—Mini Review—

Aging of the Female Reproductive System

Dieter Egli[1] and Hidenori Akutsu[2]*

[1]The New York Stem Cell Foundation Laboratory, New York, USA
[2]Department of Reproductive Biology, Center for Regenerative Medicine, National Research Institute for Child Health and Development, Tokyo 157-8535, Japan

Abstract: Women in Japan and elsewhere are increasingly delaying childbearing. The percentage of Japanese women giving birth to their first child at the age of 30 and above has increased from 28.9% in 1980 to 58.9% in 2009. As a result, women seeking to conceive one or more children increasingly do so when the effects of reproductive aging first become noticeable. Female fertility begins to decrease after 30 years of age, falls markedly after the age of 35 in association with an increased risk of complications during pregnancy and of chromosomal abnormalities in the offspring, and most women become infertile after the age of 40. In parallel, the number of assisted reproductive technology (ART) treatment cycles in Japan is steadily increasing, from 37,455 in 1997 to 190,613 in 2008, according to a survey by the Japan Society of Obstetrics and Gynecology. Because of the high emotional and social toll of infertility on couples and the demographic consequences of a decrease in fecundity, it is imperative to better understand the biology of reproductive aging. Here we review current knowledge about the decline of female fertility during aging and discuss the implications for infertility treatments.

Key words: Aging, Oocyte, Chromosomal abnormality, Oocyte donation

Introduction

Delayed childbearing is a characteristic trend in developed countries, and it is related to socioeconomic, cultural and environmental factors. It also correlates with an increase in couples seeking treatment for infertility, and a decrease in the number of births per woman from 1.75 in 1980 to 1.37 in 2009 in Japan, according to The Ministry of Health, Labour and Welfare (Fig. 1) [1]. It is well known that advanced maternal age has a negative effect on fecundity and increases the risk of adverse outcomes during pregnancy [2, 3]. The decrease in fertility and the increase of fetal loss with maternal age occur irrespective of reproductive history [4–6]. A hallmark of advanced maternal age is an increase in karyotypic abnormalities in oocytes [7], preimplantation embryos [8], and spontaneous abortuses as well as in live-born infants [9]. While this is well documented in the literature and known to contribute to female infertility, the molecular cause of these abnormalities is not well understood. Further research is required to increase our understanding of oocyte biology and to advance novel treatment options for infertility.

Development and Maturation of Human Oocytes

In humans, primordial germ cells arising extragonadally migrate to the ovarian rudiment where they continue to proliferate as oogonia. The germ cells complete their differentiation process into oocytes after a final round of DNA replication and enter meiosis. These oocytes arrest their cell cycle at the prophase of meiosis after initiating recombination between paired homologous chromosomes, a stage termed the diplotene stage. Oocytes at the diplotene stage become organized in primordial follicles composed of mesenchymal cells and pregranulosa cells. A finite store of primordial follicles is established around the time of birth, after which no new germ cells or follicles are formed. The oocytes in the primordial follicles remain arrested at the prophase for many years until the final stages of folliculogenesis prior to ovulation. Under hormonal control at puberty and throughout the reproductive life span, some primordial follicles are

Received: July 15, 2011
Accepted: July 25, 2011
*To whom correspondence should be addressed.
e-mail: hakutsu@nch.go.jp

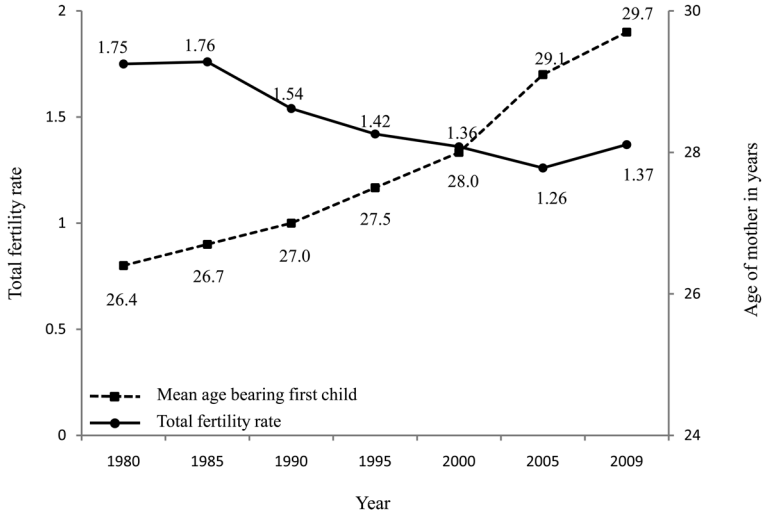

Fig. 1. Total fertility rate and mother's age when bearing the first child in Japan
The figure shows the average number of children per woman in Japan from 1980 to 2009 (line with circles), and the average age of the women at the age of the first child (line with squares). For many women, the potential birth of a second or third child - which is required to maintain population levels - falls into the period of declining ability to conceive (see Fig. 2).

recruited to form follicles with several layers of granulosa cells and a zona pellucida surrounding the oocyte. Oocyte enlargement begins with further proliferation of granulosa cells and theca cell differentiation. During follicle maturation, these consecutive changes eventually result in a large antral follicle characterized by a pool of extracellular fluid and differentiation of granulosa cell layers into mural cells and cumulus cells. Twenty-four (24) to 36 h prior to ovulation triggered by a surge of luteinizing hormone, the oocyte resumes the cell cycle and progresses from the prophase of meiosis I to the metaphase of meiosis II where the cell cycle is once more arrested until fertilization. Typically, a single oocyte is ovulated each month and the remaining incompletely matured follicles and oocytes undergo cell death, a process termed atresia.

The Oocyte Pool Decreases during Aging

Repeated follicle recruitment and atresia with each menstrual cycle result in a gradual decrease in the number of oocytes. The decrease in the number of available germ cells in fact already begins before birth; about 20 % of the germ cells die prenatally due to apoptosis, and at puberty approximately 400,000 follicles remain [10–12]. In each menstrual cycle, approximately 1,000 oocytes are lost. The rate of oocyte decline appears to follow a biphasic pattern; it remains relatively constant from birth and much of the reproductive lifespan, then accelerates in the late 30s, eventually resulting in menopause [13]. At menopause, only about 1,000 oocytes remain [14]. Using this biphasic model of ovarian decline and measurements of ovarian size it is possible to estimate the ovarian reserve and predict the age of onset of ovarian exhaustion [15]. More recently, a new model of the gradual decline in oocyte numbers was proposed based on modern stereology methods [16]. The new model by Hansen *et al.*, is biologically plausible because physiological aging processes tend to be gradual, even though the oocyte count at any particular age is similar to the model of Faddy and Gosden [17]. In either model, the fundamental aspect of reproductive aging in women is a decrease in the number of ovarian follicles and ultimately their depletion, resulting in menopause. Menopause sets the upper age limit of female fertility, with an average onset at the age of about 51 years [18]. More precise estimates of the total number of remaining follicles in the ovary and the ability to predict the remaining reproductive life span would be very informative for decisions regarding infertility treatments.

Fig. 2. Live births per ART cycle using own oocytes and donor oocytes
Comparison of the percentages of transfers resulting in live births in ART cycles using embryos from donor eggs and own eggs in relation to age. It shows that the percentage of transfers resulting in live births for cycles using embryos from own eggs declines as women age (line with circles). In contrast, the percentage of transfers that resulted in live births for cycles using embryos from donor eggs (line with squares) remained fairly constant at or above 50% for all ages. Data were drawn from the 2008 Centers for Disease Control and Prevention (CDC) ART report [27].

Interestingly, female fertility is much reduced when still sizable numbers of oocytes are present in the ovary, and years before menstrual cycles become irregular, suggesting that follicle counts are not the primary limit to conception. Rather, other age-associated changes, such as the functionality of the oocyte may be important determinants of female fertility. The approximately 10–15 years of decreasing fertility leading up to the onset of menopause is clinically most relevant, as most women seeking treatment for infertility fall in this group.

Oocyte Quality or Uterine Environment?

To distinguish between the effects of aging on oocytes and the other parts of the reproductive system, Talbert and Krohn performed an elegant experiment in mice: they transferred embryos from young mice (2–7 months of age) into old hosts (13–24 months of age), and the converse, transferred embryos obtained from old mice into young hosts [19]. Embryos from young mice did not survive well in old hosts, but embryos from old and young mice survived equally well in young hosts. They concluded that the decline of litter size in aged mice is due to an unfavorable uterine environment, not to a decrease in oocyte function. Consistent with that, older mice were subsequently shown to have fewer implantation sites and a high rate of embryo resorption, possibly because of decreased hormonal support [20, 21].

The effects of aging on oocytes and other parts of the reproductive system were clarified in humans when oocyte donation was first used for the treatment of infertility. The result was unlike in mice. Remarkably, embryos generated from oocytes of young women implanted efficiently into women above the age of 40 [22, 23] or even into post-menopausal women [24]. In countries with permissive legislation, oocyte donation has become an established part of clinical practice, especially in the USA, where more than 8,000 cycles of oocyte donations are performed each year [25]. Extensive clinical experience of oocyte donation to women of advanced age confirms the conclusions made in the initial studies, that the ability of the human uterus to support a pregnancy remains high well beyond the limits of natural conception and that a decrease in oocyte quality leads to the decline of female fertility (Fig. 2) [26].

Frequent Fetal Loss due to Aneuploidy

Schwartz and colleagues found that in women of 25 years of age and less, artificial insemination resulted in 11% of women becoming pregnant [2]. Assuming that fertilization by sperm is efficient *in vivo*, this suggests that even in young and healthy women, the majority of fertilized zygotes is spontaneously lost [28]. As many as 31% of pregnancies are lost after implantation, most of them without being detected [29]. Meulenbreuk and Geraedts analyzed the karyotypes of cells explanted from 81 spontaneous abortuses, and found that 50 of them (62%) had an abnormal number of chromosomes [30]. Other studies similarly found frequent numerical aneuploidies [31], and a large body of literature now estimates the incidence of aneuploidy at 35% in spontaneous abortions and 4% in stillbirths [32, 33]. This establishes chromosomal aneuploidy as a major cause of fetal loss after implantation. Fetal loss is more frequent with advanced maternal age [34], irrespective of reproductive history such as the number of previous miscarriages and parity [4–6]. In parallel, the incidence of numerical chromosomal abnormalities increases dramatically with advanced maternal age [34].

Chromosome Segregation in Human Oocytes

For accurate chromosome segregation, the maintenance of physical connections in homologous chromosomes and the attachment of centromeres of sister chromatids to the same spindle poles are required during the meiotic process. Although meiosis proceeds according to the same principle in both males and females, there are important differences between the sexes: meiosis and meiotic recombination is initiated in females before birth and the cell cycle arrests until just before ovulation, while in males, spermatogonial stem cells undergo frequent divisions in the adult, and meiosis is completed within 25 days [35]. The prolonged cell cycle arrest in oocytes may lead to an accumulation of cell division defects the oocyte is unable to correct, while in sperm, defects in chromosome segregation are selected against by frequent cell divisions. Pellestor and colleagues analyzed more than 3,000 unfertilized human MII oocytes of patients undergoing IVF treatment and found that the percentage of oocytes with numerical aneuploidies remained at 10% or below until about 34 years of age, and then increased until at the age of 45, 100% of oocytes were aneuploid [7]. This strongly suggests that the first meiotic cell division is a primary cause for karyotypic abnormalities. Two mechanisms explaining age-related chromosomal aneuploidy in oocytes and embryos have been proposed: non-disjunction and predivision [36]. Non-disjunction is the failure to segregate homologous chromosomes during meiosis I. Non-disjunction may also occur at meiosis II or subsequent embryonic mitoses. Predivision is the premature separation of homologs before anaphase I, leading to physically unconnected chromatids in the meiosis I oocyte [37, 38]. Both non-disjunction and predivision would result in numerical chromosomal aberrations in the oocyte and the embryo.

Using genetic variants it has become possible to identify the origin of additional or lost chromosomes and point to the stage of meiosis at which it occurred [32]. The reductional division at meiosis I will normally result in hemizygosity and the loss of parental heterozygosity in centromeric markers. Trisomies resulting from non-disjunction at meiosis I are expected to carry centromeric markers originating from three grand-parents. Trisomies originating due to chromosome segregation errors at meiosis II or in subsequent embryonic mitoses would result in cells with centrometic markers originating from 2 grand-parents (1 passed on maternally, 1 passed on paternally), one of which is present in two copies. Hassold and colleagues analyzed the origin of trisomies in spontaneous abortuses and found that depending on the chromosome, between 33 % (chromosome 18) and 100 % (chromosome 16) were caused by errors occurring in meiosis I of the oocyte [39, 40]. The contribution of meiosis I in sperm was comparatively low, with 0 % for most autosomes, and 50 % for the sex chromosomes. These findings establish chromosomal segregation defects at meiosis I as a hallmark of oocyte aging.

Interestingly, chromosomal imbalance is a rare phenomenon in most lower species such as yeast (1 in 10,000) and Drosophila (1 in 6,000–7,000), and even in mice, the number of chromosomally abnormal oocytes is much lower, suggesting that frequent errors during meiosis are specific to human [33]. However, in these other species, the period between initiation of meiosis and reproduction is days or months, and is not nearly as long as in humans where it is up to 50 years.

Chromosomal Abnormalities during Embryonic Mitosis

The finding that chromosomal segregation errors occur frequently in human oocytes led to the perception that most if not all common segregation errors occur

during meiosis [33]. Because the time point of karyotype analysis in these studies was months after fertilization, there was inevitably selection which eliminated karyotypes incompatible with cellular viability. More recent data obtained from human preimplantation embryos days after fertilization have revealed frequent errors in chromosome segregation during mitosis. Chromosomal aneuploidies in human preimplantation embryos were first described in the early nineties [41]. Munne and colleagues found that aneuploidies were frequent and often mosaic, meaning they were not found in all blastomeres, an indication that they arose during mitosis. A subsequent study examining chromosomes X, Y, 13, 15, 16, 17, 18, 21 and 22 in more than 6,000 embryos of IVF patients found that 80% of embryos from women aged 41 years or older were abnormal for these chromosomes [42]. This number was lower for women aged 35 years or younger, with 60% karyotypically abnormal embryos. Interestingly, the majority of these abnormalities was mosaic and had occurred in mitosis. These studies used fluorescent probes for a subset of chromosomes, limiting the analysis to only part of the genome. More recently, array based technologies have allowed the examination of copy number variation across the entire genome. Vanneste and colleagues found that 91% of human embryos in embryos of good morphology, obtained from women under 35 years of age, contained blastomeres with chromosomal abnormalities. In addition to numerical abnormalities, they also found frequent deletions, duplications and amplifications that were reciprocal in sister blastomeres [43]. They concluded that post-zygotic chromosome instability explains low human fecundity and is a frequent cause of chromosomal disorders. Karyotypic abnormalities are significantly less frequent at the blastocyst stage, than at the early cleavage stages [44], suggesting some chromosomal abnormalities are negatively selected because they are incompatible with development to the blastocyst stage. Most abnormal karyotypes appear to be incompatible with development or cellular viability and are gradually eliminated, leading to the observed decrease in karyotypic abnormalities as development proceeds.

Determining the karyotype of preimplantation embryos has become a common part of clinical practice. However, because karyotypic abnormalities are so prevalent and often mosaic in cleavage stage embryos, the benefit of karyotyping to increase the rate of successful pregnancies has been limited to ART patients with recurrent miscarriages, in particular couples carrying chromosomal translocations [45]. Determining karyotypes at the blastocyst stage may be effective at eliminating karyotypes compatible with cellular viability, but may be incompatible with development to term.

Altered Cell Cycle Control in Aged Oocytes

Laboratory studies of the mouse have demonstrated that the capacity of an oocyte for competence in both fertilization and embryonic development is acquired in a stepwise fashion during the late stages of folliculogenesis [46]. Entry into meiosis is controlled by MPF (maturation-promoting factor) complex composed of Cdk1/cyclin B through differential phosphorylation. During maturation, the levels of MFP increase, eventually leading to nuclear envelope breakdown, chromosome condensation and spindle assembly. Once all chromosomes are aligned at the metaphase plate, cyclin B is degraded, the chromosomes begin to segregate and the polar body is extruded. Meiotic maturation, including spindle formation, polar body formation, and meiosis II arrest, also requires the activation of MAP (mitogen-activated protein kinase) kinases [47]. It was reported that oocytes of aged females tend to progress to anaphase I earlier than those of young females [48]. A common defect in aged oocytes (both human and mouse) involve spindle-shortening or disorganization and failure of spindle-assembly and chromosome alignment [49, 50]. Also oocytes from aged women have a less stringent spindle checkpoint and fail to detect misaligned chromosomes on the spindle. These observations suggest that alterations in cell cycle control are responsible for the increased rate of errors in chromosome segregation and non-disjunction and predivision in aged mammalian oocytes [36].

Aging of the Oocyte Cytoplasm

Defects in chromosome segregation are major, but it is unlikely to be the only factor involved in the decline of oocyte quality. In human oocytes, not only nuclear defects, but also the cytoplasmic condition may impact on embryo development during reproductive aging. Cytoplasmic aging has been associated with a decline in mitochondrial functions and a reduction in the content of transcripts from genes involved in spindle assembly [51, 52]. Mitochondria convert metabolites into ATP, which is essential for numerous cellular processes and for the oocyte's energy. It has been postulated that an

accumulation of deletions or mutations in mitochondrial DNA occurs in normal oocytes during the long arrest in meiosis I [53]. A deficiency in quantity and quality of mitochondria might compromise ATP-dependent energy supply and spindle assembly. This perturbation of the fidelity of chromosome segregation could be related to the likelihood of aneuploidy. Although mitochondrial function may deteriorate during aging, it is worth noting that the content of mitochondrial DNA varies considerably in individual human oocytes, even when collected from the same person [54]. A possible way to distinguish between nuclear defects and cytoplasmic defects of aged oocytes would be to transfer the nucleus of an aged oocyte into a 'young' oocyte. Mitsui *et al*. transferred oocyte genomes from mice aged 10–12 months into oocytes of young mice aged 3–5 months [55]. They found that development to term increased from 6.3% for aged oocytes to 27.1% for the reconstructed oocytes. Therefore, at least in mice, aging of the cytoplasm appears to be an important aspect of reproductive aging. Whether a similar manipulation would rescue the developmental potential of human oocytes is currently unclear.

Conclusion

Totipotential developmental competence, or the ability to give rise to all cell types of the body, is a unique property of human oocytes. However, we have scant knowledge about human oocytes at the molecular level. This is in part due to the difficulty in obtaining oocytes for research, which is heavily dependent on local regulations. However, a better understanding of human oocyte biology could greatly improve ART treatments, and possibly help to preserve female fertility to an older age. Such an extension is currently possible using oocytes donated by younger women, and may also be possible by cryopreserving human oocytes at a young age and using them at an older age. Both of these treatments are somewhat contentious and depend on permissive local regulations.

Ovarian aging impinges greatly upon the high prevalence of unintended childlessness. It will be important to further investigate the molecular mechanisms responsible for chromosomal abnormalities in oocytes and embryos. Furthermore, we need new approaches to study the quality of human oocytes. A host of new techniques has been developed in the last decade, such as genome-wide gene expression analysis, analysis of small RNAs, epigenetic analysis, and assays for energy homeostasis and proteomics. If these could be applied to human oocytes, we could greatly increase our understanding of human oocyte biology. Such studies might also reveal molecular markers for predicting the ability to conceive, which is not currently possible. Identification of robust and accurate markers of ovarian aging would also help in family planning and could guide decisions in ART treatment.

Acknowledgements

We thank Akihiro Umezawa, Kenji Miyado, Keiichi Yoshida, Shoko Sato, and other members of our laboratory for stimulating discussion. This work was supported by grants from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan; a grant from the Ministry of Health, Labour and Welfare Sciences (MHLW) to HA and by a Grant-in-Aid for Challenging Exploratory Research (22659304) to HA. D.E. is a NYSCF senior reserch fellow. The authors declare no conflict of interest.

References

1) Statistics Bureau, Director-General for Policy Planning (Statistical Standards) and Statistical Research and Training Institute: The Statistical Handbook of Japan 2010. http://www.stat.go.jp/english/data/handbook/pdf/c02cont.pdf
2) Schwartz, D. and Mayaux, M.J. (1982): Female fecundity as a function of age: results of artificial insemination in 2193 nulliparous women with azoospermic husbands. Federation CECOS. N. Engl. J. Med., 306, 404–406.
3) Menken, J., Trussell, J. and Larsen, U. (1986): Age and infertility. Science, 233, 1389–1394.
4) Fretts, R.C., Schmittdiel, J., McLean, F.H., Usher, R.H. and Goldman, M.B. (1995): Increased maternal age and the risk of foetal death. N. Engl. J. Med., 333, 953–957.
5) Berkowitz, G.S., Skovron, M.L., Lapinski, R.H. and Berkowitz, R.L. (1990): Delayed childbearing and the outcome of pregnancy. N. Engl. J. Med., 322, 659–664.
6) Nybo Andersen, A.M., Wohlfahrt, J., Christens, P., Olsen, J. and Melbye, M. (2000): Maternal age and fetal loss: population based register linkage study. BMJ, 320, 1708–1712.
7) Pellestor, F., Andréo, B., Arnal, F., Humeau, C. and Demaille, J. (2003): Maternal aging and chromosomal abnormalities: new data drawn from in vitro unfertilized human oocytes. Hum. Genet., 112, 195–203.
8) Munné, S., Chen, S., Colls, P., Garrisi, J., Zheng, X., Cekleniak, N., Lenzi, M., Hughes, P., Fischer, J., Garrisi, M., Tomkin, G. and Cohen, J. (2007): Maternal age, morphology, development and chromosome abnormalities in over 6,000 cleavage-stage embryos. Reprod. Biomed.

Online, 14, 628–634.
9) Hook, E.B., Cross, P.K. and Schreinemachers, D.M. (1983): Chromosomal abnormality rates at amniocentesis and in live-born infants. JAMA, 249, 2034–2038.
10) Faddy, M.J. and Gosden, R.G. (2003): Modelling the dynamics of ovarian follicle utilization throughout life. In: Biology and Pathology of the Oocyte (Trounson, A.O. and Gosden, R.G., eds.), pp.44–52, Cambridge University Press, Cambridge.
11) Hsueh, A.J., Billig, H. and Tsafriri, A. (1994): Ovarian follicle atresia: a hormonally controlled apoptotic process. Endocr. Rev., 15, 707–724.
12) McGee, E.A. and Hsueh, A.J. (2000): Initial and cyclic recruitment of ovarian follicles. Endocr. Rev., 21, 200–214.
13) Faddy, M.J., Gosden, R.G., Gougeon, A., Richardson, S.J. and Nelson, J.F. (1992): Accelerated disappearance of ovarian follicles in mid-life: implications for forecasting menopause. Hum. Reprod., 7, 1342–1346.
14) Faddy, M.J. and Gosden, R.G. (1996): A model conforming the decline in follicle numbers to the age of menopause in women. Hum. Reprod., 11, 1484–1486.
15) Wallace, W.H., and Kelsey, T.W. (2004): Ovarian reserve and reproductive age may be determined from measurement of ovarian volume by transvaginal sonography. Hum. Reprod., 19, 1612–1617.
16) Hansen, K.R., Knowlton, N.S., Thyer, A.C., Charleston, J.S., Soules, M.R. and Klein, N.A. (2008): A new model of reproductive aging: the decline in ovarian non-growing follicle number from birth to menopause. Hum. Reprod., 23, 699–708.
17) Broekmans, F.J., Soules, M.R. and Fauser, B.C. (2009): Ovarian aging: mechanisms and clinical consequences. Endocr. Rev., 30, 465–493.
18) McKinlay, S.M., Brambilla, D.J. and Posner, J.G. (1992): The normal menopause transition. Maturitas, 14, 103–115.
19) Talbert, G.B. and Krohn, P.L. (1966): Effect of maternal age on viability of ova and uterine support of pregnancy in mice. J. Reprod. Fertil., 11, 399–406.
20) Harman, S.M. and Talbert, G.B. (1970): The effect of maternal age on ovulation, corpora lutea of pregnancy, and implantation failure in mice. J. Reprod. Fertil., 23, 33–39.
21) Holinka, C.F., Tseng, Y.C. and Finch, C.E. (1979): Reproductive aging in C57BL/6J mice: plasma progesterone, viable embryos and resorption frequency throughout pregnancy. Biol. Reprod., 20, 1201–1211.
22) Navot, D., Bergh, P.A., Williams, M.A., Garrisi, G.J., Guzman, I., Sandler, B. and Grunfeld, L. (1991): Poor oocyte quality rather than implantation failure as a cause of age-related decline in female fertility. Lancet, 337, 1375–1377.
23) Sauer, M.V., Paulson, R.J. and Lobo, R.A. (1990): A preliminary report on oocyte donation extending reproductive potential to women over 40. N. Engl. J. Med., 323, 1157–1160.
24) Sauer, M.V., Paulson, R.J. and Lobo, R.A. (1993): Pregnancy after age 50: application of oocyte donation to women after natural menopause. Lancet, 341, 321–323.
25) Technology, S.f.A.R. (2007): Assisted reproductive technology in the United States: 2001 results generated from the American Society for Reproductive Medicine/ Society for Assisted Reproductive Technology registry. Fertil. Steril., 87, 1253–1266.
26) Sauer, M.V. and Klein, J.U. (2011): Egg Donation and Surrogacy. In: Infertility (Emre, S., ed.), pp.135–145, Wiley-Blackwell, Oxford.
27) Centers for Disease Control and Prevention (2010): 2008 Assisted reproductive technology success rates: National Summary and Fertility Clinic Reports. http://www.cdc.gov/art/ART2008/sect4_fig46-50.htm#f47
28) Kline, J., Stein, Z. (1985): Very early pregnancy. In: Reproductive toxicology (Dixon, R.L., ed.), pp. 251–265, Raven, New York.
29) Wilcox, A.J., Weinberg, C.R., O'Connor, J.F., Baird, D.D., Schlatterer, J.P., Canfield, R.E., Armstrong, E.G. and Nisula, B.C. (1988): Incidence of early loss of pregnancy. N. Engl. J. Med., 319, 189–194.
30) Meulenbroek, G.H. and Geraedts, J.P. (1982): Parental origin of chromosome abnormalities in spontaneous abortions. Hum. Genet., 62, 129–133.
31) Carr, D.H. (1963): Chromosome studies in abortuses and stillborn infants. Lancet, 2, 603–606.
32) Hassold, T., Abruzzo, M., Adkins, K., Griffin, D., Merrill, M., Millie, E., Saker, D., Shen, J. and Zaragoza, M. (1996): Human aneuploidy: incidence, origin, and etiology. Environ. Mol. Mutagen., 28, 167–175.
33) Hassold, T. and Hunt, P. (2001): To err (meiotically) is human: the genesis of human aneuploidy. Nat. Rev. Genet., 2, 280–291.
34) Hassold, T. and Chiu, D. (1985): Maternal age-specific rates of numerical chromosome abnormalities with special reference to trisomy. Hum. Genet., 70, 11–17.
35) Heller, C.G. and Clermont, Y. (1963): Spermatogenesis in Man: An Estimate of Its Duration. Science, 140, 184–186.
36) Eichenlaub-Ritter, U. (2003): Aneuploidy in ageing oocytes and after toxic insult. In: Biology and Pathology of the Oocyte (Trounson, A.O. and Gosden, R.G., eds.), pp.220–257, Cambridge University Press, Cambridge.
37) Angell, R.R. (1991): Predivision in human oocytes at meiosis I: a mechanism for trisomy formation in man. Hum. Genet., 86, 383–387.
38) Angell, R. (1997): First-meiotic-division nondisjunction in human oocytes. Am. J. Hum. Genet., 61, 23–32.
39) Hall, H.E., Surti, U., Hoffner, L., Shirley, S., Feingold, E. and Hassold, T. (2007): The origin of trisomy 22: evidence for acrocentric chromosome-specific patterns of nondisjunction. Am. J. Med. Genet. A., 143A, 2249–2255.
40) Hassold, T., Hall, H. and Hunt, P. (2007): The origin of human aneuploidy: where we have been, where we are going. Hum. Mol. Genet., 16, R203–208.
41) Munné, S., Lee, A., Rosenwaks, Z., Grifo, J. and Cohen, J. (1993): Diagnosis of major chromosome aneuploidies in human preimplantation embryos. Hum. Reprod., 8, 2185–2191.
42) Munné, S., Chen, S., Colls, P., Garrisi, J., Zheng, X.,

Cekleniak, N., Lenzi, M., Hughes, P., Fischer, J., Garrisi, M., Tomkin, G., Cohen, J. (2007): Maternal age, morphology, development and chromosome abnormalities in over 6,000 cleavage-stage embryos. Reprod. Biomed. Online, 14, 628–634.

43) Vanneste, E., Voet, T., Le Caignec, C., Ampe, M., Konings, P., Melotte, C., Debrock, S., Amyere, M.,Vikkula, M., Schuit, F., Fryns, J.P., Verbeke, G., D'Hooghe, T., Moreau, Y. and Vermeesch, J.R. (2009): Chromosome instability is common in human cleavage-stage embryos. Nat. Med., 15, 577–583.
44) Fragouli, E., Lenzi, M., Ross, R., Katz-Jaffe, M., Schoolcraft, W.B. and Wells, D. (2008): Comprehensive molecular cytogenetic analysis of the human blastocyst stage. Hum. Reprod., 23, 2596–2608.
45) Lalioti, M.D. (2008): Can preimplantation genetic diagnosis overcome recurrent pregnancy failure? Curr. Opin. Obstet. Gynecol., 20, 199–204.
46) Eppig, J.J. (1996): Coordination of nuclear and cytoplasmic oocyte maturation in eutherian mammals. Reprod. Fertil. Dev., 8, 485–489.
47) Heikinheimo, O. and Gibbons, W.E. (1998): The molecular mechanisms of oocyte maturation and early embryonic development are unveiling new insights into reproductive medicine. Mol. Hum. Reprod., 4, 745–756.
48) Eichenlaub-Ritter, U. (2000): The determinants of non-disjunction and their possible relationship to oocyte ageing. In: Studies in Profertility Series 9: Female Reproductive Ageing (Te Velde, E.R., Pearson, P.L. and Broekmans, F.J., eds.), pp. 149–184, Parthenon, New York.
49) Liu, L. and Keefe, D.L. (2002): Ageing-associated aberration in meiosis of oocytes from senescence-accelerated mice. Hum. Reprod., 17, 2678–2685.
50) Battaglia, D.E., Goodwin, P., Klein, N.A., and Soules, M.R. (1996): Influence of maternal age on meiotic spindle assembly in oocytes from naturally cycling woman. Hum. Reprod., 11, 2217–2222.
51) Eichenlaub-Ritter, U., Vogt, E., Yin, H. and Gosden, R. (2004): Spindles, mitochondria and redox potential in ageing oocytes. Reprod. Biomed. Online, 8, 45–58.
52) Steuerwald, N., Cohen, J.,Herrera, R.J., Sandalinas, M. and Brenner, C.A. (2001): Association between spindle assembly checkpoint expression and maternal age in human oocytes. Mol. Hum. Reprod., 7, 49–55.
53) Schon, E.A., Kim, S.H., Ferreira, J.C., Magalhaes, P., Grace, M., Warburton, D., and Gross, S.J. (2000): Chromosomal non-disjunction in human oocytes: is there a mitochondrial connection. Hum . Reprod., 15(Suppl 2), 160–172.
54) Reynier, P., May-Panloup, P., Chrétien, M.F., Morgan, C.J., Jean, M., Savagner, F., Barrière, P. and Malthièry, Y. (2001): Mitochondrial DNA content affects the fertilizability of human oocytes. Mol. Hum. Reprod., 7, 425–429.
55) Mitsui, A., Yoshizawa, M., Matsumoto, H. and Fukui, E. (2009). Improvement of embryonic development and production of offspring by transferring meiosis-II chromosomes of senescent mouse oocytes into cytoplasts of young mouse oocytes. J. Assist. Reprod. Genet., 26, 35–39.