J Assist Reprod Genet (2013) 30:269–274
DOI 10.1007/s10815-012-9905-9

GENETICS

A study of meiotic segregation in a fertile human population following ovarian stimulation with recombinant FSH-LH

Esther Velilla · Silvia F. Fernández · Jordi Suñol · Marisa López-Teijón

Received: 14 September 2012 / Accepted: 25 November 2012 / Published online: 10 January 2013

Abstract
Objective The aim of the study is to investigate the meiotic segregation in fresh eggs from anonymous egg donors and to analyze the baseline levels of aneuploidy in this population.
Results The study includes the largest series of donor eggs so far studied: 203 eggs from donors aged between 20 and 31 years. No diagnosis was obtained in 10.8 % of cases (22/203). The biopsy of the first and second polar bodies was completed in a sequential manner on day 0 and day 1 of embryo development. Chromosomes 13, 16, 18, 21 and 22 are analyzed by means of the FISH test. The diagnosable fertilized eggs gave an aneuploidy rate of 19.1 % (31/162), with 83.8 % (26/31) of the errors produced during meiosis I, 12.9 % (4/31) produced during meiosis II, and 3.2 % (1/31) produced during both meiosis I and II. The premature division of sister chromatids is the main source of meiotic error during Meiosis I, resulting in the creation of oocyte aneuploidy.
Conclusions FISH analysis of the first and second polar body in donor oocytes gave an aneuploidy rate of 19.1 %. This study shows the majority of errors occur during Meiosis I.

Capsule FISH analysis of the first and second polar body in donor oocytes gave an aneuploidy rate of 19.1%. This study shows the majority of errors (83.8%) occur during Meiosis I.

E. Velilla (✉) · S. F. Fernández · J. Suñol
Institut Marquès, C/Manuel Girona nº 33
La Masia- Clínica CIMA,
Barcelona 08034, Spain
e-mail: esther.velilla@institutomarques.com

M. López-Teijón
Institut Marquès and Leonardo Marquès
Foundation, Barcelona, Spain

Keywords Aneuploidy · Egg donation · Polar body analysis · IVF · PGD

Introduction

The incidence of aneuploidy in the eggs of young and fertile women has not been extensively studied. We present an observational study using Preimplantation Genetic Diagnosis for aneuploidy screening (PGD) where the incidence of aneuploidy in donor eggs is assessed by examining the first and second polar bodies by means of the FISH technique. The application of this technique to eggs from a donor program offers us real insights into the true incidence of aneuploidy in young women with no known fertility problems.

Earlier studies have shown that, in general, autosomal aneuploidy occurs during the meiotic divisions of the oocytes, and principally in meiosis I [13, 20]. The gradual degradation of the cohesins during meiosis I could be one of the factors that leads to the creation of aneuploid oocytes and this mechanism is dependent upon maternal age [24]. Some papers have also described an interchromosomal effect [14], where the presence of chromatids isolated during the meiotic process could affect the normal segregation process of the other chromosomes.

However, although the percentage of aneuploidy increases in relation to maternal age, the prevalence of aneuploidy in a young and potentially fertile population is not known. Nor is it known which is the most frequent chromosomal abnormality seen, nor the significance of these abnormalities in cases of infertility. In order to study the fertile population, some authors have focused their attention on the embryos created following gamete donation and analysed these using PGD. These authors report a 56–57 % incidence of chromosomal anomalies in this population [19, 25]. However, this high percentage of chromosomal alterations could be due to two main factors: (i) the paternal contribution, and (ii) the mitotic errors that can occur

in the development of an embryo. Reis Soares et al. [25] attributes it to the aggressive stimulation protocol used for the egg donors and concludes that when a high number of eggs are obtained, these eggs show a high level of aneuploidy.

Currently the most accurate way to assess aneuploidy in oocytes is to analyze their two polar bodies by means of preimplantation genetic diagnosis for aneuploidy screening. This method gives a result that is specific to each oocyte, and if embryo aneuploidy is diagnosed thereafter this indirectly allows the paternal and mitotic contribution to be assessed.

Some groups have focused their work on the genetic study of donated oocytes that come from couples undergoing assisted reproduction for problems of infertility [6, 7, 9]. Donated immature oocytes that were subsequently matured in Vitro have been the subject of other studies [6, 7, 9–11]. However, the majority of these studies analyzes only the first polar body and therefore only studies the first phase of the meiotic process, and do not evaluate what may be happening during Meiosis II.

Furthermore, it is important to point out the biased nature of the study populations, given that the women providing the oocytes are themselves patients undergoing assisted reproduction, a large proportion of whom carry a high risk of having chromosomal anomalies secondary to advanced maternal age, and who therefore are not representative of the fertile population in general. The in vitro maturation process itself has also been shown to occasionally induce abnormal chromosomal segregation, and therefore in this group there is a risk of overestimating the level of genetic abnormality [18, 26, 33].

Recently the Kuliev Group, pioneers in the study of oocytes and the application of this technology, published the largest review of oocyte studies to date, covering results from 20,986 oocytes [12]. In this work they report that for 30.4 % of the oocytes analyzed, meiotic errors occurred during meiosis I (detected in the first polar body), for 39.8 % meiotic errors only occurred during meiosis II (detected by assessment of the second polar body) and for 29.8 % the meiotic errors occurred in both meiosis I and II (chromosomal anomalies were detected in both the first and the second polar bodies). This result implies that the study of both polar bodies is of vital importance, given that only 60.2 % of the chromosomal alterations that exist in a fertilized egg would have been detected if only one is studied. Recently Fragouli et al. [8] published a study of all chromosomes in oocytes originating from donors by means of array CGH. In this study they analyzed both the first polar body and the oocyte at the Metaphase II stage, and were therefore able to create a complete amplification of the genome. This enabled them to make a diagnosis in 64 % of the cases (7 % in the polar body and 55 % in the oocytes). The main limitation of this study is the partial analysis of the meiotic segregation process, given that they do not analyze the second polar body. The aneuploidy rate calculated following the end of first meiotic division was 2.8 %.

The objective of our study was therefore to investigate the process of meiotic segregation in egg donors who provide oocytes to the Egg Donation program at our clinic, and to analyze the baseline levels of aneuploidy in this population. This study provides the largest series yet studied of fresh oocytes in Metaphase II that originate from donors. In assessing the chromosomal load of both polar bodies the appropriate methodology is used to obtain a complete assessment of meiosis in these oocytes.

Materials and methods

Study population

A total of 17 anonymous donors from our Egg Donation program from January 2010 to March 2011 were included in this study. The average age was 26.06 years, with a range of 20–31 years. Prior to accepting them onto the donation program each donor underwent blood tests, clinical examination, and a complete history was taken regarding their own and familial medical history. This is routine practice in our centre, and complies with the demands made by Spanish law. Each donor completed one single cycle of donation for this study.

Ovarian stimulation

Ovarian stimulation took place using recombinant FSH and LH gonadotropins (Pergoveris®; Merck Serono, Geneva, Switzerland) at 225 IU of FSH and 112.5 IU of LH daily from the third day of the cycle. Recombinant gonadotrophins were used to induce the development of multiple follicles in the ovaries and to potentiate the maturation of the oocytes within. The high degree of purity of the medication used also prevented any additional substances from having effects which we could not control. FSH 225 IU was applied daily, a dose that was both appropriate for a young population and normal ovary morphology.

When the dominant follicle reached 14 mm in diameter (Fig. 1), GnRH antagonist treatment was started with Cetrotide 0,25 mg in subcutaneous (SC) injections a day (Cetrotide®; Merck Serono, Geneva, Switzerland). Ovulation was provoked using 0.3 mg SC injections of triptorelin acetate (Decapeptyl, Ipsen Pharma-biotech Ltd, Signes-France). Egg collection occurred 36 h later under sedation using an ultrasound guided transvaginal probe. This day-case procedure followed our standard technique and protocol for ambulatory minor surgery. The oocytes obtained were taken to the IVF laboratory, were cumulus cells were removed and then we proceeded with preimplantation genetic diagnosis for aneuploidy screening of the oocyte: two sequential biopsies were performed to extract the two polar bodies.

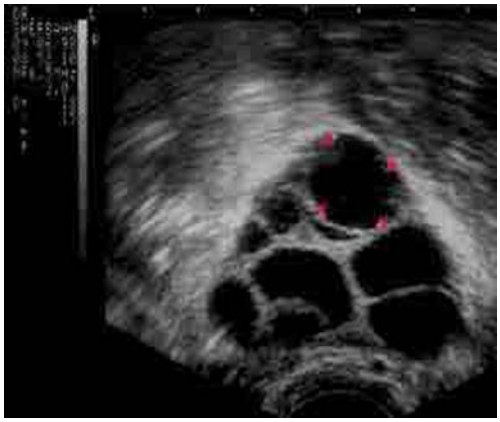

Fig. 1 Ovarian ultrasound. Ovarian stimulation: Scan control of follicular growing

Polar body analysis

The first polar body biopsy (PB-1) took place on the day of the egg collection but after ICSI had been performed, and the second biopsy (PB-2) took place 16–18 h later, once the fertilization rate had been calculated. The polar bodies were fixed using a solution of methanol and acetic acid in a 3:1 concentration.

The hybridization technique was performed for chromosomes 13, 16, 18, 21 and 22 (Abbot, Downers Grove, IL) and subsequent analysis took place according to the protocol described by [31].

In order to optimize the technique a second and third round of hybridization with subtelomeric probes was applied to those embryos for which it was not possible to obtain a result in the first round, either because there was no information obtained regarding a certain chromosome due to hybridization failure, or because the exact chromosomal load could not be calculated (Fig. 2).

The BX61 Olympus microscope was used to perform the analysis, with specific filters for DAPI/Green/Red, FITC, Texas red, Aqua, Blue, Orange and Gold. For the analysis of the hybridization signals the criteria described by Verlinsky and Kuliev [31] were used.

Statistical analysis

To compare the incidence of aneuploidy in the oocytes that were not fertilized with those that had been fertilized, a G-squared test was used ($p<0.05$). All statistical analyses were performed using the software package SPSS 15.0 and R.2.8.0.

Results

The doses of GnRH antagonists and the protocol followed to stimulate the ovaries did not create any side-effects nor induce hyperstimulation of the ovaries. Menstruation occurred a few days after egg collection (4 – 7 days). This protocol offers in our hands good results regarding tolerance and ease for the donors, safety in the treatment, and recovery of a acceptable quantity of mature oocytes.

From the 17 egg collections that took place, 268 oocytes were obtained and 231 (86.2 %) of these were in Metaphase II. The average number of oocytes collected per donor was 15.7, and the average number of oocytes in Metaphase II per donor was 13.5. Two hundred and three oocytes were included in that study and were biopsied.

Of the 203 oocytes that were biopsied, a diagnosis was obtained for 89.2 % of them (181/203). It was not possible to establish a diagnosis in 10.8 % (22/203) of the oocytes due to the presence of incomplete nuclei, secondary to the fragmentation of the polar bodies. Of the oocytes that provided a diagnosis, 10.5 % (19/181) did not fertilize, and therefore only the first polar body was biopsied. 89.5 % (162/181) fertilized correctly (creating zygotes) and in these cases both the first and second polar bodies were biopsied (Table 1). The results of the polar body analyses using the FISH technique is presented in Table 2. Of the 19 eggs that did not fertilize, 57.9 % were euploid (11/19) and 42.1 % were aneuploid (8/19). Whereas of the 162 oocytes that were successfully fertilized, 80.9 % were euploid (131/162) and 19.1 % were aneuploid (31/162). Although there was a higher rate of chromosomal alterations in the non fertilized oocytes when compared with those that fertilized, this difference was not statistically significant ($p>0.05$).

Following the study of segregation of the 8 abnormal oocytes and the 31 abnormal zygotes it was noted that 87.2 % of them (34/39) presented meiotic anomalies produced during Meiosis I.

But after fertilization, 83.8 % of the zygotes (26/31) presented meiotic errors produced during Meiosis I, 12.9 % of the zygotes (4/31) presented errors produced during Meiosis II and 3.23 % (1/31) showed errors produced both in Meiosis I and II.

Following the analysis of the errors produced during Meiosis I and II, 100 % of the errors produced during Meiosis I (34/34) were due to the premature separation of the sister chromatids, whilst 100 % (4/4) of the errors produced during Meiosis II were due to non disjunction of the sister chromatids (Table 2). Theoretical representation of the errors produced in meiosis I and II are shown in Fig. 3.

Discussion

In general, chromosomal anomalies that occur in the embryo prior to implantation halt that embryo's development and do not allow a gestation to take place. However, small proportions of embryos with chromosomal anomalies do evolve and allow us to perform prenatal diagnoses. Following the

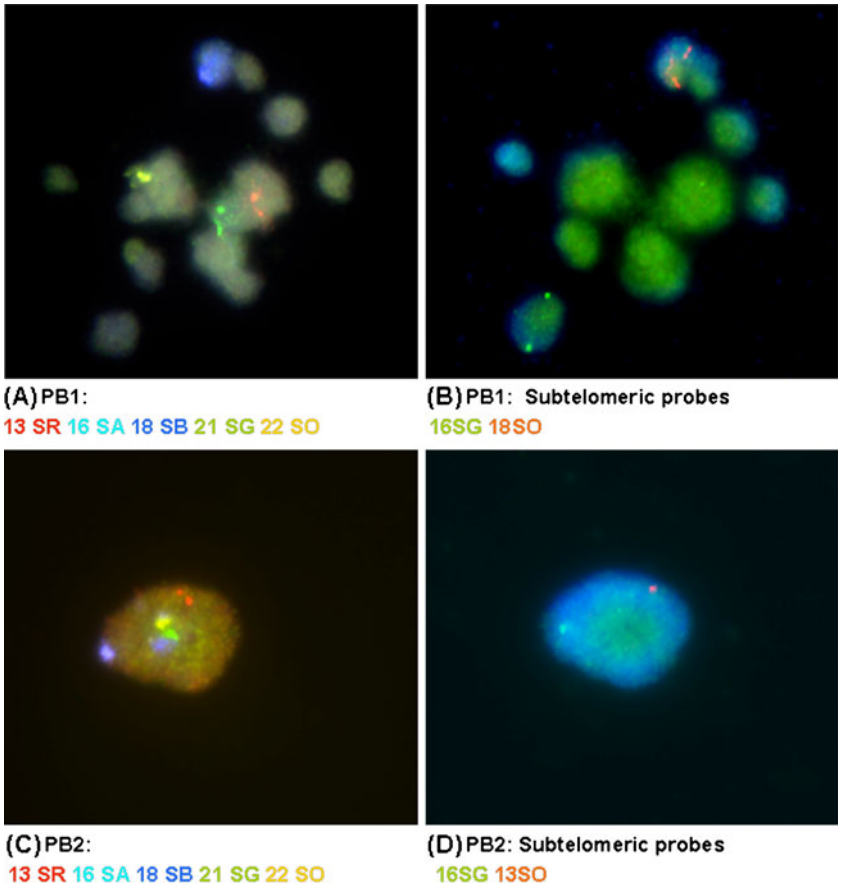

Fig. 2 Polar body hybridization. **a**: PB-1 first round. **b**: PB-1: second round with subtelomeric probes. **c**: PB-2: first round. **d**: PB-2: second round with subtelomeric probes

study of embryo remains after a miscarriage, it can be seen that the majority of trisomies and monosomies detected in these prenatal studies originate from the maternal meiotic process [12] and that the incidence of these anomalies is directly correlated to the increase in maternal age [8].

Until recently, genetic studies of the female gamete usually analyzed oocytes donated by patients who had undergone IVF for conjugal infertility. This means the oocytes may well present the genetic anomalies mentioned above, may be immature, or have a lower potential for correct fertilization [6, 7, 9, 11, 17].

Significant methodological differences are also seen regarding the number of chromosomes studied and the technique used (CGH, FISH, cem-FISH), and in the majority of studies, only the first polar body is analyzed, and therefore only the first phase of meiosis is examined. To these factors the diversity of the study population must be added, with IVF being indicated for different etiologies. Also different protocols and doses of medication will have been used during ovarian stimulation. For all these reasons the results regarding the percentage of abnormalities detected varies

Table 1 General results obtained in polar body biopsy

	Total	%
Biopsied oocytes	203	
Oocytes with no diagnosis	22	10.8
Oocytes with diagnosis	181	89.2
Not fertilized: PB-1	19	10.5
Fertilized (Zygotes): PB-1 and PB-2	162	89.5

Table 2 FISH results obtained following the analysis of PB-1 and PB-2 by FISH for chromosomes 13, 16, 18, 21 and 22, presented according to the result of fertilization

	Total	%
Non fertilized oocytes: PB-1	19	
Normal result (Euploid)	11	57.9 %
Abnormal result (Aneuploid) [a]	8	42.1 %
Fertilized oocytes (Zygotes): PB-1 and PB-2	162	
Normal result (euploid)	131	80.9 %
Abnormal result (Aneuploid)	31	19.1 %
Error produced at meiosis I [a]	26	83.8 %
Error produced at meiosis II [b]	4	12.9 %
Error produced at meiosis I and II	1	3.3 %

[a] .100 % of the alterations in Metaphase I were due to premature separation

[b] .100 % of the alterations in Metaphase II were due to non disjunction

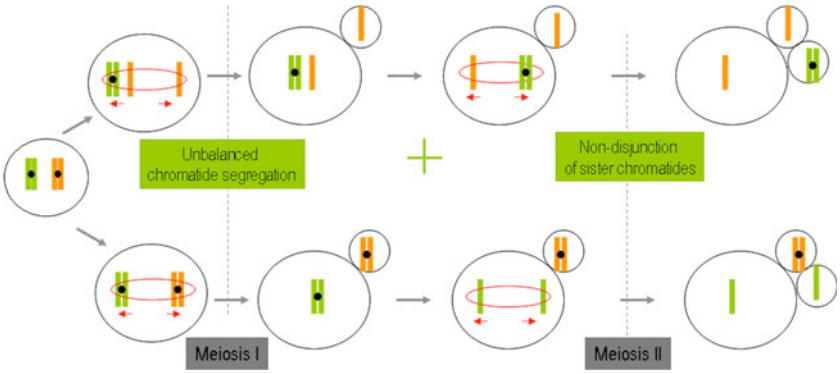

Fig. 3 Meiosis I and II errors. Scheme of the different kinds of errors produced in Meiosis I and II

considerably between the published studies [6, 9, 11, 22–24], ranging from 22 % [6] to 57.1 % [11].

In their large series, Verlinsky and Kuliev [31] used a single biopsy to analyze 10317 oocytes originating from 1027 patients and 1551 IVF cycles with an average age of 38.5 years. They were able to obtain an interpretable diagnosis in 79.6 % of the oocytes and they argue that this low result was either due to the failure of hybridization or to the loss of chromatin during the process of embryo biopsy or fixation. One third of the aneuploidies observed by this group were only detectable in the second polar body. It is known that following the extrusion of the polar body the polar body itself starts to deteriorate, and with time starts the apoptotic process of cell death. This process can affect the quality of the hybridization signals and lead to the creation of diagnostic error.

To solve this problem we performed sequential biopsies of polar body I and II on two separate occasions rather than a single biopsy on day one of embryo development. This allowed us to make a complete assessment of the meiotic process. Using this approach we reduced the percentage of non diagnostic oocytes from 20.4 % [24] to 10.8 %.

Few published studies have reported chromosomal anomalies in fresh donor eggs. Sher et al. [28] used CGH to analyze 92 oocytes obtained from donors aged between 23 and 29. They also analyzed the first and second polar bodies and reported an aneuploidy incidence rate of 65 %. This same research group presented in the same year a series of 132 oocytes with an incidence of aneuploidy of 56 % [16]. Two years later [8] presented a study of 106 donor oocytes analyzing the first and second polar bodies by means of CGH, and showed a much lower aneuploidy incidence: 2.8 %. These differences were attributed by other authors to problems with the techniques used in the earlier studies [16, 28].

In 2010, [21] found a 17.85 % aneuploidy rate following the analysis of the first polar body. In our study the overall incidence of aneuploidy in zygotes is 19.1 %. We have to consider that our study is based on 17 egg donors and 5 chromosomes has been studied. Aneuploidy rate could be higher if all chromosomes had been analyzed.

The differences between aneuploidy rates could be explained in part by the different stimulation protocols used, or differences between the study populations, but in order to determine that a comparative study should be performed.

In the literature, two mechanisms of non disjunction in unfertilized oocytes have been described: (i) non disjunction of the whole chromosome (ND) in which 2 homologous chromosomes are segregated to the same cell pole [17], and (ii) predivision of sister chromatids (PC) [1–3, 34], where the 2 sister chromatids created from one chromosome are separated, and only one of these chromatids is segregated (Fig. 3). Some authors describe the appearance of both factors during meiosis I in similar proportions [1, 4, 6, 32]. Angel et al. [2] postulated that the PC could appear at the end of meiosis I in up to 50 % of cases. This hypothesis has been confirmed by Fragouli et al. [7].

In our study group this percentage increases considerably given that we observed that all the errors produced in Meiosis I were due to PC (100 %; 34/34), and this result concurs with the results presented by other authors where this phenomenon was detected in 91.4 % of cases [5, 29, 30, 34]. We therefore note that the premature division of the sister chromatids is the principal mechanism of the meiotic error that occurs during Meiosis I, giving rise to oocyte aneuploidy. Some authors suggest that the aging of the oocytes in culture could predispose to this type of meiotic abnormality [15, 27]. We could speculate that this could occur when the biopsy of the first polar body occurs on day 1 of embryo development, at the same time as the biopsy of the second polar body, given that at that stage the first polar body would have aged. However, in our study this was not the case because the biopsy of the first polar body was done immediately post ICSI.

In conclusion, in our hands, having used a protocol of ovarian stimulation that used recombinant FSH-LH, we can conclude:

1) The prevalence of oocyte aneuploidy in donor eggs following the stimulation protocol used and after the analysis of meiosis I and II segregation was 19.1 %.
2) For the study of aneuploidy in oocytes it is necessary to analyze both polar bodies given that 83.8 % of the meiotic errors are produced during Meiosis I, 12.9 %

during Meiosis II and 3.2 % of the meiotic errors are produced in both in meiosis I and II.

3) The premature division of sister chromatids is the main source of meiotic error during Meiosis I, resulting in the creation of oocyte aneuploidy.

Acknowledgments Leonardo Marques Foundation
Funding Merck Serono

References

1. Angell RR, Xian J, Keith J, Ledger W, Baird DT. First meiotic division abnormalities in human oocytes: mechanism of trisomy formation. Cytogenet Cell Genet. 1994;65(3):194–202.
2. Angell RR, Xian J, Keith J. Chromosome anomalies in human oocytes in relation to age. Hum Reprod. 1993;8(7):1047–54.
3. Angell RR. Predivision in human oocytes at meiosis I: a mechanism for trisomy formation in man. Hum Genet. 1991;86(4):383–7.
4. Clyde JM, Gosden RG, Rutherford AJ, Picton HM. Demonstration of a mechanism of aneuploidy in human oocytes using Multifluor fluorescence in situ hybridization. Fertil Steril. 2001;76(4):837–40.
5. Cupisti S, Conn CM, Fragouli E, Whalley K, Mills JA, Faed MJ, et al. Sequential FISH analysis of oocytes and polar bodies reveals aneuploidy mechanisms. Prenat Diagn. 2003;23(8):663–8.
6. Fragouli E, Wells D, Thornhill A, Serhal P, Faed MJ, Harper JC, et al. Comparative genomic hybridization analysis of human oocytes and polar bodies. Hum Reprod. 2006;21(9):2319–28.
7. Fragouli E, Wells D, Whalley KM, Mills JA, Faed MJ, Delhanty JD. Increased susceptibility to maternal aneuploidy demonstrated by comparative genomic hybridization analysis of human MII oocytes and first polar bodies. Cytogenet Genome Res. 2006;114(1):30–8.
8. Fragouli E, Escalona A, Gutierrez-Mateo C, Tormasi S, Alfarawati S, Sepulveda S, et al. Comparative genomic hybridization of oocytes and first polar bodies from young donors. Reprod Biomed Online. 2009;19(2):228–37.
9. Gutiérrez-Mateo C, Benet J, Wells D, Colls P, Bermúdez MG, Sánchez-García JF, et al. Aneuploidy study of human oocytes first polar body comparative genomic hybridization and metaphase II fluorescence in situ hybridization analysis. Hum Reprod. 2004;19 (12):2859–68.
10. Gutiérrez-Mateo C, Benet J, Starke H, Oliver-Bonet M, Munné S, Liehr T, et al. Karyotyping of human oocytes by cenMFISH, a new 24-colour centromere-specific technique. Hum Reprod. 2005;20 (12):3395–401.
11. Gutiérrez-Mateo C, Wells D, Benet J, Sánchez-García JF, Bermúdez MG, Belil I, et al. Reliability of comparative genomic hybridization to detect chromosome abnormalities in first polar bodies and metaphase II oocytes. Hum Reprod. 2004;19(9):2118–25.
12. 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(3):167–75. Review.
13. Hassold T, Hunt P. To err (meiotically) is human: the genesis of human aneuploidy. Nat Rev Genet. 2001;2(4):280–91. Review.
14. Hunt P, LeMaire R, Embury P, Sheean L, Mroz K. Analysis of chromosome behavior in intact mammalian oocytes: monitoring the segregation of a univalent chromosome during female meiosis. Hum Mol Genet. 1995;4(11):2007–12.
15. Kamiguchi Y, Rosenbusch B, Sterzik K, Mikamo K. Chromosomal analysis of unfertilized human oocytes prepared by a gradual fixation-air drying method. Hum Genet. 1993;90(5):533–41.
16. Keskintepe L, Sher G, Keskintepe M. Reproductive oocyte/embryo genetic analysis: comparison between fluorescence in-situ hybridization and comparative genomic hybridization. Reprod Biomed Online. 2007;15(3):303–9.
17. Kuliev A, Zlatopolsky Z, Kirillova I, Spivakova J, Cieslak, Janzen J. Meiosis errors in over 20,000 oocytes studied in the practice of preimplantation aneuploidy testing. Reprod Biomed Online. 2011;22(1):2–8.
18. Li Y, Feng HL, Cao YJ, Zheng GJ, Yang Y, Mullen S, et al. Confocal microscopic analysis of the spindle and chromosome configurations of human oocytes matured in vitro. Fertil Steril. 2006;85(4):827–32.
19. Munné S, Ary J, Zouves C, Escudero T, Barnes F, Cinioglu C, et al. Wide range of chromosome abnormalities in the embryos of young egg donors. Reprod Biomed Online. 2006;12(3):340–6.
20. Nicolaidis P, Petersen MB. Origin and mechanisms of non-disjunction in human autosomal trisomies. Hum Reprod. 1998;13 (2):313–9. Review.
21. Obradors A, Rius M, Cuzzi J, et al. Errors at mitotic segregation early in oogenesis and at first meiotic division in oocytes from donor females: comparative genomic hybridization analyzes in metaphase II oocytes and their first polar body. Fertil Steril. 2010;93:675–9.
22. Pellestor F, Anahory T, Hamamah S. Effect of maternal age on the frequency of cytogenetic abnormalities in human oocytes. Cytogenet Genome Res. 2005;111(3–4):206–12.
23. Pellestor F, Andréo B, Anahory T, Hamamah S. PRINS as an efficient tool for aneuploidy assessment in human oocytes and preimplantation embryos. Methods Mol Biol. 2006;334:151–60.
24. Pellestor F, Andréo B, Arnal F, Humeau C, Demaille J. Maternal aging and chromosomal abnormalities: new data drawn from in vitro unfertilized human oocytes. Hum Genet. 2003;112(2):195–203.
25. Reis Soares S, Rubio C, Rodrigo L, Simón C, Remohí J, Pellicer A. High frequency of chromosomal abnormalities in embryos obtained from oocyte donation cycles. Fertil Steril. 2003;80(3):656–7.
26. Requena A, Bronet F, Guillen A, Agudo D, Bou C, García-Velasco JA. The impact of in-vitro maturation of oocytes on aneuploidy rate. Reprod Biomed Online. 2009;18(6):777–83.
27. Sandalinas M, Márquez C, Munné S. Spectral karyotyping of fresh, non-inseminated oocytes. Mol Hum Reprod. 2002;8 (6):580–5.
28. Sher G, Keskintepe L, Keskintepe M, et al. Oocyte karyotyping by comparative genomic hybridization [correction of hybrydization] provides a highly reliable method for selecting 'competent' embryos, markedly improving in vitro fertilization outcome: a multiphase study. Fertil Steril. 2007;87:1033–40.
29. Verlinsky Y, Cieslak J, Ivakhnenko V, Evsikov S, Wolf G, White M, et al. Chromosomal abnormalities in the first and second polar body. Mol Cell Endocrinol. 2001;183 Suppl 1:S47–9.
30. Verlinsky Y, Cieslak J, Ivakhnenko V, Evsikov S, Wolf G, White M, et al. Prevention of age-related aneuploidies by polar body testing of oocytes. J Assist Reprod Genet. 1999;16(4):165–9.
31. Verlinsky Y, Kuliev A. Atlas of preimplantation genetic diagnosis. Second edition. Taylor and Francis Group. 2005.
32. Verlinsky Y, Lerner S, Illkevitch N, Kuznetsov V, Kuznetsov I, Cieslak J, et al. Is there any predictive value of first polar body morphology for embryo genotype or developmental potential? Reprod Biomed Online. 2003;7(3):336–41.
33. Vlaisavljevic V, Krizancic Bombek L, Vokac NK, Kovacic B, Cizek-Sajko M. How safe is germinal vesicle stage oocyte rescue? Aneuploidy analysis of in vitro matured oocytes. Eur J Obstet Gynecol Reprod Biol. 2007;134(2):213–9.
34. Zenzes MT, Casper RF. Cytogenetics of human oocytes, zygotes, and embryos after in vitro fertilization. Hum Genet. 1992;88 (4):367–75. Review.