J Assist Reprod Genet
DOI 10.1007/s10815-010-9516-2

ASSISTED REPRODUCTION TECHNOLOGIES

# Are poor responders patients at higher risk for producing aneuploid embryos in vitro?

**Amanda Souza Setti · Daniela Paes de Almeida Ferreira Braga · Rita de Cássia Savio Figueira · Matheus de Castro Azevedo · Assumpto Iaconelli Jr. · Edson Borges Jr.**

Received: 3 September 2010 / Accepted: 17 November 2010


**Abstract**
*Purpose* To test the hypothesis that aged women with poor ovarian response express an increase on embryo chromosomal alterations when compared to aged women who presented normal response.
*Methods* Couples undergoing intracytoplasmic sperm injection cycles with preimplantation genetic screening, were subdivided into two groups: Poor Responder group ($n$=34), patients who produced ≤4 oocytes; and Normoresponder group ($n$=50), patients who produced ≥5 oocytes. Groups were compared regarding cycles' outcomes and aneuploidy frequency.
*Results* There were no significant differences between and groups regarding the fertilization rate ($p$=0.6861), clinical pregnancy ($p$=0.9208), implantation ($p$=0.6863), miscarriage ($p$=0.6788) and the percentage of aneuploid embryos ($p$=0.270). Embryo transfer rate was significantly lower on poor responder group ($p$=0.0128) and logistic regression confirmed the influence of poor response on the chance of embryo transfer ($p$=0.016).
*Conclusions* Aged females responding poorly to gonadotrophins are not at a higher risk for producing aneuploid embryos in vitro.

**Keywords** Intracytoplasmic sperm injection · Ovarian stimulation · Poor ovarian response · Preimplantation genetic screening

*Capsule* Aged females responding poorly to gonadotrophins are not at a higher risk for producing aneuploid embryos in vitro.

A. S. Setti · D. P. A. F. Braga · E. Borges Jr.
Sapientiae Institute – Educational and Research Center in Assisted Reproduction,
Rua Vieira Maciel, 62,
São Paulo, SP, Brazil 04503-040

D. P. A. F. Braga · R. C. S. Figueira · M. C. Azevedo · A. Iaconelli Jr. · E. Borges Jr. (✉)
Fertility – Assisted Fertilization Center,
Av. Brigadeiro Luis Antônio, 4545,
São Paulo, SP, Brazil 01401-002
e-mail: edson@fertility.com.br

E. Borges Jr.
Department of Gynecology and Obstetrics,
Botucatu Medical School/UNESP,
Distrito de Rubião Junior,
18618-970, Botucatu, SP, Brazil

## Introduction

Despite advances in assisted conception, poor ovarian response to gonadotrophin stimulation remains a significant problem in modern fertility treatment, especially, in vitro fertilization (IVF) where a large number of oocytes is desirable. Low follicular recruitment following ovarian stimulation may be observed in 9 to 24% of the IVF cycles [1]. Poor response is associated with a significant decline in the success rate of fertility treatment [2], thus, poor responders management is of extreme importance; nevertheless, it is one of the most difficult challenges to those carrying out assisted reproductive techniques (ART).

Poor response phenomenon was first described by Garcia et al. [3], who defined these patients as having a peak E2 level of <300 pg/mL after standard stimulation with human menopausal gonadotrophin. Despite the widespread use of the term "poor response", no standard definition exists. The number of developed follicles and/or number of oocytes retrieved after ovarian stimulation are two of the most important criteria for defining poor ovarian response. The proposed number varies among different authors, and ranges from less than three to less than five

Published online: 26 November 2010

dominant follicles on the day of human chorionic gonadotrophin (hCG) administration [4], and/or less than three to less than five retrieved oocytes [5]. However, the definition of poor response is still a matter of debate.

The poor outcome after an initial low response cycle, regardless of the quality of embryos transferred or the age of the patient, suggests that embryos of good morphological quality may still have a low pregnancy potential in this group of patients. Poor responder patients appear to be at greater risk for fetal aneuploidy compared to patients with normal ovarian reserve [6, 7]. Previous studies have demonstrated that women with a reduced follicular ovarian pool are at risk of a trisomic pregnancy, independent of age [8] and suggested that poor ovarian reserve is a primordial factor in the genesis of Down Syndrome [9]. Moreover, these patients also have significantly higher rates of pregnancy loss compared to patients with normal ovarian reserve [10].

It is well known that ovarian poor response to stimulation protocols increases as women get older [2]. Those patients are usually stimulated with high doses of gonadotrophins, however, few numbers of oocytes are retrieved, few number of usually low-quality embryos are transferred and, consequently, low pregnancy rates are expected [11].

The reduction of fecundity for women with advanced maternal age is attributed to reduction of ovarian reserve and deterioration of oocyte quality [12]. Despite the well established relationship between advanced age and diminished ovarian response to gonadotrophins, it is still unknown if, considering only patients with advanced maternal age, there is a difference between the incidence of chromosomally abnormal embryos between women with normal response and women with poor response to ovarian stimulation. The aim of this study was to chromosomally compare embryos originated from aged women with normal and with poor response to ovarian stimulation protocol.

## Material and methods

### Experimental design, patients and inclusion criteria

Using our center's computerized database we retrospectively identified cycles performed from January 2007 to December 2009 which fulfilled the following inclusion criteria: indication for intracytoplasmic sperm injection (ICSI) associated with preimplantation genetic aneuploidy screening (PGS) as a result of advanced maternal age (female age >35 years old). Only first attempt cycles were included in this study. All cases of severe male factor were excluded. A total of 84 ICSI cycles were identified and split into two groups according to the number of retrieved oocyte following ovarian stimulation: Normoresponder (NR group, $n$=50), patients who produced more than four oocytes; and Poor Responder (PR group, $n$=34), patients in which up to four oocytes were retrieved.

### Controlled ovarian stimulation

Controlled ovarian stimulation was achieved by long-term pituitary down-regulation using a gonadotropin-releasing hormone agonist (GnRH agonist, Lupron Kit™, Abbott S. A Societé Française des Laboratoires, Paris, France). This procedure was followed by ovarian stimulation with recombinant follicle-stimulating hormone (FSH) (Gonal-F®, Serono, Geneve, Switzerland). Oocyte retrieval was performed 35 h after the administration of recombinant human chorionic gonadotrophin (rhCG, Ovidrel™, Serono, Geneve, Switzerland), through transvaginal ultrasonography.

### Intracytoplasmic sperm injection, assessment of fertilization and embryo development

Oocytes were transferred into a micro-injection dish, prepared with 4 μL droplets of buffered medium (HEPES, Irvine Scientific, Santa Ana, USA), and covered with mineral oil on a heated stage at 37.0±0.5°C of an inverted microscope. Approximately 16 h after ICSI, fertilization was confirmed by the presence of two pronuclei and the extrusion of the second polar body. Embryos were kept in a 50 μl drop of HTF medium supplemented with 10% HSA covered with mineral oil in a humidified atmosphere under 6% CO2 at 37°C until transfer.

### Embryo biopsy

Embryos reaching at least the 5-cell stage on day 3 of development were biopsied by laser zona drilling using a 1.48 μm Infrared Diode Laser (Octax Laser Shot System, MTG, Bruckberg, Germany) and returned to culture. Only one blastomere was removed per embryo. The definition of a successful biopsy was the removal of a cell without lysis, so that the cell could be used for fixation and analysis. If the blastomere were not successfully biopsied or fixed, a second blastomere was taken, but only if the embryo initially had ≥6 blastomeres.

### Blastomere fixation and fluorescent in situ hybridization (FISH)

The blastomere of an embryo was fixed on a slide according to the previously described HCI/Tween 20 method [13]. A two-round FISH procedure was performed, which allowed for the detection of chromosomes X, Y, 13,

18 and 21 (Multivision PGT Probe Panel; Vysis, Downers Grove, IL, USA) in the first round and chromosomes 16 and 22 in the second round. The hybridization solution for the second round was prepared by mixing a probe for chromosome 16 (Vysis, Satellite II DNA/D16Z3 probe, Spectrum Orange) and a probe for chromosome 22 (Vysis, LSI 22, 22q11.2, Spectrum Green). The FISH procedure was performed as previously described [14]. The results of the first and second round were analyzed by an observer using a fluorescence microscope.

### FISH scoring criteria

At diagnosis, embryos were considered normal when two gonosomes and two chromosomes 13, 16, 18, 21 and 22 were present; were considered trisomic or monosomic if, respectively, an extra or a missing signal was observed and were considered haploid, triploid or polyploidy if, respectively, one, three or more copies of the set of chromosomes were present. The presence of two or more chromosomal abnormalities within the same blastomere was characterized as multiple abnormalities and this embryo was never considered for transfer. Several FISH procedure-related factors were responsible for no or no reliable result: damaged or incomplete nuclei, nuclei covered with debris or nuclei without clear signals (FISH failure).

### Embryo transfer

Embryo transfer was performed on day 5 by using a soft catheter with transabdominal ultrasound guidance. Only embryos found to be chromosomally normal were considered for embryo transfer, and up to a maximum of three embryos were transferred.

### Clinical follow-up

A pregnancy test was performed 12 days after embryo transfer; a positive pregnancy test was considered to define a biochemical pregnancy. All women with a positive test had a transvaginal ultrasound scan at 2 weeks after the positive test; a clinical pregnancy was diagnosed when fetal heartbeat was detected. Pregnancy rates were calculated per transfer. Miscarriage was defined as spontaneous abortion before 20 weeks' gestation.

### Statistical analysis

Groups were compared regarding ICSI outcome and incidence of embryo aneuploidy. Results are expressed as means ± standard deviation (SD) for numeric variables and proportions (%) for categorical variables. The proportions were compared by the Chi-squared test. Influence of poor ovarian response on aneuploidy rates was assessed using logistic regression analysis, and results are expressed as odds ratio (OR), confidence intervals (CI) and *p*-values. Results were considered to be significant at the 5% critical level ($p<0.05$). Data analysis was carried out using GraphPad Prism version 4.0 Statistical Program.

Written informed consent was obtained, in which patients agreed to share the outcomes of their cycles for research purposes. The study was approved by the local Institutional Review Board.

## Results

### Cycle's general characteristics

Cycle's general characteristics are shown in Table 1.

### Embryo biopsy and FISH diagnosis

A total of 511 embryos were biopsied in NR and PR groups. The removal of an extra blastomere was necessary in 11 embryos. Due to technical issues, including failure of hybridization, signal overlapping yielding false-negative results and split or diffused signals, embryo biopsy failure occurred in 26 cells in NR and PR group. These results are shown in Table 2.

### Embryo aneuploidy in NR and PR groups

There were no significant differences between NR and PR groups regarding the percentage of aneuploid embryos (51.0% [$n=213$] vs 58.2% [$n=39$], $p=0.270$), embryos showing autosomal aneuploidy (45.9% [$n=192$] vs 50.7% [$n=34$], $p=0.2659$), sexual aneuploidy (15.1% [$n=63$] vs 14.9% [$n=10$], $p=0.4355$) and multiple abnormalities (21.1% [$n=88$] vs 26.8% [$n=18$], $p=0.2368$).

### Influence of poor response on embryo aneuploidy

Logistic regression analyses showed no influence of poor response on the incidence of embryo aneuploidy (OR: 1.33, CI: 0.65–2.75, $p=0.271$), embryo sexual aneuploidy (OR: 0.9, CI: 0.32–2.50, $p=0.836$) and autosomal aneuploidy (OR: 1.23, CI: 1.23–2.53, $p=0.566$). However, poor response was determinant of the likelihood of embryo multiple abnormalities occurrence (OR: 2.15, CI: 0.88–5.25, $p=0.0017$).

### Influence of poor ovarian response on ICSI outcomes

Although fertilization (83.9% vs 87.1%, $p=0.6861$), clinical pregnancy (28.0% vs 29.4%, $p=0.9208$) and implanta-

**Table 1** Cycle's general characteristics

| Variable | NR Group ($n$=50) | PR Group ($n$=34) | $p$ value |
|---|---|---|---|
| Mean female age (SD) | 40.7 (2.6) | 41.3 (1.5) | 0.2094 |
| Mean total dose of FSH (SD) | 2948 (856.7) | 2858 (838.1) | 0.4534 |
| Oocyte yield (%) | 75.8 | 74.2 | 0.5574 |
| MII oocyte rate (%) | 72.8 | 78.3 | 0.1153 |
| Mean number of retrieved oocytes (SD) | 16.4 (12.1) | 3.7 (0.4) | <0.0001 |
| Mean numbr of injected oocytes (SD) | 12.8 (7.9) | 2.7 (1.1) | <0.0001 |
| Mean number of cleaved embryos (SD) | 10.7 (5.5) | 2.4 (0.5) | <0.0001 |
| Mean number of embryos transferred (SD) | 2.0 (0.9) | 1.1 (0.8) | 0.0001 |
| Pregnancy rate (%) | 28.0 | 29.4 | 0.9208 |

*NR* normoresponder, *PR* poor responder, *SD* standart deviation, *FSH* follicle stumulating hormone

tion rates (22.6% vs 20.6%, $p$=0.6863) were not significantly different between NR and PR groups, the percentage of cycles without embryo transfer was significantly higher on PR group (4.0% [$n$=2] vs 23.5% [$n$=8], $p$=0.0128). This finding was confirmed using a binary logistic regression, showing that poor response was determinant of the likelihood of embryo transfer occurrence (OR: 7.38, CI: 2.35–2.24, $p$=0.016). Miscarriage rates were similar between NR and PR groups (8.3% vs 7.7%, $p$=0.6788).

## Discussion

In this study we attempted to associate ovarian poor response with increased occurrence of embryo chromosomal alterations in aged women. However, our results failed to demonstrate such an association, because embryos originating from poor responder cycles and from normal responder cycles presented similar aneuploidy rates.

Poor ovarian response after hormonal stimulation is a sign of diminished ovarian reserve and may be considered a sign of latent ovarian failure. It is now realized that an accelerated decline of ovarian function begins much earlier than previously thought, most like in the mid-thirties [15]. At around this time the total remaining number of follicles in the ovaries has been shown to be near 25000 and there is an accelerated loss of follicles, as well as qualitative changes in the remaining follicles. These follicles have fewer granulosa cells, decreased mitosis and increased apoptosis. There is an altered communication between the granulosa cells and the oocytes, which results in abnormal nuclear and cytoplasmic maturation within the oocyte [16].

Poor responders' oocytes has been suggested to be of poor quality because they are the last oocytes available from the ovarian pool and are at an increased risk for chromosomal abnormalities [16, 17]. Diminished ovarian reserve was reported to contribute in part to unexplained recurrent abortions [18]. It is noted in the literature that fetal aneuploidy can result in first-trimester spontaneous abortions and is more common in older women with poorer ovarian reserve [19].

With increasing age, oocyte quality becomes impaired, leading to poor embryo quality and low implantation and pregnancy rates. In addition, with advancing age there is a decrease in response to gonadotrophin stimulation [20] and more gonadotrophin is required per cycle [21]. It has been suggested that the greater the total dose of gonadotrophin required for oocyte retrieval, the lower is the pregnancy rate [22, 23]. In addition, in women with advanced maternal age, the germ cells are exposed to a longer arrested period in meiosis I and hence have a higher risk of aneuploidy [24, 25].

The blastomeres in this study were analyzed for seven chromosomes. The chromosomes selected for analysis in this study are most often associated with trisomies with potential of delivery, i.e. chromosome X, Y, 13, 18 and 21 and with trisomies frequently found in spontaneous abortions, i.e. chromosome 16 and 22 [26]. Our findings regarding the influence of poor response on the probability of embryo transfer, and the statistically lower number of transferred embryos on PR group could be attributed to the

**Table 2** Embryo biospy and FISH results

| | NR group ($n$=50) | PR group ($n$=34) | $p$ value |
|---|---|---|---|
| Cleaved embryos | 536 | 88 | 0.8614 |
| Biospied embryos | 440 (82.1%) | 71 (80.7%) | 0.7506 |
| Removal of one extra blastomere | 10 | 1 | 0.6415 |
| Biopsy failure | 22 (5.0%) | 4 (5.6%) | 0.8216 |
| Embryos with FISH diagnosis | 418 (95.0%) | 67 (94.4%) | 0.8216 |

*NR* normoresponder, *PR* poor responder, *FISH* fluorescent in situ hybridization

statistically lower number of oocytes collected and consequently, the lower number of embryos available for biopsy in this group.

The data generated from this study show that aged patients with a poor prognosis are at an equivalent risk for producing chromosomally abnormal embryos as compared with aged patients with good prognosis. It could be suggested that maternal age, by itself, already implicates in reduced rates of chromosomally normal embryos and the intensity of the response to gonadotrophin does not influence these rates. Indeed, one study suggested that poor response may be indicative mainly of oocyte quantity not quality [27]. Additionally, Weghofer et al. [28] questioned the belief that prematurely declining ovarian function represents a shift of the normal, physiologic ovarian aging curve toward younger age.

Similar implantation and pregnancy rates were observed between the two analyzed groups. This fact could be explained by the equivalent embryo morphology and chromosomal status of transferred embryos after PGS performance. Furthermore, the rates were not calculated per stated cycle. Pregnancy rates were calculated per transfer and implantation rates are related to the number of embryos transferred. Thus, the similar rates between PR and NR group could be related to the higher number of cycles without embryo transfer in PR group.

Other studies have investigated and failed to correlate poor response, regardless of age, and embryo chromosomal quality [12] or compromised pregnancy rates [29].

Despite the suggested beneficial impact of PGS on the ongoing implantation rate in patients aged ≥38 years [30], Handarson et al. [31] compared the outcomes of ICSI cycles with and without PGS in women of advanced age and observed no improvement in pregnancy rates in PGS cycles. On the contrary, the pregnancy rate was lower in the PGS group as well as the live birth rate [31]. Similarly, another study found that the effect of PGS in women of advanced maternal age did not differ in women with a high embryonic aneuploidy risk when compared with women with a low embryonic aneuploidy risk. Moreover, live birth rates were lower after cycles with PGS when compared with cycles without PGS [32].

In spite of a good experimental design, our study possesses drawbacks, as follows:

(i) The number of included subjects is very small. However, due to the unclear effect of poor response on the embryo viability, it is difficult for the physicians and/or the patients to resort to PGS in patients producing small numbers of oocytes and embryos.

(ii) We used the number of produced oocytes to characterize the patients as poor or normoresponder. It is generally accepted that the number of available and recruited follicles in one cycle may differ from the next cycle in the same patient. Indeed, some markers have been proposed to predict ovarian reserve, however, there is no consensus regarding the characterization of poor response.

(iii) We did not perform polar body chromosomal analysis; therefore, we cannot state for certain that embryonic aneuploidy was related to the oocyte. It is believed that the exclusion of patients with severe male factors may be minimized but not totally refused the impact of spermatozoa-related embryonic aneuploidy.

(iv) We performed single blastomere biopsy for embryonic chromosomal analysis, which does not take into account the risk of embryo mosaicism. However, because there is no convincing data demonstrating the safety of double-over single-blastomere biopsy [33, 34], single blastomere biopsy is performed in our center. Furthermore, we evaluated and defined aneuploidy using information from 7 chromosome probes only, which is not a comprehensive analysis of all chromosome found in humans.

There was only one finding in this study concerning the relation between poor ovarian response and embryo chromosomal abnormalities. Our results showed that embryo multiple abnormalities occurrence risk is more than two-fold higher in poor responder patients. However, this finding should not be of importance because the low rate of multiple chromosome anomalies found in spontaneous abortions suggests that severe errors, such as chaotic pre-embryos, are incompatible with sustained development [26].

In conclusion, aged females responding poorly to gonadotrophins are not at a higher risk for producing aneuploid embryos in vitro. Therefore, poor ovarian response to gonadotrophins does not justify the implementation of PGS in these patient's cycles due to the association with a statistically reduced number of cycles transferred and a lower number of embryos transferred per patient.

## References

1. Keay SD, Liversedge NH, Mathur RS, Jenkins JM. Assisted conception following poor ovarian response to gonadotrophin stimulation. Br J Obstet Gynaecol. 1997;104:521–7.
2. Veleva Z, Jarvela IY, Nuojua-Huttunen S, Martikainen H, Tapanainen JS. An initial low response predicts poor outcome in in vitro fertilization/intracytoplasmic sperm injection despite improved ovarian response in consecutive cycles. Fertil Steril. 2005;83:1384–90.
3. Garcia JE, Jones GS, Acosta AA, Wright Jr G. Human menopausal gonadotropin/human chorionic gonadotropin follicular maturation for oocyte aspiration: phase II, 1981. Fertil Steril. 1983;39:174–9.

4. Land JA, Yarmolinskaya MI, Dumoulin JC, Evers JL. High-dose human menopausal gonadotropin stimulation in poor responders does not improve in vitro fertilization outcome. Fertil Steril. 1996;65:961–5.
5. Surrey ES, Bower J, Hill DM, Ramsey J, Surrey MW. Clinical and endocrine effects of a microdose GnRH agonist flare regimen administered to poor responders who are undergoing in vitro fertilization. Fertil Steril. 1998;69:419–24.
6. 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.
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.
8. Haadsma ML, Mooij TM, Groen H, Burger CW, Lambalk CB, Broekmans FJ, et al. A reduced size of the ovarian follicle pool is associated with an increased risk of a trisomic pregnancy in IVF-treated women. Hum Reprod. 2010;25:552–8.
9. Bartmann AK, Araujo FM, Iannetta O, Paneto JC, Martelli L, Ramos ES. Down syndrome and precocious menopause. J Assist Reprod Genet. 2005;22:129–31.
10. Levi AJ, Raynault MF, Bergh PA, Drews MR, Miller BT, Scott Jr RT. Reproductive outcome in patients with diminished ovarian reserve. Fertil Steril. 2001;76:666–9.
11. Kumbak B, Kahraman S, Karlikaya G, Lacin S, Guney A. In vitro fertilization in normoresponder patients with endometriomas: comparison with basal simple ovarian cysts. Gynecol Obstet Investig. 2008;65:212–6.
12. Thum MY, Abdalla HI, Taylor D. Relationship between women's age and basal follicle-stimulating hormone levels with aneuploidy risk in in vitro fertilization treatment. Fertil Steril. 2008;90:315–21.
13. 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.
14. Staessen C, Tournaye H, Van Assche E, Michiels A, Van Landuyt L, Devroey P, et al. PGD in 47, XXY Klinefelter's syndrome patients. Hum Reprod Update. 2003;9:319–30.
15. Nikolaou D, Lavery S, Turner C, Margara R, Trew G. Is there a link between an extremely poor response to ovarian hyperstimulation and early ovarian failure? Hum Reprod. 2002;17:1106–11.
16. De Sutter P, Dhont M. Poor response after hormonal stimulation for in vitro fertilization is not related to ovarian aging. Fertil Steril. 2003;79:1294–8.
17. Nasseri A, Mukherjee T, Grifo JA, Noyes N, Krey L, Copperman AB. Elevated day 3 serum follicle stimulating hormone and/or estradiol may predict fetal aneuploidy. Fertil Steril. 1999;71:715–8.
18. Trout SW, Seifer DB. Do women with unexplained recurrent pregnancy loss have higher day 3 serum FSH and estradiol values? Fertil Steril. 2000;74:335–7.
19. Wilcox AJ, Weinberg CR, O'Connor JF, Baird DD, Schlatterer JP, Canfield RE, et al. Incidence of early loss of pregnancy. N Engl J Med. 1988;319:189–94.
20. Piette C, de Mouzon J, Bachelot A, Spira A. In-vitro fertilization: influence of women's age on pregnancy rates. Hum Reprod. 1990;5:56–9.
21. Jacobs SL, Metzger DA, Dodson WC, Haney AF. Effect of age on response to human menopausal gonadotropin stimulation. J Clin Endocrinol Metab. 1990;71:1525–30.
22. Shulman A, Ghetler Y, Fejgin M, Kaneti H, Weinstein S, Beyth Y, et al. Relationship between the threshold of ovarian sensitivity to human menopausal gonadotrophin stimulation and in-vitro fertilization treatment outcome. Hum Reprod. 1995;10:3198–201.
23. Stadtmauer L, Ditkoff EC, Session D, Kelly A. High dosages of gonadotropins are associated with poor pregnancy outcomes after in vitro fertilization-embryo transfer. Fertil Steril. 1994;61:1058–64.
24. Delhanty JD. Mechanisms of aneuploidy induction in human oogenesis and early embryogenesis. Cytogenet Genome Res. 2005;111:237–44.
25. Munne S, Sandalinas M, Escudero T, Marquez C, Cohen J. Chromosome mosaicism in cleavage-stage human embryos: evidence of a maternal age effect. Reprod Biomed Online. 2002;4:223–32.
26. Ziebe S, Lundin K, Loft A, Bergh C, Nyboe Andersen A, Selleskog U, et al. FISH analysis for chromosomes 13, 16, 18, 21, 22, X and Y in all blastomeres of IVF pre-embryos from 144 randomly selected donated human oocytes and impact on pre-embryo morphology. Hum Reprod. 2003;18:2575–81.
27. Toner JP. Age = egg quality, FSH level = egg quantity. Fertil Steril. 2003;79:491.
28. Weghofer A, Barad D, Li J, Gleicher N. Aneuploidy rates in embryos from women with prematurely declining ovarian function: a pilot study. Fertil Steril. 2007;88:90–4.
29. Kailasam C, Keay SD, Wilson P, Ford WC, Jenkins JM. Defining poor ovarian response during IVF cycles, in women aged <40 years, and its relationship with treatment outcome. Hum Reprod. 2004;19:1544–7.
30. Gianaroli L, Magli MC, Ferraretti AP, Munne S. Preimplantation diagnosis for aneuploidies in patients undergoing in vitro fertilization with a poor prognosis: identification of the categories for which it should be proposed. Fertil Steril. 1999;72:837–44.
31. Hardarson T, Hanson C, Lundin K, Hillensjo T, Nilsson L, Stevic J, et al. Preimplantation genetic screening in women of advanced maternal age caused a decrease in clinical pregnancy rate: a randomized controlled trial. Hum Reprod. 2008;23:2806–12.
32. Twisk M, Mastenbroek S, Hoek A, Heineman MJ, van der Veen F, Bossuyt PM, et al. No beneficial effect of preimplantation genetic screening in women of advanced maternal age with a high risk for embryonic aneuploidy. Hum Reprod. 2008;23:2813–7.
33. Van de Velde H, De Vos A, Sermon K, Staessen C, De Rycke M, Van Assche E, et al. Embryo implantation after biopsy of one or two cells from cleavage-stage embryos with a view to preimplantation genetic diagnosis. Prenat Diagn. 2000;20:1030–7.
34. Edwards RG, Hansis C. Initial differentiation of blastomeres in 4-cell human embryos and its significance for early embryogenesis and implantation. Reprod Biomed Online. 2005;11:206–18.