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Working in close quarters: biparental meiosis in the oocyte

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***In vitro* fertilization (IVF) methods involve fertilizing haploid oocytes arrested in meiosis II with haploid sperm. An experimental IVF method had been developed in mice involving injection of diploid sperm nuclei into equally diploid oocytes (biparental meiosis) to increase the chance of reproduction in cases where haploid sperm cannot be obtained. However, this method had been shown to be highly error prone. In this issue of *EMBO Reports*, Ogonuki *et al* show that reducing ooplasm volume by half reduces the segregation errors and increases the likelihood of producing viable offsprings in mice (Ogonuki *et al*, 2022).**

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See also: **N Ogonuki *et al*** (July 2022)

Fertilization for sexual reproduction requires a spermatozoon to meet an oocyte, and their fusion to form the zygote, the first cell of the embryo. The challenge of this process lies in the preparation of the two gametes through meiosis, during which germ cells halve their genomes. The resulting haploid gametes then merge and create a diploid embryo (El Yakoubi & Wassmann, 2017). In mammals, there are crucial differences between male and female gametogenesis, the most important one being distinct timing. While sperm is continually produced throughout a male's life, oocytes are formed in the female fetus and cell cycle-arrested after meiotic recombination, before resuming meiosis to divide their genetic material. Hence, females are already born with their reserve of oocytes in the ovary, arrested in Germinal Vesicle (GV-) stage at prophase of the first meiotic division (meiosis I). Upon hormonal stimulation, some oocytes may resume meiosis and complete the first division with the segregation of their homologous chromosomes. One copy of the genetic material stays in the oocyte, while the other copy is discarded in a smaller cell named polar body. The oocyte then enters the second meiotic division (meiosis II) and stays arrested in metaphase II. Once fertilization takes place, the second division can ensue with separation of sister chromatids, formation of the female pronucleus, and fusion with the male pronucleus. Sperm, however, does not observe this meiosis II arrest but rather is already haploid when it fertilizes the oocyte (El Yakoubi & Wassmann, 2017). Another important difference between female and male gametes is their size, with the oocyte being much bigger than the sperm. For most common *in vitro* fertilization (IVF) methods, oocytes arrested in meiosis II awaiting fertilization are harvested following hormonally stimulated ovulation. Oocytes are then fertilized with haploid sperm *in vitro*.

A study by Ogonuki *et al* (2022) of *EMBO Reports* deals with a different *in vitro* fertilization method in mouse oocytes: injection of diploid sperm nuclei before they undergo the first meiotic division, into equally diploid, GV-stage oocytes. The logic behind this approach is that the oocyte cytoplasm should be able to support the meiotic divisions not only of female, but also of male chromosomes, should they be present (i.e., biparental meiosis). If true, both male and female chromosomes should undergo a reductional first meiotic division with the segregation of chromosomes and extrusion of a polar body, now containing not only half of the female but also half of the male chromosomes. The second meiotic division with the segregation of male and female sister chromatids and second polar body extrusion should then lead to the generation of the zygote containing the correct, diploid genome content (Fig 1). While this approach appears feasible at the first glance, segregation of chromosomes was found to be extremely error prone in biparental meiosis I (Kimura *et al*, 1998; Ogura *et al*, 1998). In this study, the authors built on previous work and tested the hypothesis that the large oocyte cytoplasm may interfere with the segregation of double the number of chromosomes in meiosis I (Kyogoku & Kitajima, 2017; Lane & Jones, 2017). Using high-resolution live imaging, the authors show that indeed, the big size of the oocyte is disadvantageous for biparental meiosis. When sperm nuclei were injected into normal-sized GV-stage oocytes, both paternal and maternal chromosomes were able to align, segregate, and enter meiosis II. In line with previous studies, normal segregations were only observed for 2% of biparental first meiotic divisions. The majority of these errors (86%) were due to precocious sister chromatid segregation in meiosis I, and surprisingly, most errors were derived from paternal chromosomes. In an attempt to reduce these errors, the authors tested whether halving the ooplasm size improved segregation. Halved oocytes were once again injected with sperm nuclei and monitored throughout meiotic maturation. Indeed, errors were significantly reduced, and 21% of segregations in meiosis I appeared to take

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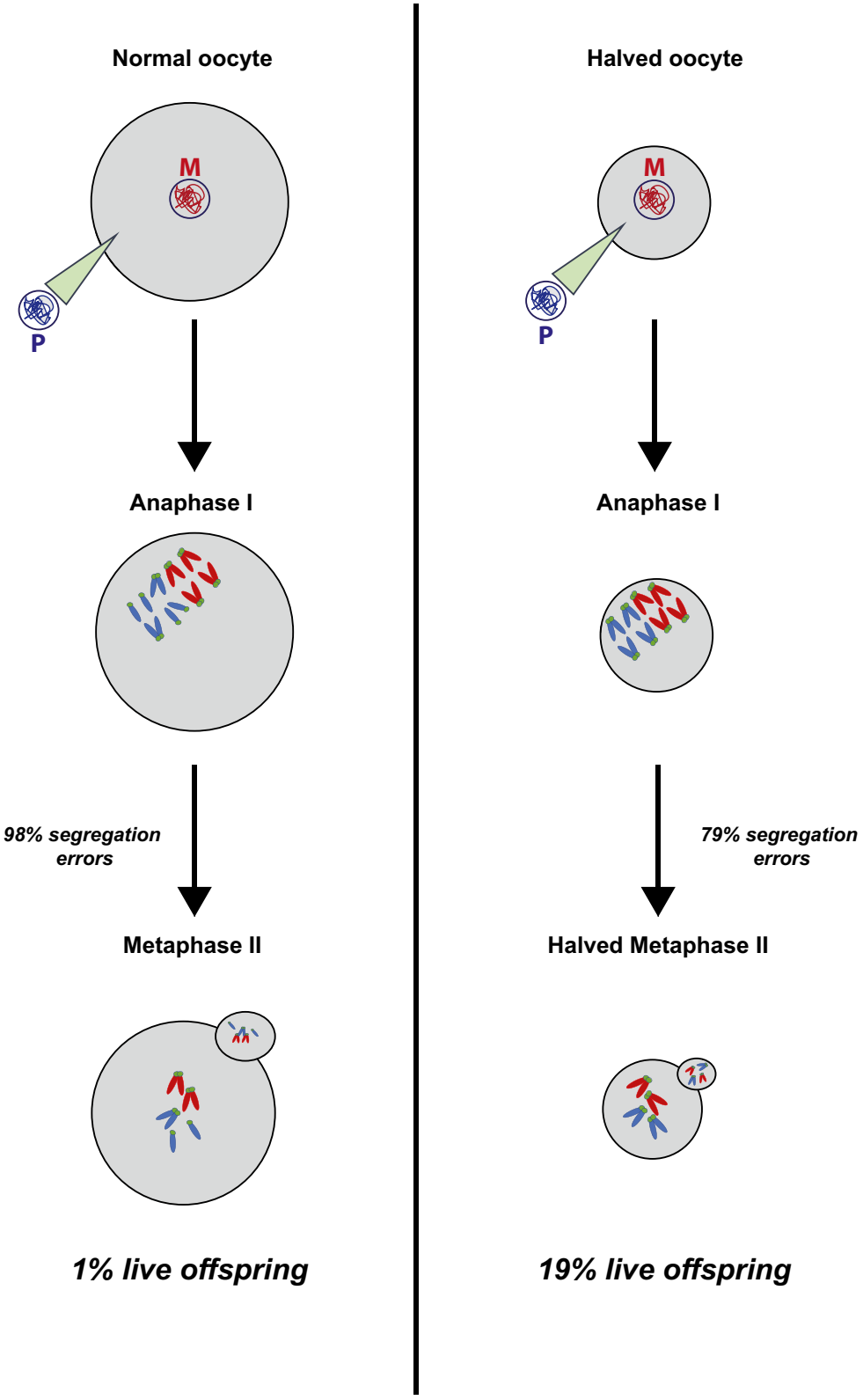

Figure 1. Halving the oocyte volume increases fidelity of biparental meiosis.

On the left, normal-sized oocytes injected with paternal nuclei undergoing meiosis with high rate of segregation errors and low numbers of live offspring. On the right, halved biparental oocytes undergo meiosis with less errors and a higher number of live births. P: paternal, M: maternal diploid prophase I nucleus.

place normally (Fig 1). Of note, the authors did not analyze meiosis II in those oocytes. To verify that these halved oocytes can produce viable embryos, they were re-implanted into foster mothers. Only 1% of normal-sized oocytes undergoing biparental meiosis gave offspring, while 19% of the halved oocytes developed into live pups clearly showing an improvement with reduction in oocyte size. The authors put their strategy to test using two mouse models with azoospermia due to meiotic arrest. Male chromosomes derived from prophase I nuclei injected into oocytes with halved cytoplasm were able to segregate and live births were obtained, though with low success rate. Chromosome karyotype analysis of these mice when they reached adulthood showed the occurrence of sex chromosome aneuploidies. However, these are the only aneuploidies that allow mice to survive up to adulthood (Hernandez & Fisher, 1999); hence, high rates of autosomal aneuploidies that led to spontaneous abortions are likely.

At this point, the authors cannot yet provide an explanation of why reducing the cytoplasmic volume of oocytes increases fidelity of chromosome segregation when an extra set of chromosomes is present. The authors show that missegregations also occur at high rates when an additional set of chromosomes coming from another oocyte is present; thus, aneuploidy rates in biparental meiosis I are not solely due to the presence of male chromosomes. The authors speculated that the key protein required for the protection of centromeric cohesin, namely Sgo2, was absent from paternal chromosomes, leading to the precocious separation of sister chromatids observed, but this turned out not to be the case (Lee *et al*, 2008; Llano *et al*, 2008). The authors had shown previously that proper checkpoint control by the spindle assembly checkpoint (SAC) is affected by oocyte size, being more efficient in smaller, and less efficient in bigger oocytes (Kyogoku & Kitajima, 2017). However, inefficient SAC control also turned out not to be the reason for the high error rate in biparental meiosis. Hence, reducing cell size improves the segregation of additional chromosomes in biparental meiosis by some unknown mechanism.

Through this groundbreaking work, the authors succeeded in obtaining zygotes and viable offspring from oocytes that completed the meiotic divisions not only for their own genome, but also for that of the sperm. Crucially, the success rate achieved was much higher compared with previous studies, due to halving the oocyte cytoplasmic volume. Obviously, this has major implications for human reproductive medicine, because it may represent a means to obtain offspring from individuals suffering from azoospermia due to spermatid arrest in prophase I (Hunt & Hassold, 2002). However, and as mentioned by the authors, the significant aneuploidy rate observed here, and missing knowledge on potential consequences on health of the offspring when male chromosomes segregate in oocytes, still requires a large amount of research before this technique might be applied in the clinic.

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