Published in final edited form as:

Fertil Steril. 2009 April ; 91(4): 1230–1237. doi:10.1016/j.fertnstert.2008.01.075.

Chromosomal instability in rhesus macaque preimplantation embryos

Catherine Dupont, M.Sc.^a, Lutz Froenicke, PhD^b, Leslie A. Lyons, PhD^b, Barry D. Bavister, PhD^{c,d}, and Carol A. Brenner, PhD^a

^a Departments of Obstetrics & Gynecology and Physiology, CS Mott Center for Human Growth and Development, Wayne State University, School of Medicine, Detroit, MI 48201, USA

^b Department of Population Health and Reproduction, School of Veterinary Medicine, University of California-Davis, Davis, CA 95616, USA

^c Department of Biological Sciences, University of New Orleans, New Orleans, LA 70148, USA

^d Caribbean Primate Research Center, Sabana Seca, PR 00952, USA

Abstract

Objective—To establish a relevant animal model to systematically investigate chromosomal instability in human oocytes and preimplantation embryos.

Design—Prospective rhesus monkey IVF study.

Setting—Academic laboratory, Oregon National Primate Research Center and Caribbean Primate Research Center.

Patients—Young Rhesus macaque females.

Interventions—*In vitro* produced (IVP) entire rhesus macaque preimplantation embryos were cytogenetically assessed using a 5-color fluorescent *in situ* hybridization (FISH) assay developed for rhesus macaque chromosomes homologous to human chromosomes 13, 16, 18, X and Y, using human bacterial artificial chromosome (BAC) probes.

Main Outcome Measure(s)—Chromosomal abnormality rates in preimplantation embryos from young rhesus macaque females were established.

Result(s)—Fifty preimplantation embryos, displaying good morphology and normal development, from 11 young rhesus macaque females were analyzed. Overall, 27 embryos (54%) were normal, 11 embryos (22%) mosaic, 3 embryos (6%) chaotic, 2 embryos (4%) aneuploid, 3 embryos (6%) haploid and 4 embryos (8%) triploid.

Conclusion(s)—These data indicate that IVP rhesus macaque and human preimplantation embryos exhibit similar numerical chromosomal aberrations. Rhesus macaques appear to be a suitable animal model for investigating the origin of chromosomal instability observed in human preimplantation embryos.

Reprint requests: Catherine Dupont, M.Sc., Departments of Obstetrics & Gynecology and Physiology, CS Mott Center for Human Growth and Development, Wayne State University, School of Medicine, 275 E Hancock St, Detroit, MI 48201, USA, (FAX: 313-577-8554; e-mail: cdupont@med.wayne.edu).

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Keywords

Aneuploidy; ART; Cytogenetics; Embryo; FISH; IVF; Non-human primate; PGD

INTRODUCTION

Most chromosomal aneuploidies are lethal to cells, though a significant percentage originating during gametogenesis or further embryonic development are tolerated. It has been estimated that approximately 25–30% of all human conceptuses are lost prior to implantation, while another 30% are lost during the post-implantation period before clinical recognition (1,2). An additional 10–15% are spontaneously aborted (between 6 and 28 weeks gestation) and 1% are stillborn (after 28 weeks through to term) (1,2). This equates to an approximately 30% live birth rate (1,2). Chromosomal aberrations, originating pre- or post-zygotically, are believed to be a major underlying cause for this low reproductive success rate. Approximately 48% of spontaneous abortions and 6% of stillbirths are cytogenetically abnormal; displaying primarily trisomic errors (27% and 4%, respectively), followed by polyploidies (10% and 0.6%, respectively) and monosomy X (9% and 0.25%, respectively) (2). Except for 1% of spontaneous abortions and 0.5% of stillbirths, the observed chromosomal errors are present in all fetal cells analyzed by karyotyping (2–5).

The frequency of preimplantation losses has been estimated using retrospective data generated from infertile patients in *in vitro* fertilization (IVF) clinics. Most studies screening human preimplantation embryos for abnormalities only assess 1 or 2 blastomeres, which may not accurately reflect the chromosome complement of the remaining blastomeres because of significant incidences of chromosomal mosaicism (6–13). Published studies generally concur on the cytogenetic classification of haploid, polyploid, normal and aneuploid human embryos. All blastomeres of normal embryos display a normal diploid chromosome complement, and abnormal non-mosaic (aneuploid) embryos exhibit all blastomeres with a specific monosomy or trisomy. However, the literature differs slightly on the classification of abnormal embryos with non-homogeneously distributed errors (6,7). Reported abnormality rates in human preimplantation embryos range from 19.3–81%, with 20–54% being mosaic and/or chaotic, 1.5–37.2% being aneuploid, 1.1–45.5% being polyploid and 0–7% being haploid. Chromosomal abnormality rates fluctuate and correlate with maternal age (7–11), recurrent pregnancy loss (10,12), failed IVF cycles (9,13), and poor embryo quality (7,8,11). In general, during preimplantation genetic diagnosis (PGD), single blastomeres removed from 8-cell human embryos are analyzed for chromosomal abnormalities that frequently occur in newborns (X, Y, 13, 18, 21) and in spontaneous abortions (X, Y, 13, 16, 18, 21 and 22) (2,3).

Due to ethical and practical limitations, representative data on chromosomal abnormality rates in human preimplantation embryos are difficult to obtain. Therefore, the most commonly studied model used to investigate the impact of assisted reproductive technologies (ART) on chromosomal defects, in both oocytes and preimplantation embryos, has been the mouse (14). However, since the overall incidence of aneuploidy (trisomy or monosomy) among mouse oocytes does not exceed 1–2%, it is not a suitable translational animal model (15). A non-human primate model would be far more representative due to physiological, hormonal and karyotypical similarities. In the past, the use of non-human primates has been hampered by restricted accessibility and the high cost of ART procedures. Cytogenetic data are also limited in non-human primate newborns and are non-existent for non-human primate preimplantation embryos. The few published reports of abnormal newborn non-human primates have identified trisomies involving human homologous chromosomes 13, 18, 21 and X (16–20) in chimpanzee, baboon, bonobo, long-tailed

macaque, rhesus macaque, pigtailed macaque and lowland gorilla and coincide with most cytogenetic abnormalities in human newborns. This study focuses on cytogenetic analysis of *in vitro* produced (IVP) rhesus macaque preimplantation embryos, using a five-color FISH assay, to establish the rhesus macaque as a model for evaluating chromosomal instability in human embryos produced by ART.

MATERIALS AND METHODS

Unless otherwise stated, chemicals and reagents were purchased from Sigma-Aldrich (St Louis, MO, USA).

Controlled hormonal ovarian stimulation

All procedures were performed according to the institutional animal care and animal use committee (IACUC) protocols approved at the Oregon National Research Primate Center (ONPRC) and the Caribbean Primate Research Center (CPRC) in Puerto Rico. The ovarian stimulation protocol has been described previously (21). Briefly, to support follicular growth, all rhesus macaques hormonally stimulated at the ONPRC and the CPRC received a sequential regime of recombinant human gonadotropins. Young female macaques (aged under 18 years) at the ONPRC received recombinant human follicle stimulating hormone (rhFSH, Organon, Oss, The Netherlands; 30 IU per injection, twice daily, i.m.) for 7 days, followed by 2 days of rhFSH and recombinant human luteinizing hormone (rhLH, EMD Serono, Rockland, MA, USA; 30 IU rhFSH and 30 IU rhLH per injection, twice daily, i.m.). During the final three days of the rhFSH treatment, animals also received the Gonadotropin Releasing Hormone (GnRH) antagonist Acycline (0.075 mg/kg animal body weight) to prevent a spontaneous LH surge. Ovarian stimulation of rhesus macaques situated at the CPRC was performed by administration of rhFSH (37.5 IU per injection, twice daily, i.m.; Organon, Roseland, NY, USA) for 8 days, followed by 2 days of rhFSH and recombinant human chorionic gonadotropin (rhCG, EMD Serono, Rockland, MA, USA; 37.5 IU rhFSH and 100 IU rhCG per injection, twice daily, i.m.). At both primate centers, on the final day of rhFSH treatment, 32–33 hours before follicular aspiration, oocyte maturation was induced with a single injection of rhCG (750–1000 IU, i.m.).

Oocyte and sperm collection, insemination and embryo culture

Procedures for oocyte recovery, sperm collection, insemination and embryo culture have been described previously (22–24). Briefly, follicular fluid aspirates were collected in TALP-Hepes containing 0.3% bovine serum albumin (BSA). The aspirates were sifted through a cell strainer (Becton-Dickinson, Franklin Lakes, NJ; Falcon, 70 μ m pore size) and oocytes were collected after rinsing the filter. After cumulus removal using hyaluronidase (0.03%), oocytes were rinsed and placed in TALP-medium culture drops supplemented with BSA (25) and incubated in 5% CO₂ in air at 37 °C until insemination. Semen was obtained via electro-ejaculation (26). Seminal plasma was removed according to standard protocols and obtained spermatozoa were activated with cyclic adenosine monophosphate (cAMP) and caffeine (1 mM each) and used for insemination at a final concentration of 1.5–2.0 10^5 sperm/ml or intracytoplasmic injection (ICSI) (22,27). The presence of pronuclei was assessed 18–20 hours post-insemination or post-ICSI. Presumptive zygotes were transferred to amino acid-supplemented HECM-9 culture drops (28) and incubated under a 5% CO₂ in air atmosphere at 37 °C. The culture medium was refreshed every 48 hours.

Embryo collection and blastomere fixation

Where possible, embryos that reached the 8-cell stage between 54 and 78 hours post-insemination, with no or minor fragmentation, were selected. The zona pellucida was removed using pronase (1 mg/ml) in TL-Hepes containing 1 mg/ml polyvinyl alcohol

(PVA), after which embryos were dissociated in calcium/magnesium-free phosphate buffered saline (PBS, Invitrogen, San Diego, CA, USA). Nuclei were fixed slowly on a slide using 0.01 M HCl/0.1% Tween 20 (29). After dehydration in an ethanol series, slides were kept at -20°C until required for analysis.

DNA Probes

DNA probes identifying macaque chromosomes X, Y, 17, 18, and 20 (homologous to human chromosomes X, Y, 13, 18, and 16 respectively) (30) were generated from previously described pooled human bacterial artificial chromosomes (BAC) (31). BAC DNAs were extracted using the PhasePrep BAC DNA kit, after which samples were pooled and directly labeled by nick translation. The nick translation mixture contained a 1x enzyme mix from a BioNick translation kit (Invitrogen, San Diego, CA, USA), nucleotides (0.02 mM each of dATP, dCTP and dGTP and 0.01mM dTTP, Promega, Madison, WI, USA), 50 mM reaction buffer (Tris-HCl pH 7.8, 5 mM MgCl_2 , 10 mM β -mercaptoethanol, 10 $\mu\text{g/ml}$ nuclease-free BSA) and 0.01mM fluorophore labeled dUTPs (Alexa488-dUTP, Invitrogen, San Diego, CA, USA; Cy3-dUTP, Amersham-GE Healthcare, Piscataway, NJ, USA). Reaction mixtures were incubated for 2 hours at 16°C , followed by an inactivation for 10 min at 65°C . Differentially labeled probes were combined and co-precipitated with Cot-1 DNA and ssDNA as previously described (32). Resulting precipitated DNA was resuspended in hybridization solution (50% deionized formamide, 10% dextran sulphate, in 2x SSC).

Fluorescence *In Situ* Hybridization (FISH)

The five-probe FISH assay was carried out in two consecutive hybridization experiments. Slides were thawed, fixed in ice-cold Carnoy's fixative for 1 minute, then rinsed in PBS. After dehydration, air-dried slides were co-denatured with probe mixture for 5 minutes at 75°C then incubated overnight at 37°C . Post-hybridization, slides were washed twice for 5 minutes in 0.05X sodium chloride-sodium citrate buffer (SSC) at 39°C , followed by 2 minutes in 2X SSC at room temperature. Finally, slides were air-dried, counterstained with DAPI and visualized and recorded using an Olympus BX41 microscope (Olympus, Tokyo, Japan). For the second hybridization slides were washed in 2X SSC for 1 minute at room temperature, ethanol dehydrated and air-dried. After a 3 minute co-denaturation and overnight incubation, slides were treated as described for the first hybridization.

FISH analysis

Slides were analyzed using an Olympus BX41 microscope equipped with single band pass filters (Semrock, Rochester, NY, USA) for DAPI, FITC and Cy3 and recorded using an Olympus DP71 Digital Camera and its associated software. After the first round of FISH, probes for human homologous chromosomes X, Y and 13, labeled respectively with Alexa488, Alexa488+Cy3 and Cy3, were visualized. Human homologous chromosomes 16 and 18, labeled respectively with Cy3 and Alexa488, were assessed after the second round of FISH. Recorded images were after acquisition superimposed and recolored to enable visualization of the probes in false colors. Scoring criteria were dependent on the diameter of fixed nuclei. If the nucleus had a diameter of $35\text{ }\mu\text{m}$ or more, then two signals of the same color were considered a split signal when they were separated by a distance of less than the size of one signal; while if the signals were separated by at least one signal in size, they were scored as two signals. However, when the nucleus diameter was smaller than $35\text{ }\mu\text{m}$, two signals were always scored as two signals and not split, due to the condensed nature of the nucleus. Results from metaphase spreads and nuclei with over-denatured chromatin were omitted. After analysis of each blastomere, embryos were classified as normal, aneuploid, mosaic, chaotic, polyploid or haploid (classifications are listed in Table 1; modified from

Delhanty et al., 1997;6). Furthermore, when the total complement of all nuclei within a multinucleated blastomere (MNB) was normal diploid, the blastomere was scored as normal.

RESULTS

Eleven rhesus macaque females were included in this study. Three females were located at the CPRC and eight females at the ONPRC. The average female age was eight years old (range 4 to 13), and at the time of stimulation no female had more than 4 previous hormonal stimulations. On average 42 oocytes per IVF cycle were collected.

Fifty rhesus macaque preimplantation embryos were removed from culture 54–78 hr post-insemination. Where possible, embryos that reached the 8-cell stage with no or minor fragmentation were selected. Whole embryo dissociation yielded 384 individual blastomeres, of which 348 were successfully fixed. Data from metaphase spreads or nuclei with overdenatured chromatin were omitted. Overall, 341 blastomeres were analyzed with probes targeting the homologs of human chromosomes X (Alexa488), Y (Alexa488-Cy3) and 13 (Cy3) in the first hybridization. Of these, 325 blastomeres could be successfully rehybridized for macaque chromosomes homologous to human 16 (Cy3) and 18 (Alexa488) in the second hybridization. The Y-probe produced comparatively small signals, which made Y-signals in some male blastomeres difficult to interpret. After analysis of each blastomere embryos were classified as normal, mosaic, chaotic, aneuploid, haploid or polyploid according to the definitions listed in Table 1.

Out of 50 embryos, 27 (54%) were considered diploid-normal (Fig 1), 11 (22%) were diploid-mosaic, 3 (6%) diploid-chaotic, 2 (4%) diploid-aneuploid (Fig 2a–b), 3 (6%) haploid-mosaic/chaotic (Fig 2c) and 4 (8%) triploid-normal/mosaic (Fig 2d; summarized in Table 2). Three embryos with MNB were classified as normal since the combined chromosomal complement was normal diploid (Table 3). Overall, from the 11 diploid-mosaic embryos, 45 blastomeres were normal, 24 abnormal, 1 blastomere was multinucleated with an abnormal diploid complement and 5 blastomeres displayed inconclusive results (Table 3).

DISCUSSION

To investigate the chromosomal abnormality frequency in rhesus macaque preimplantation embryos, a 5-color FISH assay was developed. Most assessed chromosomes in human preimplantation embryos are those commonly implicated in abnormal spontaneous abortions (chromosomes 13, 16, 18, 21, 22, X and Y) and chromosomally abnormal newborns (chromosomes 13, 18, 21, X and Y). Therefore, a comparable assay was developed for cytogenetic analysis of rhesus macaque preimplantation embryos. However, since the rhesus macaque homologs of human chromosomes 21 and 22 are fused to much larger chromosomes (33,34) they were not included in this study. Custom made pools of directly labeled human BAC probes were generated for human chromosomes 13, 16, 18, X and Y; targeting rhesus macaque chromosomes 17, 20, 18, X and Y, respectively (31).

All analyzed IVP preimplantation embryos were generated from oocytes from young rhesus macaque females. The majority of embryos analyzed exhibited good morphology and embryo development prior to blastomere dissociation for FISH analysis. The high frequency of aneuploidy in human preimplantation embryos has been associated with increasing maternal age (7–11). Similarly, embryos that display poor development and/or morphology may exhibit more chromosomal abnormalities (7,8,11). The abnormality rates in rhesus macaque preimplantation embryos are similar to chromosomal anomaly rates observed in 'high quality' IVF human preimplantation embryos collected from young women (7,11).

From a total of 50 rhesus macaque preimplantation embryos analyzed by FISH, 27 (54%) were normal. In the remaining group of 23 embryos, 11 (22%) were mosaic, 3 (6%) chaotic, 2 (4%) aneuploid, 3 (6%) haploid and 4 (8%) triploid (Table 2). Munne et al. (1995) (7) reported 50% normal and 50% abnormal embryos, within which 40% mosaic/chaotic, 4% were aneuploid, 2% haploid and 4% polyploid. On the other hand, Marquez et al. (2000) (11) described 25% mosaic/chaotic, 1.4% aneuploid, 7% haploid embryos and 5.6% polyploid, with the remaining 61% being normal. While the mosaic/chaotic data from Munne et al. (1995) (7) differ slightly from those presented here, the abnormality rates reported by Marquez et al. (2000) (11) are similar to those we observed in rhesus macaque preimplantation embryos. Haploid embryos (Fig 2c) were only found following ICSI in the present study. Thus it is likely that these oocytes were parthenogenetically activated. Because ICSI is commonly used in human ART, this suggests that a significant proportion of oocytes develop into embryos that appear morphologically normal, without contribution of the paternal genome.

Chromosomal errors of prezygotic origin were observed in three embryos, although only two embryos were classified as aneuploid. Specifically, one aneuploid embryo had a monosomy X (Fig 2a), while another embryo revealed a monosomy of rhesus macaque chromosome 17 (human 13; Fig 2b). The third embryo, categorized as mosaic, originated from a rhesus macaque trisomy 20 (human 16) zygote, since the total chromosomal complement of all blastomeres for chromosome 20 indicated the embryo was trisomic (Munne et al., 1994). Post-zygotic segregation errors involving the chromosome allowed the presence of a normal blastomere. However, more embryos may exhibit errors attributable to a meiotic origin since the zygotic complement was difficult to determine when embryos divided chaotically or where not all blastomeres were available.

In this study, chromosomal abnormality rates in embryos from young fertile monkeys may have been underestimated because only a fraction of the genome was analyzed and data from small and/or over-denatured nuclei were omitted or considered inconclusive. Additionally, MNB in the present study were scored as normal when their total complement was normal diploid (Fig 1c and 1d). Although embryos with MNB are generally scored as abnormal, it is unclear whether these blastomeres continue to contribute to the embryo as it develops, because they may represent fragmented nuclei undergoing apoptosis. Although FISH has been applied extensively to identify chromosome abnormalities in embryos, its usefulness is restricted because only a limited number of chromosomes can be investigated in each embryo. A common approach is to analyze several chromosomes simultaneously by re-hybridizing the same sample multiple times. This study, as well as others (35), shows that repeated FISH hybridizations on blastomere nuclei cause the target DNA to degenerate and reduce the signal intensity. However analyzing a large part of the genome is limited while minimizing rehybridizations. Since detection of embryo abnormality rates increase when more chromosomes are assessed (36), chromosome selection needs careful consideration. Ideally, all chromosomes should be identified in a single blastomere during PGD. To date, one of the most successful approaches for complete chromosomal analysis of single human cells is comparative genomic hybridization (35, 37, 38).

One of the major debates in selecting chromosomes for PGD in human IVF is whether certain chromosomes are more susceptible to mis-segregation during meiosis and mitosis (39), or whether all chromosomes are equally as susceptible but that specific aneuploidies are more viable than others (40,41). Human preimplantation embryos display a wide range of aneuploidies (36,42), however a smaller range is frequently observed in spontaneous abortions (frequently chromosomes 13, 15, 16, 18, 21, 22, X and Y) or in newborns (commonly chromosomes 13, 18, 21, X and Y). This indicates that while all chromosomes

are susceptible to segregation errors to a certain extent, only certain errors, like those generally assessed during PGD, are favorable to result in a clinical pregnancy.

Aneuploidy frequencies occurring during human conception are believed to be higher than in any other assessed species, contributing to 48% of the spontaneous abortions (2) and 6% of stillbirths (2), and affect 19.3–81% of preimplantation embryos analyzed in IVF clinics (7–13). Because of the high rates of chromosomal abnormalities found in embryos from both young and older women, a fundamental question to answer is whether ovarian stimulation and/or culture conditions used in ART may in fact be responsible for inducing some of these genetic errors. There are few data on whether gonadotrophin regimens and culture conditions impact non-human primate oocyte and embryo quality. Recent studies in the rhesus monkey suggest that the hormonal ovarian stimulation in rhesus monkeys affect the quality of recovered oocytes and embryo development (43,44). Using BAC-FISH probes as reported in the present study, it will now be possible to study the effects of ARTs on chromosomal abnormality rates in rhesus monkey oocytes and embryos.

The rhesus macaque is an excellent animal model in which to study human reproduction because it displays menstrual cycles with similar hormonal profiles. Ovaries from rhesus monkeys with advanced maternal age also exhibit a reproductive decline and follicular depletion similar to that observed in humans (45,46). In addition, the hypothalamic-pituitary-gonadal (HPG) function declines gradually, resulting in more irregular menstrual cycles and intermittent incidences of ovulation (47). Using a 5-color FISH assay for human chromosomes 13, 16, 18, X and Y, representing rhesus macaque chromosomes 17, 20, 18, X and Y respectively, baseline levels of chromosomal error rates have now been established in preimplantation embryos from young monkeys. Data from the present study indicate that the rhesus macaque is a suitable model to investigate the contribution of ART to the high incidence of cytogenetic instabilities in human preimplantation embryos. Future studies will provide insight into fundamental biological mechanisms of aging that could affect fertility.

Acknowledgments

This study was supported by National Institutes of Health (1R01HD045966-01A2 and 5R24RR015395-04 awarded to B.D. Bavister, 1R03HD046553-01A1 and 1R21RR021881-01 awarded to C.A. Brenner, R24 RR017584 awarded to L.A. Lyons and RR03640 awarded to the Caribbean Primate Research Center). We thank Drs. A.J. Harvey and D.R. Armant for critical evaluation of the manuscript and Dr. S.M. Nichols for technical assistance at the Caribbean Primate Research Center.

Financial support Supported by National Institutes of Health (1R01HD045966-01A2 and 5R24RR015395-04 awarded to B.D. Bavister, 1R03HD046553-01A1 and 1R21RR021881-01 awarded to C.A. Brenner, R24RR017584 awarded to L.A. Lyons and RR03640 awarded to the Caribbean Primate Research Center).

References

1. Macklon NS, Geraedts JP, Fauser BC. Conception to ongoing pregnancy: the 'black box' of early pregnancy loss. *Hum Reprod Update*. 2002; 8:333–43. [PubMed: 12206468]
2. Jacobs PA. The chromosome complement of human gametes. *Oxf Rev Reprod Biol*. 1992; 14:47–72. [PubMed: 1437215]
3. Hassold TJ, Jacobs PA. Trisomy in man. *Annu Rev Genet*. 1984; 18:69–97. [PubMed: 6241455]
4. Hassold T, Abruzzo M, Adkins K, Griffin D, Merrill M, Millie E, et al. Human aneuploidy: incidence, origin, and etiology. *Environ Mol Mutagen*. 1996; 28:167–75. [PubMed: 8908177]
5. Goddijn M, Leschot NJ. Genetic aspects of miscarriage. *Baillieres Best Pract Res Clin Obstet Gynaecol*. 2000; 14:855–65. [PubMed: 11023805]
6. Delhanty JD, Harper JC, Ao A, Handyside AH, Winston RM. Multicolour FISH detects frequent chromosomal mosaicism and chaotic division in normal preimplantation embryos from fertile patients. *Hum Genet*. 1997; 99:755–60. [PubMed: 9187668]

7. Munne S, Alikani M, Tomkin G, Grifo J, Cohen J. Embryo morphology, developmental rates, and maternal age are correlated with chromosome abnormalities. *Fertil Steril.* 1995; 64:382–91. [PubMed: 7615118]
8. Benadiva CA, Kligman I, Munne S. Aneuploidy 16 in human embryos increases significantly with maternal age. *Fertil Steril.* 1996; 66:248–55. [PubMed: 8690111]
9. Magli MC, Gianaroli L, Munne S, Ferraretti AP. Incidence of chromosomal abnormalities from a morphologically normal cohort of embryos in poor-prognosis patients. *J Assist Reprod Genet.* 1998; 15:297–301. [PubMed: 9604763]
10. Pellicer A, Rubio C, Vidal F, Minguez Y, Gimenez C, Egozcue J, et al. In vitro fertilization plus preimplantation genetic diagnosis in patients with recurrent miscarriage: an analysis of chromosome abnormalities in human preimplantation embryos. *Fertil Steril.* 1999; 71:1033–9. [PubMed: 10360906]
11. Marquez C, Sandalinas M, Bahce M, Alikani M, Munne S. Chromosome abnormalities in 1255 cleavage-stage human embryos. *Reprod Biomed Online.* 2000; 1:17–26. [PubMed: 12804207]
12. Rubio C, Simon C, Vidal F, Rodrigo L, Pehlivan T, Remohi J, et al. Chromosomal abnormalities and embryo development in recurrent miscarriage couples. *Hum Reprod.* 2003; 18:182–8. [PubMed: 12525464]
13. Voullaire L, Collins V, Callaghan T, McBain J, Williamson R, Wilton L. High incidence of complex chromosome abnormality in cleavage embryos from patients with repeated implantation failure. *Fertil Steril.* 2007; 87:1053–8. [PubMed: 17418829]
14. Bean CJ, Hassold TJ, Judis L, Hunt PA. Fertilization in vitro increases non-disjunction during early cleavage divisions in a mouse model system. *Hum Reprod.* 2002; 17:2362–7. [PubMed: 12202426]
15. Hassold T, Hunt P. To err (meiotically) is human: the genesis of human aneuploidy. *Nat Rev Genet.* 2001; 2:280–91. [PubMed: 11283700]
16. Howell KH, Hubbard GB, Moore CM, Dunn BG, von Kap-Herr C, Raveendran M, et al. Trisomy of chromosome 18 in the baboon (*Papio hamadryas anubis*). *Cytogenet Genome Res.* 2006; 112:76–81. [PubMed: 16276093]
17. Ruppenthal GC, Moore CM, Best RG, Walker-Gelatt CG, Delio PJ, Sackett GP. Trisomy 16 in a pigtailed macaque (*M. nemestrina*) with multiple anomalies and developmental delays. *Am J Ment Retard.* 2004; 109:9–20. [PubMed: 14651446]
18. Dudley CJ, Hubbard GB, Moore CM, Dunn BG, Raveendran M, Rogers J, et al. A male baboon (*Papio hamadryas*) with a mosaic 43, XXY/42, XY karyotype. *Am J Med Genet A.* 2006; 140:94–7. [PubMed: 16315270]
19. Moore CM, Hubbard GB, Dick E, Dunn BG, Raveendran M, Rogers J, et al. Trisomy 17 in a baboon (*Papio hamadryas*) with polydactyly, patent foramen ovale and pyelectasis. *Am J Primatol.* 2007
20. Ruppenthal GC, Caffery SA, Goodlin BL, Sackett GP, Vigfusson NV, Peterson VG. Pigtailed macaques (*Macaca nemestrina*) with trisomy X manifest physical and mental retardation. *Am J Ment Defic.* 1983; 87:471–6. [PubMed: 6682289]
21. Zelinski-Wooten MB, Hutchison JS, Hess DL, Wolf DP, Stouffer RL. Follicle stimulating hormone alone supports follicle growth and oocyte development in gonadotrophin-releasing hormone antagonist-treated monkeys. *Hum Reprod.* 1995; 10:1658–66. [PubMed: 8582957]
22. Bavister BD, Boatman DE, Leibfried L, Loose M, Vernon MW. Fertilization and cleavage of rhesus monkey oocytes in vitro. *Biol Reprod.* 1983; 28:983–99. [PubMed: 6683110]
23. Bavister BD, Boatman DE, Collins K, Dierschke DJ, Eisele SG. Birth of rhesus monkey infant after in vitro fertilization and nonsurgical embryo transfer. *Proc Natl Acad Sci U S A.* 1984; 81:2218–22. [PubMed: 6326113]
24. Wolf DP, Vandervoort CA, Meyer-Haas GR, Zelinski-Wooten MB, Hess DL, Baughman WL, et al. In vitro fertilization and embryo transfer in the rhesus monkey. *Biol Reprod.* 1989; 41:335–46. [PubMed: 2508776]
25. Bavister BD, Yanagimachi. The effects of sperm extracts and energy sources on the motility and acrosome reaction of hamster spermatozoa in vitro. *Biol Reprod.* 1977; 16:228–37. [PubMed: 831847]

26. Mastroianni L Jr, Manson WA Jr. Collection of monkey semen by electroejaculation. *Proc Soc Exp Biol Med.* 1963; 112:1025–7. [PubMed: 13933495]
27. Boatman DE, Bavister BD. Stimulation of rhesus monkey sperm capacitation by cyclic nucleotide mediators. *J Reprod Fertil.* 1984; 71:357–66. [PubMed: 6086919]
28. McKiernan SH, Bavister BD. Culture of one-cell hamster embryos with water soluble vitamins: pantothenate stimulates blastocyst production. *Hum Reprod.* 2000; 15:157–64. [PubMed: 10611206]
29. Coonen E, Dumoulin JC, Ramaekers FC, Hopman AH. Optimal preparation of preimplantation embryo interphase nuclei for analysis by fluorescence in-situ hybridization. *Hum Reprod.* 1994; 9:533–7. [PubMed: 8006147]
30. Moore CM, Janish C, Eddy CA, Hubbard GB, Leland MM, Rogers J. Cytogenetic and fertility studies of a rhesus, rhesus macaque (*Macaca mulatta*) x baboon (*Papio hamadryas*) cross: further support for a single karyotype nomenclature. *Am J Phys Anthropol.* 1999; 110:119–27. [PubMed: 10502238]
31. Froenicke L, Hung PH, Vandevoort CA, Lyons LA. Development of a non-human primate sperm aneuploidy assay tested in the rhesus macaque (*Macaca mulatta*). *Mol Hum Reprod.* 2007; 13:455–60. [PubMed: 17483087]
32. Froenicke L, Anderson LK, Wienberg J, Ashley T. Male mouse recombination maps for each autosome identified by chromosome painting. *Am J Hum Genet.* 2002; 71:1353–68. [PubMed: 12432495]
33. Cambefort Y, Mounie C, Colombies P. [Chromosome band topography of *Papio hamadryas*]. *Ann Genet.* 1976; 19:269–72. [PubMed: 827970]
34. Dutrillaux B. Chromosomal evolution in primates: tentative phylogeny from *Microcebus murinus* (Prosimian) to man. *Hum Genet.* 1979; 48:251–314. [PubMed: 112030]
35. Wilton L. Preimplantation genetic diagnosis and chromosome analysis of blastomeres using comparative genomic hybridization. *Hum Reprod Update.* 2005; 11:33–41. [PubMed: 15569702]
36. Abdelhadi I, Colls P, Sandalinas M, Escudero T, Munne S. Preimplantation genetic diagnosis of numerical abnormalities for 13 chromosomes. *Reprod Biomed Online.* 2003; 6:226–31. [PubMed: 12676005]
37. Voullaire L, Slater H, Williamson R, Wilton L. Chromosome analysis of blastomeres from human embryos by using comparative genomic hybridization. *Hum Genet.* 2000; 106:210–7. [PubMed: 10746563]
38. Wells D, Delhanty JD. Comprehensive chromosomal analysis of human preimplantation embryos using whole genome amplification and single cell comparative genomic hybridization. *Mol Hum Reprod.* 2000; 6:1055–62. [PubMed: 11044470]
39. Warren WD, Gorringer KL. A molecular model for sporadic human aneuploidy. *Trends Genet.* 2006; 22:218–24. [PubMed: 16497408]
40. Warburton D, Kinney A. Chromosomal differences in susceptibility to meiotic aneuploidy. *Environ Mol Mutagen.* 1996; 28:237–47. [PubMed: 8908182]
41. Sandalinas M, Sadowy S, Alikani M, Calderon G, Cohen J, Munne S. Developmental ability of chromosomally abnormal human embryos to develop to the blastocyst stage. *Hum Reprod.* 2001; 16:1954–8. [PubMed: 11527904]
42. Bahce M, Cohen J, Munne S. Preimplantation genetic diagnosis of aneuploidy: were we looking at the wrong chromosomes? *J Assist Reprod Genet.* 1999; 16:176–81. [PubMed: 10224560]
43. Yang S, He X, Hildebrandt TB, Jewgenow K, Goeritz F, Tang X, et al. Effects of rhFSH dose on ovarian follicular response, oocyte recovery and embryo development in rhesus monkeys. *Theriogenology.* 2007; 67:1194–201. [PubMed: 17321585]
44. Gibson TC, Kubisch HM, Brenner CA. Mitochondrial DNA deletions in rhesus macaque oocytes and embryos. *Mol Hum Reprod.* 2005; 11:785–9. [PubMed: 16373367]
45. Gilardi KV, Shideler SE, Valverde CR, Roberts JA, Lasley BL. Characterization of the onset of menopause in the rhesus macaque. *Biol Reprod.* 1997; 57:335–40. [PubMed: 9241047]
46. Nichols SM, Bavister BD, Brenner CA, Didier PJ, Harrison RM, Kubisch HM. Ovarian senescence in the rhesus monkey (*Macaca mulatta*). *Hum Reprod.* 2005; 20:79–83. [PubMed: 15498779]

47. Wu JM, Zelinski MB, Ingram DK, Ottinger MA. Ovarian aging and menopause: current theories, hypotheses, and research models. *Exp Biol Med* (Maywood). 2005; 230:818–28. [PubMed: 16339746]

Figure 1.

Five-color FISH assay on rhesus macaque blastomere nuclei using pooled BAC probes. The FISH signal recordings of two subsequent hybridizations are overlaid and displayed in false colors to distinguish each chromosome. Macaque chromosomes X (green), Y (orange), 17 (red), 18 (blue), and 20 (yellow) each are identified by dot or double-dot like signals. 1A; Normal female blastomere. 1B; Normal male blastomere. 1C and 1D; Fragmented nuclei from binucleated blastomeres.

Figure 2.

Five-color FISH assay on rhesus macaque blastomere nuclei using pooled BAC probes. The FISH signal recordings of two subsequent hybridizations are overlaid and displayed in false colors to distinguish each chromosome. Macaque chromosomes X (green), Y (orange), 17 (red), 18 (blue), and 20 (yellow) each are identified by dot or double-dot like signals. 2A; Monosomy X. 2B; Male blastomere-monosomy 13. 2C; Haploid blastomere. 2D; XXY triploid blastomere.

Table 1**Definition of Embryo Aneuploidy Classifications**

A. Normal	All blastomeres normal diploid
B. Abnormal	
Haploid	Most blastomeres with a haploid complement (haploid-normal, haploid-aneuploid, haploid-mosaic or haploid-chaotic)
Diploid	
• Aneuploid	All blastomeres abnormal because of a prezygotic division error
• Mosaic	Some blastomeres normal diploid - some abnormal/not excluding a prezygotic division error
• Chaotic	All blastomeres abnormal with uncontrolled division involving all chromosomes/not excluding a prezygotic division error
Polyploid	Most blastomeres with a polyploid complement (polyploid-normal, polyploid-aneuploid, polyploid-mosaic or polyploid-chaotic)

Table 2

Rhesus macaque preimplantation embryos classified according to their chromosomal complement.

Embryo Classifications			#	%
Normal			27	54
Abnormal				
	Haploid		4	8
	Diploid	Aneuploid	2	4
		Mosaic	11	22
		Chaotic	3	6
	Triploid		3	6

Table 3
Chromosome complements of blastomeres within normal and abnormal diploid rhesus macaque embryos

	# Normal blastomeres	# Normal MNB	# Abnormal blastomeres	# Abnormal MNB	# Inconclusive
Normal Embryos (27)	169	4	0	0	12
Abnormal diploid embryos					
• Aneuploid (2)	0	0	11	0	2
• Mosaic (11)	45	0	24	1	5
• Chaotic (3)	0	0	18	3	0
Total	214	4	53	4	19