

Chromosome Aneuploid Spectrum Evolving along with Development - Relates with Gene Content in Specific Chromosome

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ABSTRACT

Human preimplantation embryos have a high incidence of aneuploidy. The occurrence of aneuploidy looks starts during meiosis continuing till day 3 of embryonic development (meiotic and mitotic errors). After day 3, embryonic genome activity is gradually activated and cell cleavage checkpoints (CCC) gradually matures and functions. In normal situation, the CCC eradicates of aneuploid blastomeres through arrest and apoptosis. At day 3, the aneuploid incidence of each chromosome is similar about 20-30% while at blastocyst stage the aneuploid incidence of each chromosome varies from 2-10%. The aneuploid rate of examined chromosomes is significant different between the examined stage ($p < 0.0001$). The aneuploid incidence is significantly reduced with development. The reduction of aneuploid incidence is different among chromosomes. The reduction efficiency appears related to the chromosomal gene content and/or genes involving with CCC or apoptosis. Chromosomal aneuploid reduction incidence is related to gene content, high gene content is purged earlier, i.e. chromosome 1 and 19, while low gene content is purged late, i.e. chromosome 13, 21, 22.

Keywords

Human, IVF, aneuploid spectrum, PGTa, Chromosomes.

Introduction

It is evident that human preimplantation embryos have a high incidence of aneuploidy and mosaicism [1-4]. Apparently, both meiotic and mitotic errors contribute the high incidence of aneuploidy. The meiosis is a process not engaging cell cycle. The main errors occur in anaphase chromosomal lagging or chromosomal non-disjunction [5,6]. The meiosis process arrests at metaphase II stage before fertilization. The mitotic errors occur post zygotic stage. In normal somatic cells, there are cell cleavage checkpoints (CCC), i.e. G1, G2, metaphase [7-10]. G1 checkpoint checks the external factors and DNA damage before entering S phase. G2 checkpoint make sure all DNA has replicated properly before entering M phase. In M phase, the spindle assembly checks all sets of chromosome alignment and spindle attachment before cleavage [11]. Failure by CCC results in arrest/apoptosis [12;13].

In humans, the embryonic genomic activation is at the 4-8 cell stage [14]. CCC is lacking before this stage. The Egg equips and supplies blastomeres with high contents of cell cleavage components to drive cleavage, bypass normal mitosis process [15]. Although blastomere contains apoptotic machinery, it does not activate until morula stage [16-18]. The uncoupling of mitotic-spindle check points with apoptosis seems to be one of the mechanisms to allow the aneuploid cells to survive and keep cleaving [12,13]. The consequences of meiotic and mitotic errors are accumulating aneuploidy and mosaic problems before CCC activation. Logically these problems cause embryo arrest/death, fetal abortion or unknown rescue mechanism which lead to give life birth of an abnormal baby.

Preimplantation Genetic Testing for aneuploidy (PGT-a) was developed to screen out those aneuploid embryos to enhance pregnancy or decrease abortion rates. The early phase of PGT-a is to screen day 3 embryos for some chromosomes, i.e. 13, 16, 18,

21, 22, gonosomes, by Fluorescent In Situ Hybridization (FISH) [19,20]. Those chromosomes are the ones frequently seen in spontaneous abortion tissue (product of conception, POC).

Even with selection of an euploid embryo to transfer after day 3 biopsy, convincing evidence has shown that the implantation rate is not improved but may be reduced [21]. With the development of molecule genetic technique and biopsy at an advance stage (blastocyst trophectoderm biopsy), it is possible to verify all 23 sets of chromosomes. There are reports showing significantly increase in viable pregnancy [22-24] with implantation rate around 60-70%. Apparently, the selection of euploid blastocyst works much better than the selections of euploid embryos at the cleavage stage [21].

Chro-mo.	1	2	3	4	5	6	7	8	9	10	11	12
NCBI	3000	2500	1900	1600	1700	1900	1800	1400	1400	1400	2000	1600
CCDS	1961	1194	1024	727	839	996	862	646	739	70.6	1224	988

Chro-mo.	13	14	15	16	17	18	19	20	21	22	X	Y
NCBI	800	1200	1200	1300	1600	600	1700	900	400	800	1400	200
CCDS	308	583	561	795	1124	261	1357	5126	215	417	804	63

NCBI: National Center Biotechnology Information. CCDS: Consensus Coding Sequence.

Since embryonic development is a symphony of thousands of genes working together [30], a blastomere with aneuploid disturbance may show sequential and differential effect, i.e. chromosome with more genes engaged in development may suffer a greater/faster impact. Chromosomes with few genes engaged in development suffer less/delay impact. Chromosomes with some genes participate in anti-apoptosis or some unknown mechanism may have blastomere continue to cleave/survive. The hypothesis of this study is that aneuploidy of chromosomes with more genes will encounter more sensitive and faster eradication and elimination.

Those aneuploid blastomeres with chromosomes containing fewer genes are more likely to be less sensitive and delay their response to CCC/verification, and continue to cleave until late stage of development or even live birth. The hypothesis predicts the spectrum of aneuploid chromosome evolves along with development in a way of chromosome with high number of genes disappear early. Those chromosomes with low number of genes will arrest/die out late, i.e. post implantation stage. Few exceptions are those chromosomes with least number of genes can escape CCC/verification or low efficiency of CCC eradication to give live birth of aneuploid baby, i.e. chromosome 13, 18, 21, or gonosome (XXY klinefelter syndrome; XO Turner syndrome).

Materials and Methods

For the day 3 cleavage category, all IVF cases during 1/1/2004 -12/31/2008 with preimplantation genetic screen with Fluorescent In-Situ Hybridization (FISH) were included. The median age was 39 with range 22 – 49. The detail of this procedure has been described previously [31]. In brief, 501 cycles with 3736 blastomeres were examined. The day 3 embryo must have at least

The recent reports show transfer of PGT-a screened aneuploid embryos result in delivery of healthy babies [25-27]. The logical explanation is either aneuploid blastomeres gradually arrest, or undergo apoptosis, and disappear or arrest/dilute out in euploid-aneuploid mosaic blastocyst and/or possible “self-correction” [1,28,29]. Either way shows the blastomere population of chromosomal euploid-aneuploid mosaic is dynamic. Due to mosaic phenomenon and trophectoderm sampling variations, the determination of euploidy/aneuploidy contains certain ambiguity. It takes large number to show a creditable trend.

With the development of human genome project, it is possible to estimate the number of genes in a specific chromosome (<https://www.ncbi.nlm.nih.gov/books/NBK22266/>). The estimate gene content of each chromosome tabulates in following table.

5 blastomeres for biopsy. A single blastomere was biopsied from each embryo. Chromosomes 13,15,16,18, 21, 22, X and Y were examined by FISH using Yysis and Cytocell kits.

For the trophectoderm biopsy category (day 5-7 blastocysts), 1366 cycles during 1/1/2014 – 3/15/2016 were included in the study. The median age of study population was 35 with range 21 to 50. Trophectoderm biopsy was performed at the expanded blastocyst or more advance stage. A few full blastocysts were biopsied at day 7. The biopsied samples were sent to Genesis Genetics (East Lansing, Michigan; Houston, Texas) for preimplantation genetic screening. A total of 4403 trophectoderm samples were analyzed by array-CGH technology. Those data without results or no specific results (only showing multiple aneuploidies) were not included in the study.

All data sets were retrospectively extracted from our in-house clinical database. No identifiable patients’ information was used. It was deemed exempt status from IRB. Since the behavior of aneuploidy by autosome or gonosome is not the same, i.e. autosomal monosomy is lethal while mono X chromosome (Turner syndrome) can have live birth. In this study, we only focus on the autosomes.

All data with results are included in the analysis. Chi-square tests used to compare the euploidy and no results rates between cleavage and blastocyst embryos. Logistic regressions were used for statistical analysis to compare the chromosomal aneuploid incidence within the same category, i.e. day 3 or blastocyst category. Logistic regression was also used for analyzing of chromosomal aneuploid incidence between the 2 categories. The

statistical significance was set at $P < 0.05$ and marginal significant was set at $0.05 < p < 0.1$.

Results

Demographic and PGS results

The patients and PGS cycle data are summarized in Table 1. Apparently the mean age of day 3 group is older than the blastocyst group. There were 3736 embryos studied in the day 3 group and 4403 embryos studied in blastocyst group. The euploid rate is significantly higher in the blastocyst group. The no result rate is significantly lower in the blastocyst group. The significant difference may just be due to the difference of age, embryo stage, and genetic test plate form.

Stage	Mean age	# cycles	# embryos	Test proto-col	# euploid	# no result
Day 3	38.0 + 4.8	501	3736	FISH	1190/3736 (31.8%)	379/3736 (10.1%)
Blastocyst	35.1 + 5.1	1366	4403	aCGH	2032/4403 (46.1%)	52/4403 (1.2%)
Analysis					$P < 0.0001$	$P < 0.0001$

Table 1: Patient and sample data.

Comparison of day 3 chromosomal aneuploidy vs. blastocyst aneuploidy

The evolving of specific chromosomal aneuploid incidence from day 3 embryo to blastocyst is summarized in Table 2. At day 3 by FISH, only chromosome 13,15,16,18,21, and 22 are examined. The evolving of these 6 chromosomes at blastocyst stage is examined. By day 3 group, there is a marginal difference ($p=0.076$) of aneuploid incidence among the studied chromosomes. Chromosomal aneuploid spectrum looks $22 > 15 > 21 > 13 > 16 > 18$. By blastocyst stage, there is a significant difference ($p < 0.0001$) of aneuploid incidence among these 6 chromosomes.

Chromosome	13	15	16	18	21	22	Overall P-value	Chromo-some-by-Day Interaction P-value
Day 3	820/3221 (25.4%)	823/3143 (26.1%)	800/3166 (25.2%)	757/3231 (23.4%)	828/3202 (25.8%)	840/3166 (26.5%)	$P=0.076$	$p < 0.0001$
Trophecto-derm	133/4195 (3.1%)	156/4196 (3.7%)	304/4194 (7.2%)	115/4196 (2.7%)	190/4196 (4.5%)	254/4196 (6.0%)	$p < 0.0001$	

Table 2: Comparison of day 3 embryo with blastocyst aneuploid incidences.

The round blue dot is the egg aneuploid incidence from 8602 oocytes. The data are adapted from Kuliev et al. [32]. The red square is the aneuploid percentage of day 3 embryos from 3736 embryos in this study. The green triangle line is the complete chromosomal aneuploid pattern of day 3 embryos from 274 embryos. The data are adapted from Rabinowitz et al. [5]. The purple X dash line is the aneuploid incidence of blastocysts from 4403 blastocysts in this study. The star magenta dot line is the aneuploid incidence of POC from 1241 samples. The data are adapted from Wang et al. [33], the figure shows a trend of aneuploid incidence decreases from oocyte to POC stage. Please see discussion for detail considerations.

Discussion

Aneuploid occurrence is a frequent event in early human embryo

The aneuploid spectrum looks $16 > 22 > 21 > 15 > 13 > 18$.

By comparing these 2 developmental stages, there is a significant difference ($p < 0.0001$). When advance from day 3 cleavage embryo to blastocyst stage, there is a significant decrease in the aneuploid incidence.

Examination of aneuploid spectrum along with development

In addition to the data in this study (day 3 and blastocyst stages), reported data at metaphase II stage [32] and at spontaneous abortion (product of conception, POC) [33] stage are modified and compiled together to have a full vision of aneuploid spectrum evolving along with development. The compiled figure is Figure 1. The Figure shows a trend of decreasing aneuploid incidence along with development. At metaphase II stage, chromosome 22, 21, and 16 have a high aneuploid incidence. At day 3, all chromosomes show fair aneuploid incidence with chromosome 16, 21, and 22 with apparent higher incidence. This trend continues to blastocyst and POC stage.

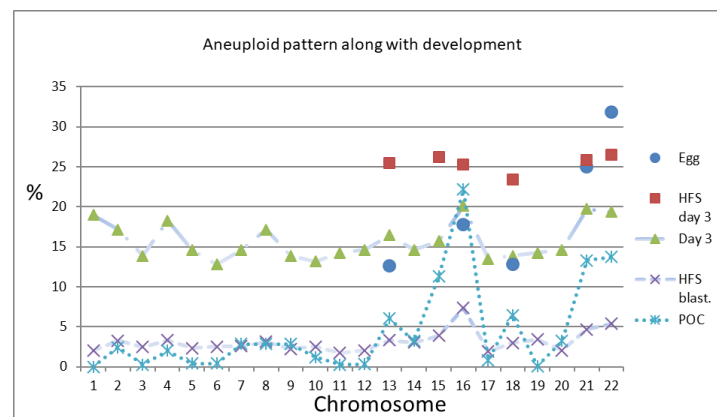


Figure 1: Composite of aneuploid pattern along with development.

development. What is the origin of meiotic and mitotic errors? Moor et al. [33] indirectly indicated the content of oocyte was the aberrant cause which is secondary to follicle cells. By mathematical model, Hardy et al. [17] also predicted the origin of aneuploidy was the egg quality. The direct evidence shows a difference of gene transcriptomics at pronuclear stage for embryos destiny to euploid or aneuploidy [35]. It coincides with the “developmental competence of embryos is already established at the zygote stage” [17]. These observations all indicate embryo aneuploidy mainly originates in the egg during folliculogenesis stage [36]. The numeric chromosomal abnormality at the oocyte stage can be examined by polar bodies. By detection Chromosome 13, 16, 18, 21, and 22 with 8602 oocytes, Kuliev et al. [32] reported the incidence of chromosomal abnormality is following the sequence

of chromosome 22>21>16>18>13. Chromosome 22 has 31.8 % of aneuploidy while chromosome 13 has 12.6% of aneuploidy. The pathway to be euploid or aneuploid appears to be already destined at oocyte stage.

In this study, the incidence of aneuploidy significantly decreases from day 3 to blastocyst stage (Table 2). It is similar to the report by Munne et al. [37] that more than 90% of aneuploidy disappeared as the embryo developed. Santos et al, 2010, observed clear decreasing of aneuploidy incidence from day 4, 5, to day 8 stage. These observations show a dynamic and evolving of aneuploid spectrum along with development. The sense of dynamic and evolving suggests a production and selection process. As reported, mitotic errors are less prone to occur after day 3 [18]. By considering aneuploid percentage decreases [28,37] and occurrence of aneuploidy less prone to happen after day 3 [18], logical deduction is that effective selection/purging mechanisms progress along with development.

The human embryo genomic activation is during 4 to 8 cell stage [14]. During the first 3 cleavages after fertilization, the cell cleavage check points are lacking and generate aneuploidy [38,39]. The cleavage of aneuploid blastomeres continues until the CCC mature. The maturation of CCC by mitosis/differentiation may trigger cell death/apoptotic mechanism to prevent the proliferation of aneuploid cells [12]. Currently neither the specific maturation timing of CCC nor the maturation timing/sequence for each specific chromosomal checkpoint is clear. The morula is the first stage of blastomere differentiation after embryo genomic activation. The Morula is also the first major stage of developmental arrest, which corresponds to first major phase of apoptotic activity in embryos [40]. The apoptotic phenomena continue to be found after the morula stage [16,40]. These observations support the concept that mitosis or/and differentiation activate/link to arrest/apoptosis mechanism [12,13] after activation of embryonic genome. The apoptotic phenomena have been described to be an active role in embryo development from pre-implantation to post-implantation stage [17,40-42]. Apparently, apoptosis is one of major mechanisms which keeps the genetic integrity of the developing human embryo.

Recently the number of genes in a specific chromosome has been reported by NCBI. (<https://www.ncbi.nlm.nih.gov/books/NBK22266/>). A Consensus Coding Sequence (CCDS) also included in the compiled table presented in the Introduction section. CCDS undergoes extensive manual review and it can consider as a subset of genes with consensus quality. Complete number of genes in each chromosome is still a working process. For the purpose of this study, the high quality CCDS data will be utilized for gene content ranking. Just By using CCDS data, the ranking of chromosome with amount of genes is by the following sequence (from high to low): 1>19>11>2>17>3>12>7>5>X>16>9>4>10>8>14>15>20>22>13>18>21>Y.

The top 3 chromosomes are 1, 19 and 11. The 3 lowest chromosomes are 13, 18, and 21 (not considering sex chromosome). From the ranking list, it shows 2 ideas: 1). The chromosomal gene content is

not following the list of chromosomal size, i.e. size of chromosome not fully corresponds to the number of genes contain within the chromosome.; 2). The main aneuploid chromosomes are seen in the lower half of the list. For the last one third of chromosomes, all 6 chromosomes (15,20,22,13,18,21) are the main chromosomes observed in abortion tissue. It reflects that aneuploidy from low gene content chromosomes can last from embryo stage, blastocyst stage, until POC stage. The delayed purging out of aneuploid blastomeres correlates with low gene content chromosomes suggests a delay/insensitive to CCC/differentiation induced arrest/apoptosis mechanism. The only exception is chromosome 16. The gene content of chromosome 16 is about at the mid of chromosomal gene ranking list. But it shows the highest presence at POC stage, this phenomenon seems suggesting that chromosome 16 expresses/contains certain anti-apoptotic or anti-arrest factor(s). This (these) factor(s) make(s) continuation cleavage of aneuploid blastomeres. It takes further differentiation until POC stage to sensitize/purging out aneuploid blastomeres.

In the early days of this study only 8 chromosomes (13, 15, 16, 18, 21, 22, X, and Y) could be tested with FISH. As the results, the incidence of aneuploidy among these chromosomes is marginal significant ($p=0.076$). Later Rabinowitz et al. [5] examined chromosomal aneuploidy with complete set of chromosomes. With 274 blastomeres, it is observed that all chromosomes had fair incidence of aneuploidy (by combination of trisomy and monosomy) with chromosomes 16, 21, 22 having a relatively higher incidence [5]. By examining 14 chromosomes with cleaving embryos, Munne et al. compiled sample size of more than one thousand blastomeres to examine. The aneuploid pattern looked similar to the results from study by Rabinowitz et al. [5]. The apparent higher incidence of aneuploidy is chromosome 22, 16, 21. The day 3 PGS data is more likely the sum of meiotic and mitotic errors. The aneuploid spectrum is more likely reflecting the occurrence of and susceptibility of specific chromosomal aneuploidy. From this study (Table 2) and Rabinowitz et al, 2012 data, the occurrence and susceptibility of aneuploid chromosome looks not showing big difference. At the blastocyst stage, there is a significantly different spectrum of chromosomal aneuploidy (Table 2). In this study, the frequency of aneuploid spectrum is chromosomes 16 > 22 > 21 > 15 > 13 > 18 which is very similar to other reports [3]. Since the intra-age, inter-center, and test platforms are not controlled, it is impossible to make direct comparison among different reports [43]. Aneuploidy of chromosome 16, 21, 22, 15, 18, and 13 has been shown as early as meiosis [32]. It shows these chromosomes are not only susceptible to aneuploidy but also insensitive/delay CCC arrest and other purging mechanisms during pre-implantation development. The consequences of these aneuploidies only show late post implantation at POC stage. Based on these observations, this study proposes a gradual eradicating of aneuploidy during development. The eradication sequence relates to specific chromosome gene content. The higher the gene content the faster the aneuploidy gets purging out. The lower the gene content the slower the aneuploidy gets demised.

The POC stage is basically the final stage to purge aneuploidy

before delivery. Those specific aneuploid chromosomes not present or rarely seen in POC must get eradicated early before POC stage, i.e. chromosome 1 and 19. Chromosome 1 and 19 contain the most genes among all chromosomes. It makes sense that aneuploidy with more gene creates more metabolic perturbation than aneuploidy with less genes. It reflects the aneuploidy involves with more genes encounters more sensitive/higher impact of eradicating force and disappears early. Those chromosomes with less genes show less interference of metabolism, slow in sensitizing CCC, and only encounter eradication after further differentiation. Chromosome 22, 21, 18, 15, and 13, with fewer genes, the impact only gradually shows up late, i.e. after implantation.

The aneuploid patterns during development are compiled and summarized in Figure 1. The aneuploid incidence of day 3 embryo by this study (red square markers) looks the highest. It supports the concept that the incidence of day 3 aneuploidy is the sum of meiotic and mitotic errors. By comparing aneuploid incidence between blastocyst and day 3 embryo (light purple cross markers), it shows a significantly lower aneuploid incidence by blastocyst stage. Logically it may be due to the gradual maturation of CCC and perturbation of developmental gene activities. At the blastocyst stage, aneuploid incidence of all chromosomes looks down to less than 5% except chromosome 16, 21, 22. The aneuploid incidences of Chromosomes 1, 16, 21, and 22 are the highest group at day 3 (green triangle marker). By just comparing the difference between day 3 and blastocyst stage, the decrease of aneuploid percentage looks similar among Chromosomes 16, 21, and 22 but not chromosome 1. Chromosome 1 has very low incidence of aneuploidy relative to Chromosomes 16, 21, and 22 at blastocyst stage. The low incidence indicates an active and effective eradication mechanism to clear up the blastomeres with chromosome 1 aneuploidy. Chromosome 1 contains highest amount of gene. It reflects gene contents reversely related to its survival.

After implantation, development can be classified into 2 subgroups in this study. One subgroup is pregnancy leading to live delivery. The other is mainly the spontaneous abortion (SAB). Wang et al. [33] reported spectrum of aneuploidy with POC tissue. The high number of observations makes the data creditable about POC tissue. But there is no aneuploid data for the delivered babies in the Wang et al. [33] study. By using the population delivery data, the percentage of genetic abnormal live birth is relative low and assumed negligible. For convenience, the aneuploid incidence is more likely concentrated in the SAB subgroup. Since Wang et al. [33] has reported the largest data for POC aneuploid spectrum, it is adapted and modified in Figure 1 (cyan star marker). By first look, it seems the aneuploid percentage of chromosome 13, 15, 16, 18, 21, and 22 increases from blastocyst to POC stage. One has to be cautious about the interpretation. The aneuploid incidence of POC is affected by denominator population for calculation of the percentage.

According to American College of Obstetricians and Gynecologists (ACOG) educational information (<http://www.acog.org/Patients/>

FAQs/Early-Pregnancy-Loss) the early pregnancy lost (first trimester loss) is roughly about 10% of total pregnant population and about half of this 10% is genetic problem (about 5% of original population). The total clinical pregnant population (the denominator population) should be 10 times of POC population. To compare apples to apples, POC aneuploid percentage is recalculated by total clinical pregnant population, the numerator is the same as incidence+ other negligible (genetic abnormality) from delivery and the denominator is the whole group (both normal delivery and SAB subgroups). Apparently, the quotient is only about 1/10th of data presented in Figure 1 (cyan star marker). By this consideration, the CCC and eradication mechanism still work. The aneuploid percentage for all chromosomes should be less than 0.5% except chromosome 13, 15, 16, 18, 21, and 22. For these chromosomes, the percentage range of aneuploidy is about 0.5-2.5%. The POC aneuploid percentage is much less than the blastocyst aneuploid percentage. This calculation does not support the idea that blastomeres with Chromosome 13, 15, 16, 18, 21, and 22 aneuploidy escape from CCC and proliferate more aneuploid blastomeres. It may just show that these chromosomal aneuploidies may not so sensitive to CCC for arrest/apoptosis but still controlled by CCC. The high percentage of chromosome 16, 21, and 22 are due their high susceptibilities to aneuploidy, starting from metaphase II stage to day 3 then to blastocyst stage. It is not due to blastomeres with these aneuploidies can escape from CCC and accelerate proliferation. Chromosomes 13, 15, and 18 aneuploidies look relative slower in response to CCC eradication, so the aneuploid percentage shows a relative increase. Exceptions are seen in the live birth of trisomy 13, 18, and 21 babies. The detail mechanism of these exceptions is not clear. The interesting evidence is Chromosome 13, 18, and 21 have least amount of genes (at the end of gene content rank). The chromosome 15, 16, and 22 contains more genes than Chromosome 13, 18, and 21. Trisomy from these 3 chromosomes (15, 16, 22) look seriously agitating the developmental gene symphony and get purged out almost completely (very rare to see live birth). As aneuploidy with highest gene content, chromosome 1, and 19 are absent from the POC aneuploid spectrum due to early eradication. The scenario looks hold for the remaining chromosomes but not as obvious as the chromosome with highest and lowest amount of genes.

Franasiak et al. [4] has reported an excellent study to examine chromosome aneuploid spectrum along with maternal age at blastocyst stage. In Figure 2 of their study, chromosome 8, 10, 12, and 19 had the highest aneuploid incidence. These relative peaks do not exist at POC stage. Chromosome 13 and 16 were at a major peak incidence at POC stage. These observations suggest the chromosome aneuploid incidence is not static. It is dynamic along with development. At day 3, the individual chromosome aneuploidy may just show the susceptibility of specific chromosome. After day 3, the gradual maturation of CCC and differentiation make some blastomere(s) with "certain chromosomal aneuploidy" gradually phasing out of proliferation cycle or engaging into apoptotic pathway. The "certain chromosomal aneuploidy" seems related to gene content of that chromosome. In Figure 3 of Franasiak et al. study [4], the chromosomal aneuploid error rate

can be categorized by karyotype or its structure. By karyotype group, the high aneuploid incidence pattern is Group G > Group E > Group D. By considering 2 main chromosomes in each group, Group G contains Chromosome 21 and 22. Group E contains chromosome 16 and 18. Group D contains chromosome 13 and 15. By adding up the chromosomal gene content ranking, it shows the similar pattern as the Karyotype group (Group G > Group E > Group D). These observations show that the gene content of each chromosome may more sensitive to karyotype. Logically the aneuploid chromosome with less number of genes gives fewer disturbances to developmental gene symphony or just a subtle mechanism to activate CCC. This consideration seems a sensible explanation of the dynamic chromosomal aneuploid spectrum along with development.

In conclusion, meiotic and mitotic errors accumulate chromosomal aneuploid incidence before embryonic genome activation. After day 3, gradual maturation of CCC and differentiation engage the aneuploid blastomeres into an eradication pathway. The aneuploid spectrum evolves through sequential differential selection. Those aneuploidies with high content of genes get purged out early while aneuploidies with low gene content get eradicated late, i.e. spontaneous abortion. Some aneuploidy elimination is incomplete/ as evidenced by live births of trisomy 13, 18, and 21.

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