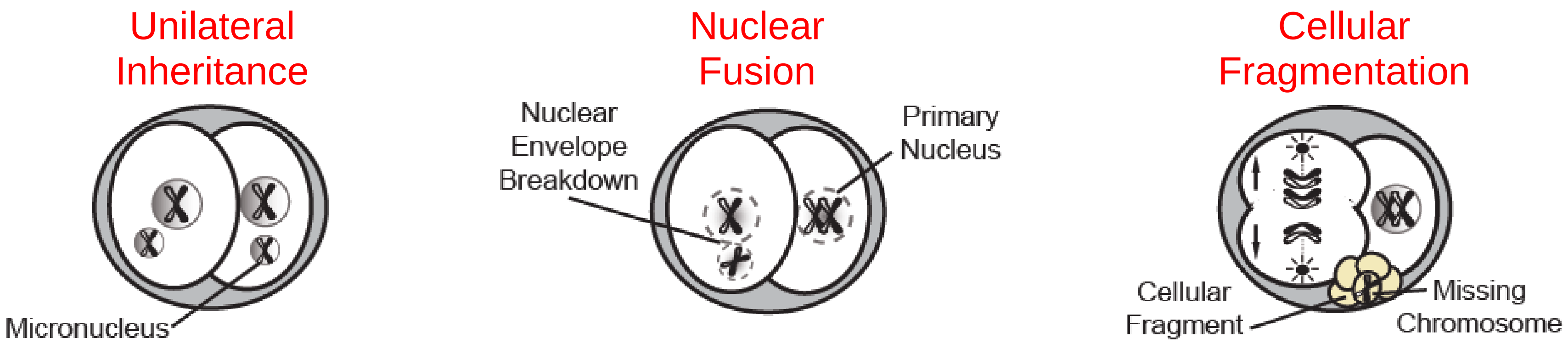
Mechanisms of mitotic mis-segregation and sequestering by micronuclei





Chavez *et al*., 2012 | Daughtry *et al*., 2019 | Brooks *et al*., 2019 | Brooks *et al*., submitted | Chavez *et al*., 2012

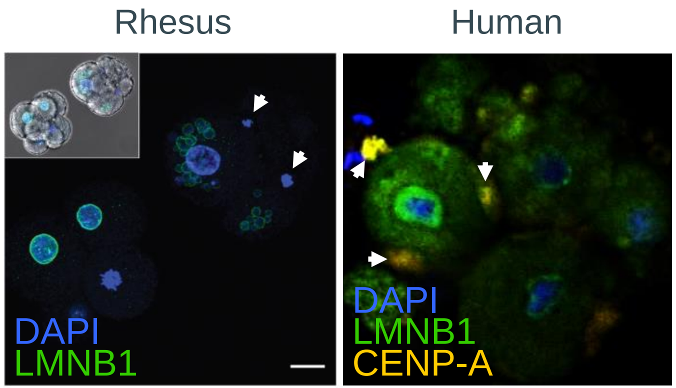
Potential fate of chromosome-containing micronuclei



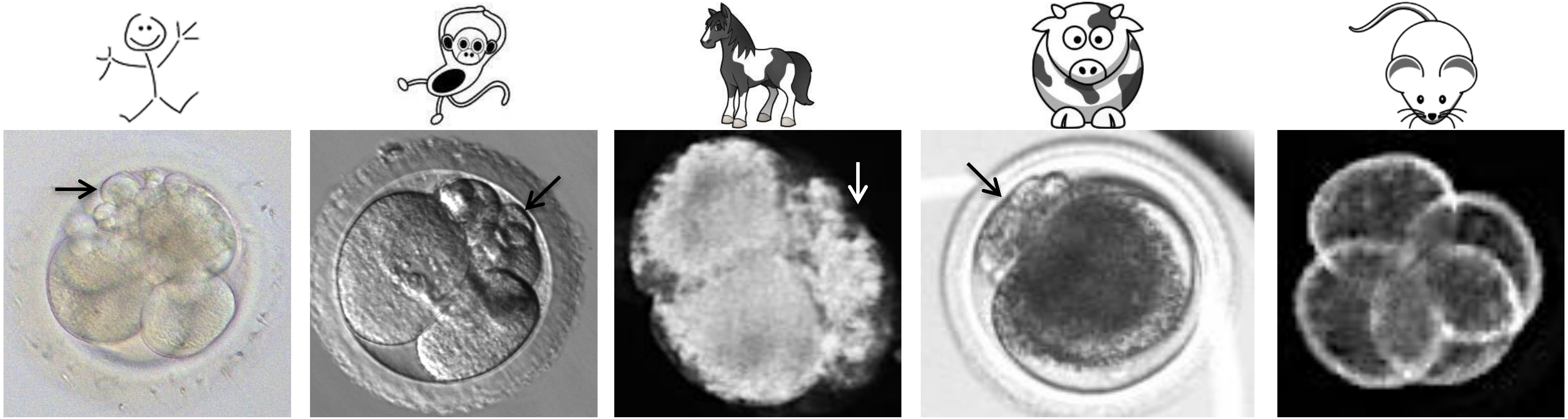
- Bovine embryos (early cleavage)
- Mouse embryos (morula stage)
- Cancer cells

- Bovine embryos (early cleavage)
- Cancer cells

(Krasta *et al*., 2012; Chavez *et al*., 2012; Zhang *et al*., 2015; Vázquez-Diez *et al*., 2016; Liu *et al*., 2018; Daughtry *et al*., 2019; Brooks *et al*., submitted)



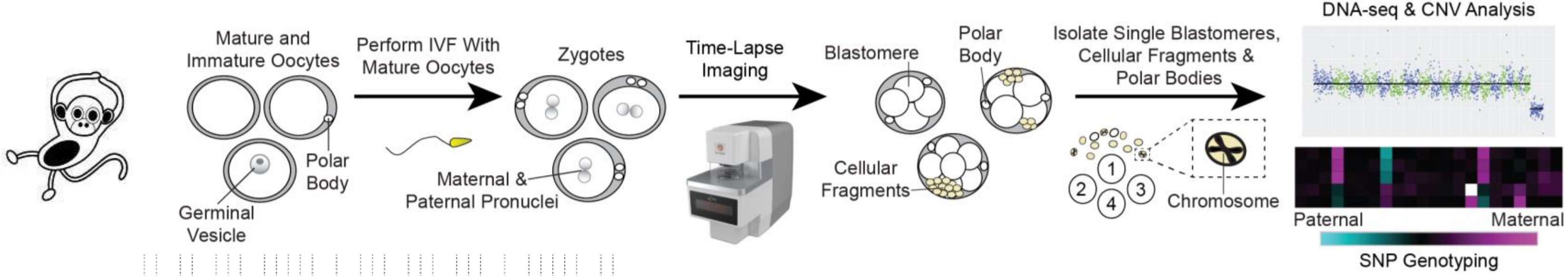
Micronuclei formation is often associated with cellular fragmentation

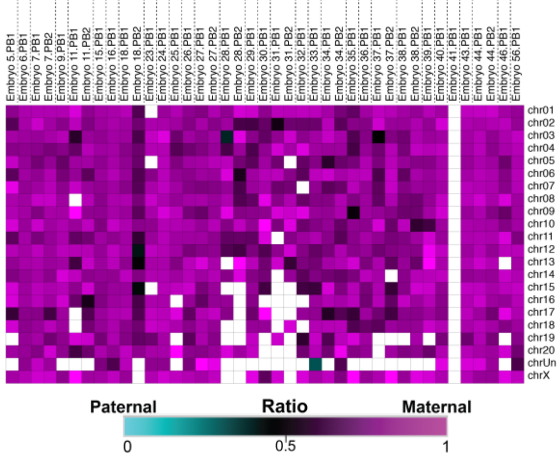


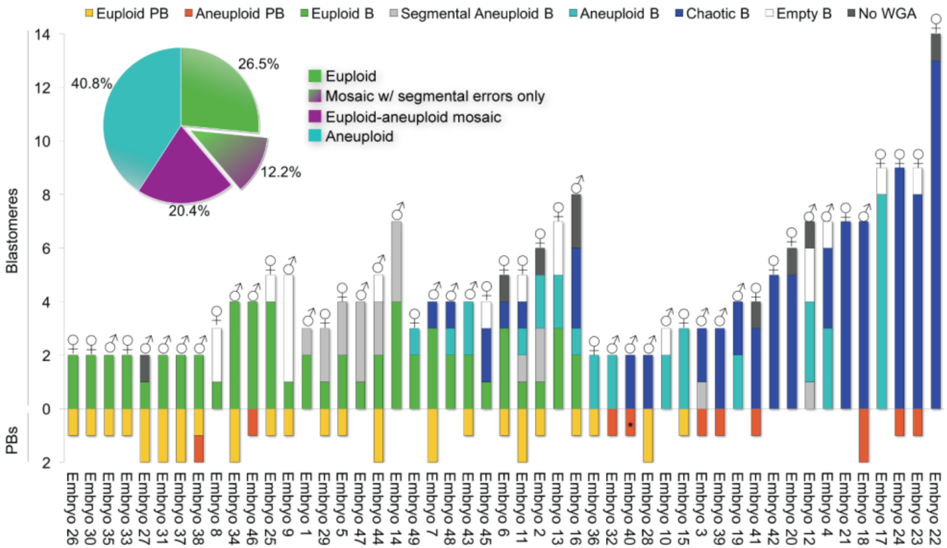
- Observed in ~50% or more of primate and equine embryos (15-20% of bovine embryos)
- Distinct from DNA fragmentation following cell death late in preimplantation development
- Evidence from multiple mammalian species, including human, that it occurs *in vivo*
- Fragmented human embryos often exhibit chromosome loss from blastomeres

(Pereda and Croxatto, 1978; Buster *et al*., 1985; Dozortsev *et al*., 1998; Antczak and Van Blerkom, 1999; Alikani, M. *et al*., 1999; Viuff *et al*., 2001; Hardy *et al*., 2001; Rambags *et al*., 2005; Chavez *et al*., 2012)

Assessment of aneuploidy and chromosome loss via fragmentation in rhesus embryos

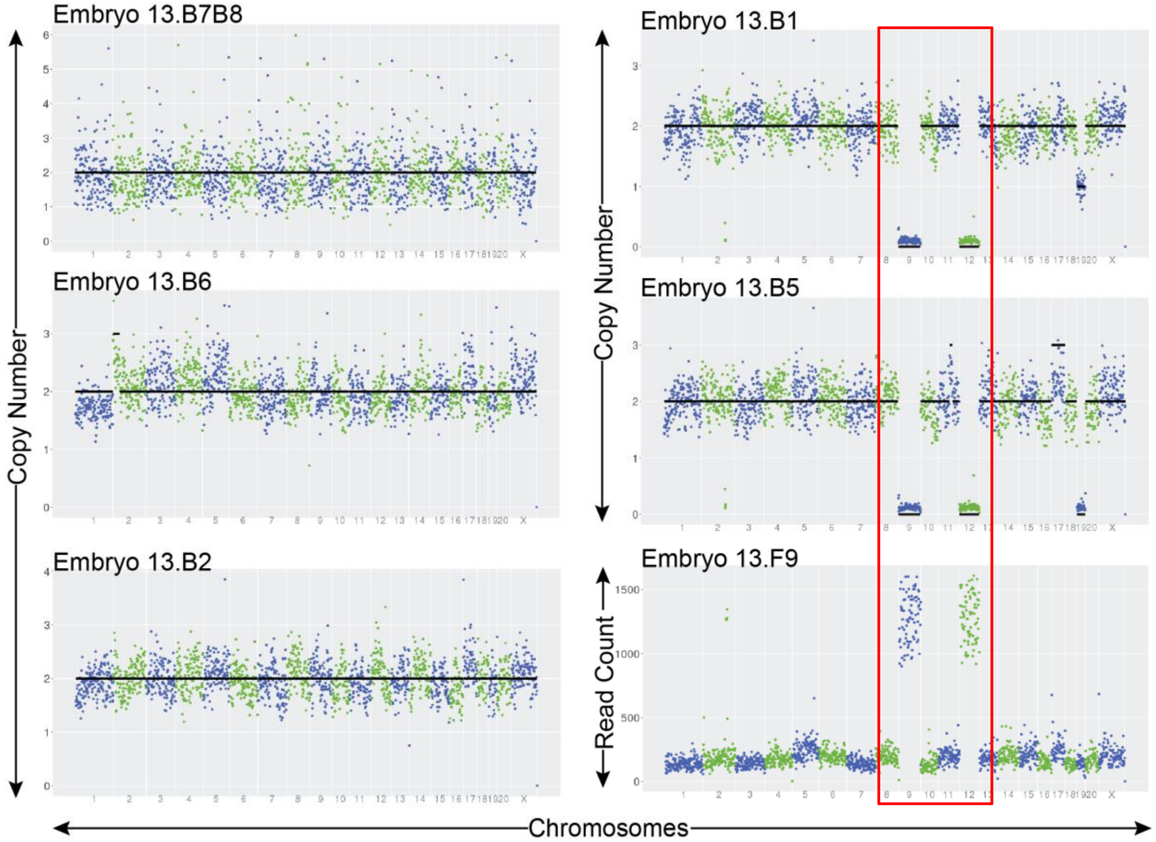


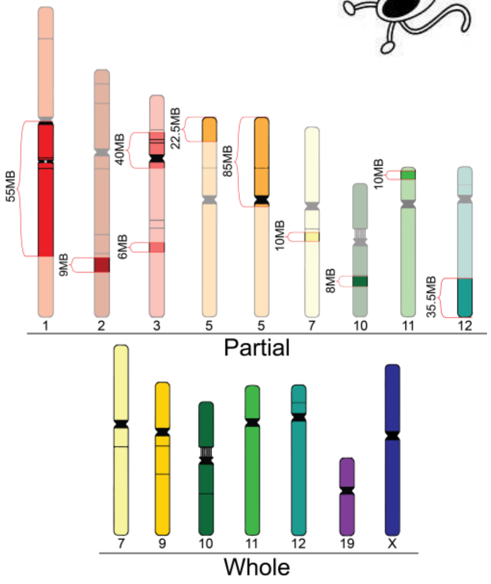




(N=50 embryos comprising 254 blastomeres; 42 polar bodies; 175 cellular fragments)

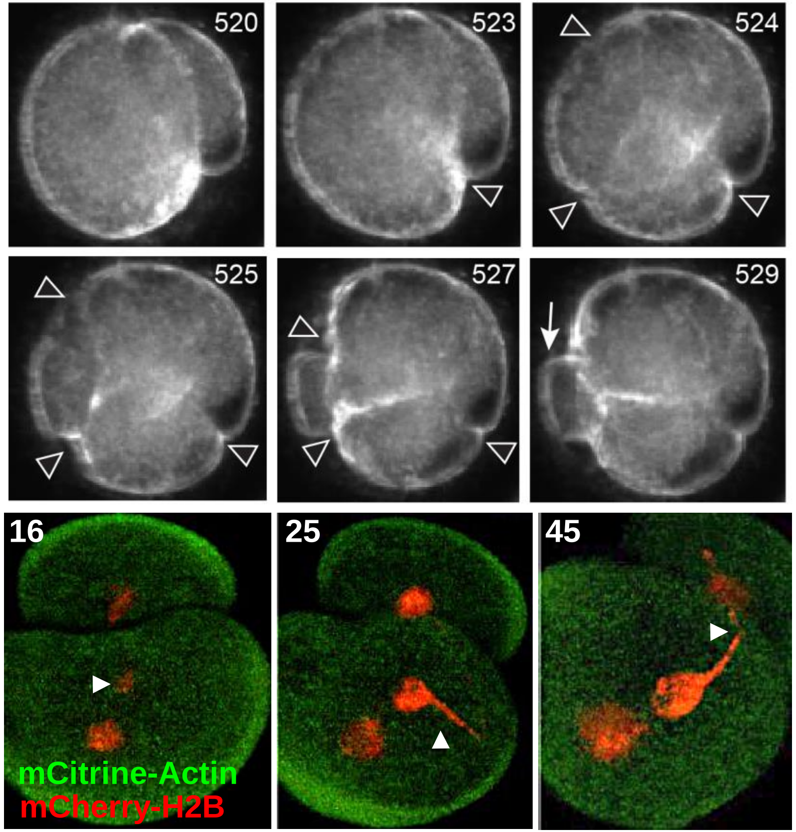
Chromosomes lost from blastomeres are contained within cellular fragments

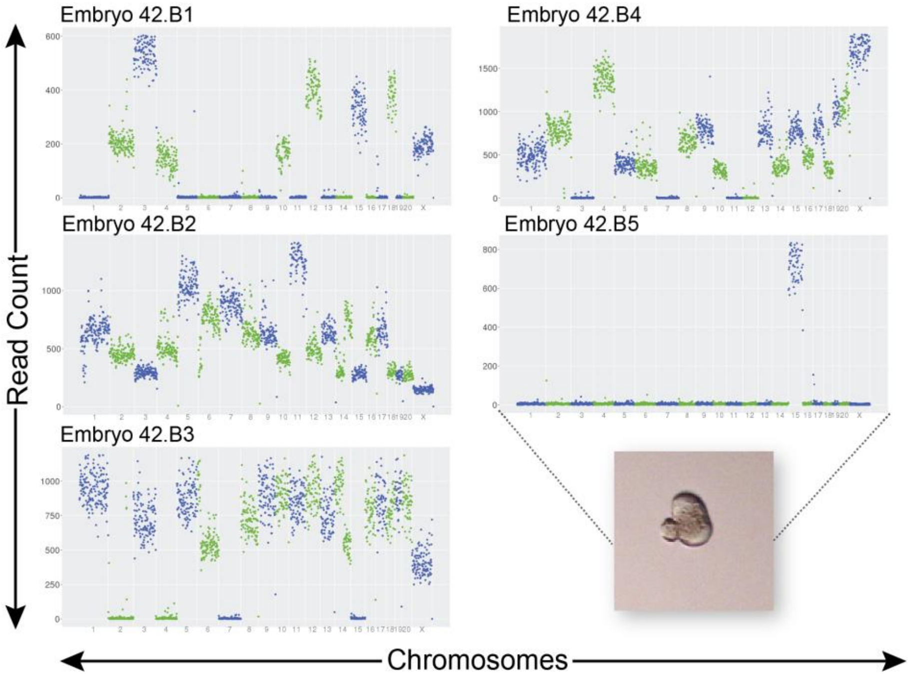




Daughtry *et al.*,
Genome Res 2019

Multipolar divisions often lead to chromosome loss and chaotic aneuploidy

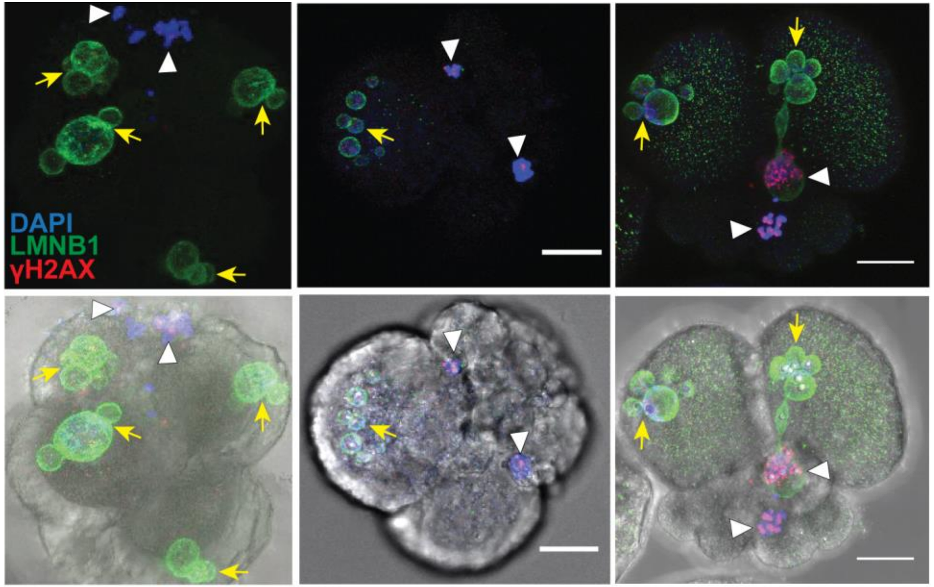




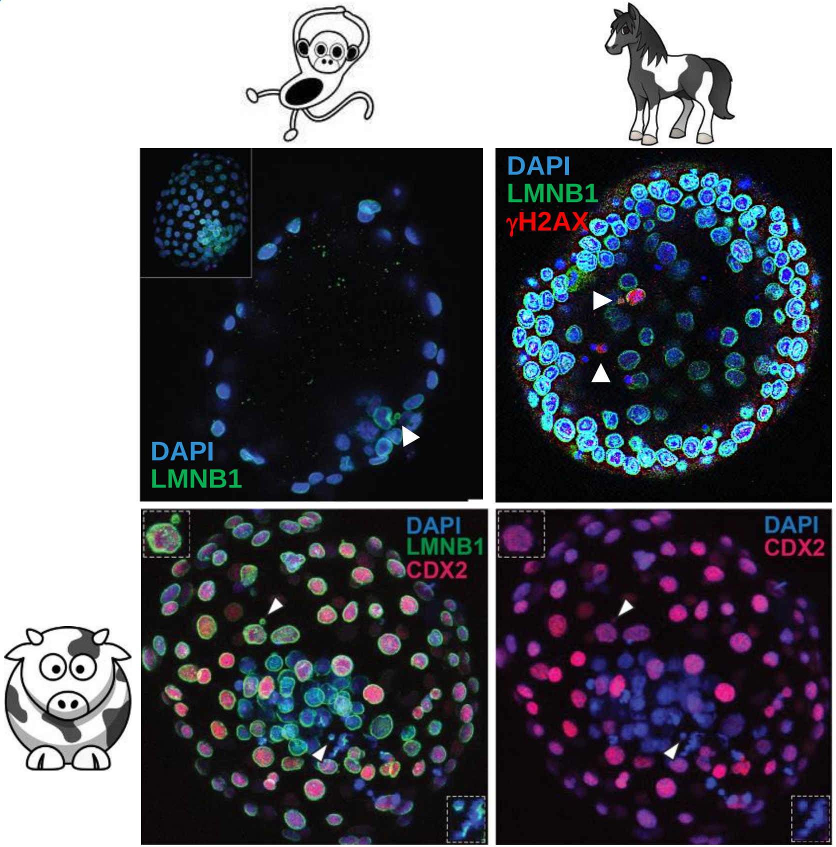
Daughtry *et al.*, Genome Res 2019

DNA damage is observed in chromosome-containing cellular fragments and micronuclei

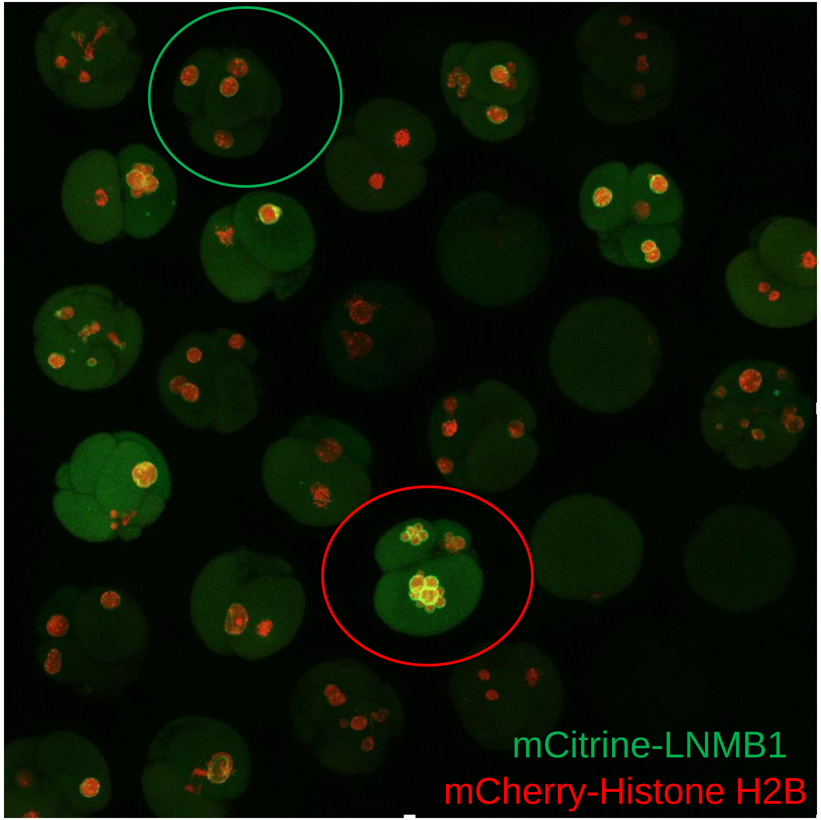
~18% of rhesus embryos had chromosome-containing fragments, but only ~6% of all fragments contained DNA detectable by sequencing

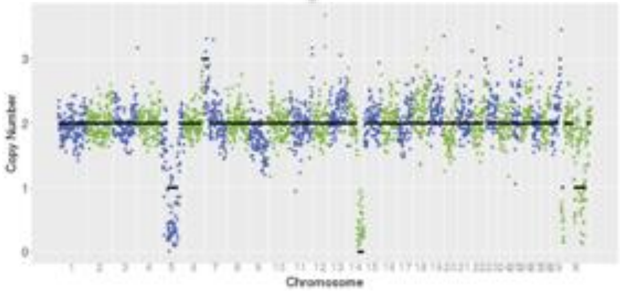


Daughtry *et al.*, Genome Res 2019



Fluorescent live-cell imaging of cleavage-stage embryos for DNA detection





Brooks, Daughtry *et al.*, unpublished

Chromosomally mosaic embryos can still lead to the birth of healthy offspring

OHSU

Healthy Babies after Intrauterine Transfer of Mosaic Aneuploid Blastocysts

N Engl J Med 2015; 373:2089-2090 | November 19, 2015 | DOI: 10.1056/NEJMc1500421

Hum Genet
DOI 10.1007/s00439-017-1797-4

Analysis of implantation and ongoing pregnancy rates following the transfer of mosaic diploid–aneuploid blastocysts

Gleicher *et al. Reproductive Biology and Endocrinology* (2016) 14:54
DOI 10.1186/s12958-016-0193-6

Reproductive Biology and Endocrinology

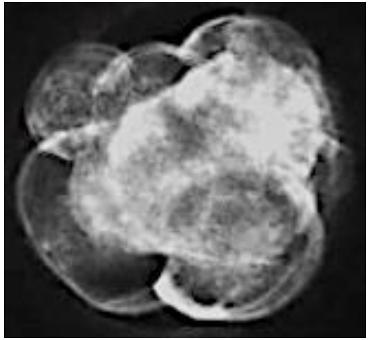
CrossMark

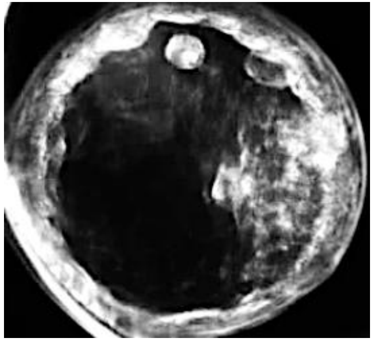
Accuracy of preimplantation genetic screening (PGS) is compromised by degree of mosaicism of human embryos

nature COMMUNICATIONS

Mouse model of chromosome mosaicism reveals lineage-specific depletion of aneuploid cells and normal developmental potential

Are there selective growth advantages for euploid cells and/or mechanism(s) for the elimination of aneuploid cells during preimplantation development?





Blastocyst formation rate	54.3% (N=50/92)
Euploid embryos	26.5% (N=13/49)
Mosaic embryos	32.6% (N=16/49)

Blastomere and cellular fragment exclusion from blastocysts

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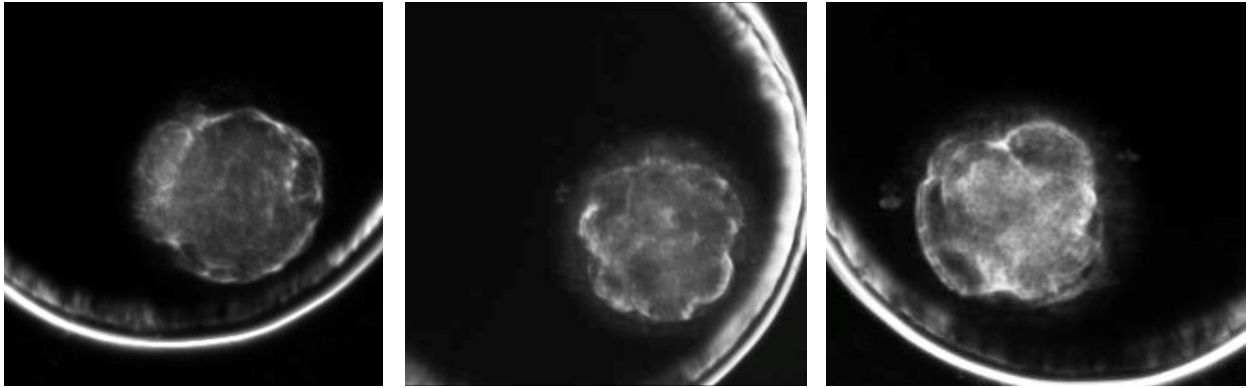
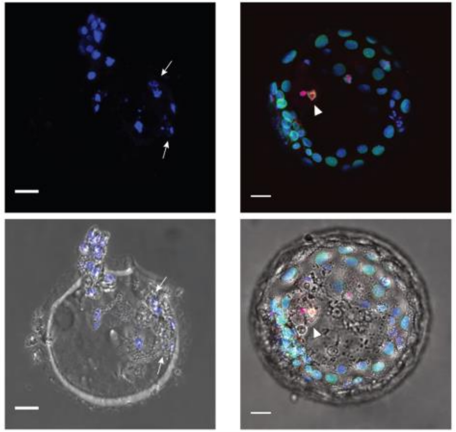
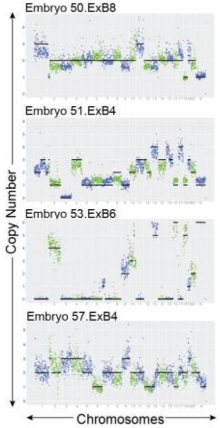
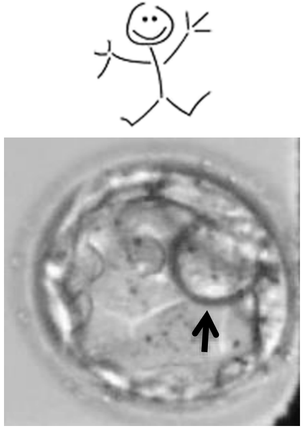
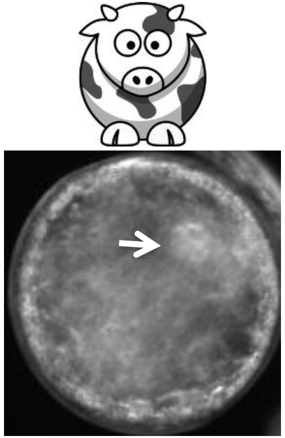


~16% of rhesus blastocysts exhibited fragment/blastomere exclusion, which may represent at least one mechanism to overcome aneuploidy during preimplantation development



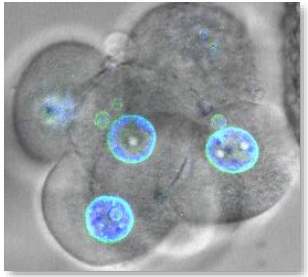






Summary & Conclusions

- Rhesus preimplantation embryos have an equal incidence of micronucleation, aneuploidy*, and cellular fragmentation as human embryos (*maternal age-related aneuploidy still needed).
- Equine and bovine embryos share some of these features at a similar frequency, but the oocytes for IVF are often *in vitro* matured.
- Cellular fragments may contain whole and/or partial chromosomes lost from blastomeres that are susceptible to DNA damage.
- Despite the high incidence of aneuploidy at the cleavage-stage, blastomere/fragment exclusion and other mechanisms may provide a means to overcome chromosome instability and continue in development.



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