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Investigation of *BAK*, *BAX* and *MAD2L1* gene expression in human aneuploid blastocysts

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Research Article

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Summary

Maintaining genomic stability is crucial for normal development. At earlier stages of preimplantation development, as the embryonic genome activation is not fully completed, the embryos may be more prone to abnormalities. Aneuploidies are one of the most common genetic causes of implantation failure or first-trimester miscarriages. Apoptosis is a crucial mechanism to eliminate damaged or abnormal cells from the organism to enable healthy growth. Therefore, this study aimed to determine the relationship between the expression levels of genes involved in apoptosis in human aneuploid and euploid blastocysts. In total, 32 human embryos obtained from 21 patients were used for this study. Trophectoderm biopsies were performed and next-generation screening was carried out for aneuploidy screening. Total RNA was extracted from each blastocyst separately and cDNA was synthesized. Gene expression levels were evaluated using RT-PCR. The statistical analysis was performed to evaluate the gene expression level variations in the euploid and aneuploid embryos, respectively. The expression level of the *BAX* gene was significantly different between the aneuploid and euploid samples. BAX expression levels were found to be 1.5-fold lower in aneuploid cells. However, the expression levels of *BAK* and *MAD2L1* genes were similar in each group. This study aimed to investigate the possible role of genes involved in apoptosis and aneuploidy mechanisms. The findings of this investigation revealed that the *BAX* gene was expressed significantly differently between aneuploid and euploid embryos. Therefore, it is possible that the genes involved in the apoptotic pathway have a role in the aneuploidy mechanism.

Introduction

The development of multicellular organisms is regulated not only by cell proliferation and differentiation but also by the elimination of unwanted cells with minimal disruption to the organism (Liu *et al*., 2000). Apoptosis is a well known mechanism by which such unwanted cells are removed and, therefore, is important in normal embryonic development as well as the elimination of abnormal cells to sustain tissue homeostasis. Aneuploidy in preimplantation embryos mostly results in implantation failure or miscarriage in the first trimester with the exception of some syndromic live births, such as Down's syndrome or Turner's syndrome (MacLennan *et al*., 2015). Therefore, any change in the number of chromosomes in the sperm or oocyte can affect the outcome of a pregnancy (MacLennan *et al*., 2015). Genome integrity is based on the homogenous distribution of replicated chromosomes to daughter cells during cell division. Apoptosis is a key process in ensuring this genomic integrity and escape from apoptotic pathways often results in neoplastic growths. The main mechanism of apoptosis is based on cytochrome *c* release from the mitochondria that is regulated tightly by Bcl2 and Bcl2 family proteins. Some members of this family, such as Bcl2, Bcl-XL and MCl-1, carry anti-apoptotic features whereas BAX and BAK are well known pro-apoptotic members of the family (Nguyen *et al*., 2007). BAX and BAK are members of the Bcl-2 family and core regulators of the intrinsic pathway of apoptosis. Upon apoptotic stimuli, they are activated and oligomerized at the mitochondrial outer membrane to mediate its permeabilization, which is considered a key step in apoptosis (Clarke and Allan, 2009).

Apoptosis is triggered when pro-apoptotic family members with a BH3 domain, such as BAD, BID, and BIM, are activated during cellular stress via changes in expression or through post-translational modifications. Then pro-apoptotic proteins, BAX and BAK, induce changes in the mitochondrial outer membrane permeability leading to the release of cytochrome *c* from the organelle's intermembrane space. Free cytochrome *c* then forms a complex with Apaf-1 called the apoptosome, which activates caspase-9 and triggers a cascade of apoptotic events (Pop *et al*., 2006; Riedl and Salvesen, 2007; Taylor *et al*., 2008; Clarke and Allan, 2009).

Mitochondria play an active role in apoptosis. First, most Bcl-2 family protein/protein interactions take place at mitochondria. Second, BAX and BAK form toroidal pores composed of proteins and lipids at the mitochondrial outer membrane, suggesting that mitochondrial

composition participates in pore formation. Third, a crucial step in apoptosis execution is the release of intermembrane space proteins to the cytosol. Fourth, BAX/BAK-induced mitochondrial outer membrane permeability in the absence of caspases triggers the release of mitochondrial DNA, leading to the production of type I interferons and causing a pro-inflammatory type of cell death. Therefore, it is likely that mitochondrial architecture, lipid composition and protein constitution are key elements in the complex regulation of apoptosis. It is well known that BAX and BAK colocalize in specific mitochondrial apoptotic foci upon activation (Clarke and Allan, 2009).

During cell division, duplicated chromosomes must be segregated accurately into daughter cells to prevent aneuploidy. The organization of microtubules into a bipolar mitotic spindle structure, the proper attachment of chromosomes to spindle microtubules at kinetochores and an appropriate length of time in mitosis, to ensure that all chromosomes correctly attach to spindle microtubules, are required for normal chromosome segregation (Orr *et al.*, 2015). The spindle assembly checkpoint (SAC) is a highly controlled signalling network that delays mitotic progression until all kinetochores are connected to spindle microtubules, therefore promoting correct chromosomal segregation. The SAC is crucial for cell and organism viability in higher eukaryotes to prevent chromosomal missegregation. However, abnormal SAC functioning allows for premature cell-cycle advancement to anaphase and significantly raises the risk of whole-chromosome missegregation, which leads to aneuploidy (Orr *et al.*, 2015). The molecular mechanism of SAC activation involves the kinetochores of both mitotic and meiotic chromosomes being enriched with BUB and MAD proteins (Maciejewska *et al.*, 2009). Mitotic arrest deficiency has been shown to be associated with MAD2, which is a key player in the SAC signal transduction cascade (Shi *et al.*, 2011). Furthermore, it has been shown that if the SAC pathway is not satisfied for an extended period of time, the members of this checkpoint are capable of triggering apoptotic signalling cascades to avoid aneuploidies and introducing genomic instability to the individual. Previous research predominantly suggests that cyclin B levels constantly drop when SAC is triggered. Once a certain threshold is obtained for cyclin B it can induce apoptosis by inducing caspase 9 and downstream apoptotic signals. Moreover, cyclin B is theorized to induce further upstream apoptotic signals that in turn activate mitochondrial pathways of apoptosis, namely BAX and BAK (Brito and Rieder, 2006; Clarke and Allan, 2009). Conversely, cyclin B is not the only SAC pathway member that can induce apoptosis, as MAD2 has also been suggested to show pro-apoptotic characteristics in addition to its role within the SAC pathway (Cheung *et al.*, 2005; Vogel *et al.*, 2007; Wang *et al.*, 2010).

In recent years, optimization of culture medium and passage numbers has improved blastocyst formation and pluripotency while reducing rates of apoptosis in IVF clinics (Park *et al.*, 2022). However, aneuploidies are still far from complete elimination in ART approaches and cause significant rates of miscarriages and/or failure of treatment due to implantation disruptions. Additionally, even in natural conception, aneuploidies can be observed in ~4–5% of clinical pregnancies, causing miscarriages (Fragouli *et al.*, 2013). While the causes of aneuploidy in IVF settings have been investigated previously, how are these aneuploidies countered by the growing embryo or how can they persist are questions remain to be firmly answered. Current theories of aneuploidy removal at the blastocyst-stage embryos involve the exclusion of aneuploid cells from compaction or 'passive' removal of aneuploid cells due to developmental arrest and stunted viability (Fragouli *et al.*, 2013; Lagalla *et al.*, 2017, 2020). Nevertheless, in somatic cells, DNA damage or aneuploidies are predominantly removed by apoptotic pathways. CHEK1-mediated cell-cycle arrest and consequent apoptosis activation are usually the first response against DNA damage. In contrast, the SAC pathway is generally the respondent during the mitotic phase and in the presence of aneuploidies (Clarke and Allan, 2009). Failure to remove abnormal cells *via* such pathways usually results in carcinogenesis. In embryos, apoptosis has been known to occur in response to DNA damage (Singla *et al.*, 2020; Zhang *et al.*, 2023). However, an apoptotic reply in response to aneuploidies is yet to be confirmed. Similarities between carcinogenic pathways and embryogenic pathways suggest that they can be within the realm of possibilities (Ma *et al.*, 2010; Smith and Sturmey, 2013; Manzo, 2019). Therefore, this study aimed to determine the relationship between the expression of genes involved in apoptosis in human aneuploid and euploid blastocysts, thereby gaining an understanding of mechanisms that may influence aneuploidies in embryos.

Materials and methods

Ethical approval was granted by the Near East University Scientific Research Ethics Committee (YDU/2021/96–1432). All patients were informed about the details of the study and provided written consent when they graciously accepted to contribute. The samples were human surplus embryos from patients attending the British Cyprus IVF Centre, Nicosia Cyprus. In total, 32 samples obtained from 21 patients were involved in this study. Of these 32 samples, 13 blastocysts were euploid (control) and 19 blastocysts were aneuploidy, respectively. Next-generation sequencing (NGS) was performed for the detection of aneuploidy via preimplantation genetic testing for aneuploidy (PGT-A). Details of the aneuploidies of samples included in this study can be found in Table 1.

Briefly, first the female partners underwent controlled ovarian stimulation and oocytes were obtained, followed by intracytoplasmic sperm injection (ICSI). The embryos that were developed to the blastocyst stage were biopsied and NGS was performed. Embryos included in this study showed no signs of structural defects such as translocations, deletions, duplications, inversions nor any other major anomalies aside from various aneuploidies.

Ovarian stimulation and embryo development

Controlled ovarian hyperstimulation (COH) was performed using a GnRH antagonist protocol. On the day of the menstrual cycle, recombinant FSH (150–300 IU, Gonal F, Serono) and/or hMG (75–150 IU, Merional, IBSA) were administered. From the sixth day of stimulation, the ovarian response was monitored by transvaginal ultrasound and by measuring the level of serum progesterone (P4) and estradiol (E2). The daily administration of 0.25 mg GnRH antagonist (Cetrotide, Serono) was applied until the day of ovulation trigger when the leading follicle size exceeded 13 mm. For the ovulation trigger, 250 mg hCG (Ovitrelle, Serono) or 0.2 mg triptorelin (Gonapeptyl, Ferrin) was administered and oocyte retrieval was planned 35 h after the trigger administration. The oocytes were denuded with 1/3 v/v hyaluronidase (hyaluronidase, 90101, Irvine Scientific, USA). Semen samples were prepared as described by Coban *et al.* (2020) and the selected sperm was injected into the oocyte. A fertilization check was performed on the next day (16–18 h post-ICSI) and two pronuclei with two polar bodies were considered to be a normal fertilization

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Table 1. List of aneuploidy status of the study sample population

Sample	Aneuploidy status	Sample	Aneuploidy status
1	+7, +8p, +14, +18, +22	17	−4p
2	−6q	18	−21
3	−10	19	X
4	−11q	20	EUPLOID
5	−9	21	EUPLOID
6	+19	22	EUPLOID
7	+5, −9q	23	EUPLOID
8	+4q	24	EUPLOID
9	−9q	25	EUPLOID
10	−19	26	EUPLOID
11	−20	27	EUPLOID
12	−16	28	EUPLOID
13	+3p	29	EUPLOID
14	−17	30	EUPLOID
15	+14	31	EUPLOID
16	X	32	EUPLOID

case (Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology, 2011). For the day 3 embryo morphology assessment, the grading system including the analysis of the number of blastomeres, blastomere evenness, degree of fragmentation, nucleus details and cytoplasmic characteristics, was used as described by Ciray *et al.* (2012). Morphological assessment of the embryos at the blastocyte stage (days 5 and 6) were performed as described by Gardner and Schoolcraft (1999). Embryos at the blastocyst stage were biopsied and the blastocysts were vitrified (Vit Kit-Freeze; 90133-SO, Irvine Scientific, USA). The biopsied samples were processed for aneuploidy screening by NGS in Igenomix. The genetic diagnosis services were carried out by IGENOMIX FZ LLC. Aneuploidy screening was performed by Ion ReproSeqPGS Kit analyzing all 24 chromosomes using Ion S5 System instruments (Thermo Fisher Scientific, Inc., MA, USA) and the data were analyzed by Ion Reporter (IRv5.4) software.

The spare aneuploid embryos and the non-transferred euploid embryos obtained from patients who had consented to research were collected for this project. Briefly, DNA and RNA were extracted from the single blastocysts and cDNA was synthesized using the RNA samples. The expression levels of genes involved in the apoptosis pathway were analyzed by real-time PCR.

Nucleic acid extraction, cDNA synthesis, and real-time PCR analysis

Nucleic acid extraction was performed using the Hibrigen total nucleic acid isolation kit (Hibrigen, Turkey, cat. No. MG-TNA-01-10) following the manufacturer's instructions. The purity and concentration of the extracted RNA were estimated using a NanoDrop spectrophotometer following the manufacturer's protocol (Thermo-Scientific, Pittsburgh, USA). The Hibrigen cDNA synthesis kit (Hibrigen, Turkey, cat. No. MD-CDNA-01–100) was used for reverse transcription following the manufacturer's instructions. The LightCycler® 480 SYBR Green I Master kit (Roche, Germany, ref. no. 04707516001) was utilized for real-time PCR following the manufacturer's protocol. Melting curve analysis was performed following each real-time PCR to differentiate between the primer dimer and the product, respectively. Primer sequences are listed in Table 2 and PCR conditions are shown in Table 3. The *ACTB* gene was used as a housekeeping gene for normalization. In cases in which an aneuploidy would affect the expression level of a gene directly, those samples were excluded from the analysis. For example, samples 6 and 10 were excluded from the analysis of *BAX* expression for having an extra chromosome 19 and missing one respectively, as *BAX* is situated on chromosome 19. Therefore, BAX expression analysis was conducted with 17 aneuploid embryos and 13 euploid samples in total.

Table 2. List of primer sequences

Genes	Forward primer	Reverse primer
BAX	GTGGTTGGGTGAGACTCCTC	GCAGGGTAGATGAATCGGGG

Table 3. Real-time PCR conditions

	PCR steps	Temperature °C/Time	Cycles
Steps	Initial denaturation	95°C/10 min	1
	Denaturation	95°C/10 s	40
	Annealing	64°C/10 s	
	Elongation	72°C/30 s	

Statistical analysis

GraphPad Prism v8 was used for all the statistical analysis.

Results

In total, 32 surplus blastocyst-stage embryos were used for this study. Trophectoderm biopsies were performed for all the blastocysts and NGS analyses were carried out. Of the 32 blastocysts, 19 were shown to have aneuploidies and the remaining 13 were classified as euploid. In this study, the possible regulatory effect of genes involved in apoptosis and the aneuploidy status of the embryos were investigated.

Briefly, the gene expression levels of *BAX, BAK,* and *MAD2L1,* which are involved in apoptosis, were analyzed by real-time PCR in euploid and aneuploid human blastocysts, respectively. The C_t values of all the analyses remained between 17–25 for *MAD2L1*, 24–28 for *BAK* and 21–29 for *BAX* as shown in Table 4. The expression level of the *BAX* gene was shown to be significantly higher in euploid compared with the aneuploid blastocysts as shown in Figure 1 ($P < 0.05$). Downregulation of BAX expression in aneuploid cells was found to be ~1.5-fold. The expression levels of the *BAK* gene were similar in samples obtained from aneuploid and euploid blastocysts samples, respectively ($P > 0.05$). Last, the expression level of *MAD2L1* was slightly higher in euploid blastocysts compared with the aneuploid blastocysts, respectively. However, this difference in the expression level was not statistically significant ($P > 0.05$).

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Table 4. Ct Value that shows the expression level of the genes

	ACTB	MAD2L1			BAK			BAX		
Sample	C_t	C_t	$\Delta\Delta C_t$	$2^{-\Delta\Delta Ct}$	C_t	$\Delta\Delta C_t$	$2^{-\Delta\Delta Ct}$	C_t	$\Delta\Delta C_t$	$2^{-\Delta\Delta Ct}$
1	22.1	19.0	0.67	0.63	28.2	2.36	0.2	26.5	5.01	0.03
2	22.7	19.5	0.57	0.68	27.8	1.36	0.39	27.0	4.91	0.03
3	22.7	21.4	2.47	0.18	28.1	1.66	0.32	27.3	5.21	0.03
4	24.5	21.4	0.67	0.63	26.8	–	2.72	27.8	3.91	0.07
5	22.2	20.9	2.47	0.18	27.4	1.46	0.36	25.8	4.21	0.05
6	23.4	20.6	0.97	0.51	27.2	0.06	0.96	26.5	3.71	0.08
7	24.1	17.5	–	7.13	26.5	–	2.53	25.7	2.21	0.22
8	23.9	20.2	0.07	0.95	–	–	–	26.6	3.31	0.10
9	22.9	20.1	0.97	0.51	27.4	0.76	0.59	27.0	4.71	0.04
10	24.1	19.0	–	2.52	27.7	–	1.10	26.6	3.11	0.12
11	24.9	18.2	–	7.64	27.6	–	2.06	28.7	4.41	0.05
12	24.9	19.9	–	2.35	27.6	–	3.12	29.7	5.41	0.02
13	22.9	19.2	0.07	0.95	27.1	0.46	0.73	27.1	4.81	0.04
14	23.3	20.7	1.17	0.45	26.6	–	1.27	26.5	3.81	0.07
15	24.4	25.9	5.27	0.03	26.8	–	2.53	26.5	2.71	0.15
16	26.4	24.0	1.37	0.39	25.6	–	23.29	28.2	2.41	0.19
17	22.4	18.4	–	1.18	26.7	0.56	0.68	27.0	5.21	0.03
18	22.8	19.6	0.57	0.68	24.5	–	4.12	27.8	5.61	0.02
19	23.4	19.6	–	1.02	25.9	–	2.36	29.3	6.51	0.01
20	23.6	19.0	–	1.78	26.9	–	1.36	21.7	–	2.45
21	22.1	18.8	0.47	0.72	26.7	0.86	0.55	23.2	1.71	0.31
22	21.7	17.6	–	1.26	26.7	1.26	0.42	22.9	1.81	0.29
23	23.2	19.5	0.07	0.95	27.4	0.46	0.73	22.1	−0.49	1.41
24	23.4	20.4	0.77	0.59	26.8	–	1.27	22.7	–	1.07
25	23.6	21.2	1.37	0.39	–	–	–	21.9	–	2.13
26	22.0	21.8	3.57	0.08	26.8	1.06	0.48	21.6	0.21	0.87
27	23.1	18.6	–	1.66	25.8	–	2.06	23.8	1.31	0.40
28	22.0	17.7	–	1.45	26.9	1.16	0.45	22.4	1.01	0.50
29	22.7	18.6	–	1.26	26.8	0.36	0.78	22.9	0.81	0.57
30	25.8	18.5	–	11.58	27.0	–	5.82	22.5	–	6.46
31	21.6	17.9	0.07	0.95	28.6	3.26	0.10	22.8	1.81	0.29
32	25.20	–	–	–	24.9	–	16.47	21.6	–	7.96

Discussion

Aneuploidy, mutations, or abnormal levels of gene expression during early preimplantation embryo development can disrupt crucial embryonic functions at specific stages and ultimately cause cleavage arrest or widespread apoptosis. Investigation of such genes and their function will surely be useful for gaining a greater understanding of the genetic circuitry of early human development. It will also provide an opportunity for the development of novel screening methods for evaluating genetic risk in reproduction. Therefore, this study aimed to investigate the relationship between the expression levels of genes involved in apoptosis in aneuploid and euploid human embryos.

Chromosome segregation failures in female meiosis result in aneuploidy in the developing oocyte and embryo, making them one of the primary genetic causes of spontaneous abortions and developmental disorders in humans. It is well acknowledged that aneuploidy of meiotic origin increases considerably as women age, and current research suggests that the majority of abnormalities originate in meiosis I. It is becoming increasingly clear that abnormalities are mostly tolerated until embryonic genome activation at the blastocyst stage. A study by Fragouli *et al.* (2013) showed that in an IVF setting aneuploidy rates can be observed in up to 83% of embryos at the morula stage that rapidly descends to approximately 60% of embryos when blastocyst-stage

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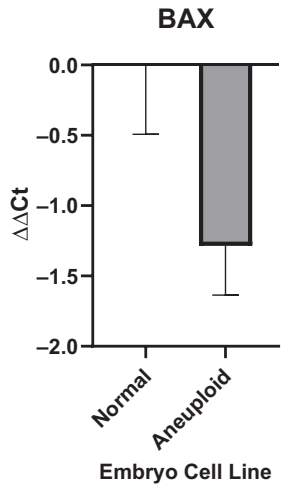

Figure 1. Relative expression of BAX in aneuploid embryos compared with euploid embryos. $\Delta\Delta C_t$ values were plotted on a logarithmic scale. When compared with euploid embryo cells, BAX expression shows an ~1.5-fold decrease in aneuploid cells, suggesting a repression of apoptotic responses in these cells.

samples are analyzed. Several studies about the mechanism of maternal age-related aneuploidy have been presented, including a failed SAC in meiosis I, failures in early meiosis, and low sister chromatid cohesion with age (Chiang *et al.*, 2012). Therefore, it is very important to investigate the expression levels of genes involved in the SAC not only in oocytes but also in the resulting embryos as potential defects may be persistent in the embryos originating from such oocytes.

Important systems for regulating genomic stability, such as cell arrest, cell-cycle checkpoints, and apoptosis, are lacking or insufficient throughout early human embryo development, resulting in an increased rate of aneuploidies (Mantikou *et al.*, 2012). Apoptosis, is required for the human body to operate properly, including cell turnover, immune system development and regulation, embryonic development, and gametogenesis (Vartak *et al.*, 2017). DNA damage has the potential to affect a wide range of biological activities, including cell-cycle control, DNA repair, and apoptosis (Bazrgar *et al.*, 2014). Numerous numbers of studies have focused on investigating the expression of genes involved in DNA repair. They discovered that it was increased in low-quality preimplantation human embryos with complicated aneuploidies (Wells *et al.*, 2005; Jaroudi and SenGupta, 2007; Jaroudi *et al.*, 2009; Kakourou *et al.*, 2013). As a result, DNA repair pathways are more activated in these embryos compared with cell-cycle control and apoptotic pathways. This suggests that DNA repair, rather than cell proliferation or apoptosis, and may be the main mechanism of DNA damage in poor-quality embryos with complicated aneuploidy (Bazrgar *et al.*, 2014). More specifically, it has been reported that the base excision repair (BER) mechanism is active in the zygote stage with sperm and oocyte BER enzymes (Lord and Aitken, 2015). Therefore, oxidative damage is predicted to be repaired. Paternal double-stranded breaks in the embryo are suggested to be repaired by a homologous repair mechanism using the female's genomic complement (Ma *et al.*, 2017). Non-homologous end joining does not seem to be active at this stage. After embryonic genome activation, the embryo may be more prone to damage and checkpoint kinase 1 (Chk1) may be involved in the control regulating the cell-cycle arrest (Ma *et al.*, 2017). DNA damage in the embryos overall may result in repair, implantation failure or passing on to the offspring (Khokhlova *et al.*, 2020). The outcome is influenced by the damage type and severity; in such severe DNA damage may result in abnormal blastocysts with embryonic loss (Shoukir *et al.*, 1998; Dumoulin *et al.*, 2000; Seli *et al.*, 2004). BCL-2 family proteins, involved in an anti-apoptotic pathway, are expressed in zygotes as well as blastocysts and therefore in cases in which their expression is impaired, implantation failure may be observed (Opferman and Kothari, 2018). Mismatch repair genes, *MSH2*, *MS3* and *PMS1*, were also shown to be expressed in human oocytes and blastocysts, suggesting that this pathway may also be active (Jaroudi *et al.*, 2009).

In contrast, Singla *et al.* (2020) discovered that aneuploid mouse cells generated at the 4–8-cell stage are progressively reduced via apoptosis from the early blastocyst stage to early post-implantation from the mosaic embryo's epiblast. In diploid cells, cellular protein quality control systems, such as the proteasome machinery and autophagy, remove the misfolded or unfolded proteins to reduce cytotoxicity and enhance healthy cell survival. After several mitotic divisions, chronic protein misfolding upregulates autophagy to the point in which it mediates cell death rather than protects the cell. This prevents the aneuploid cell from advancing further in the formation of the epiblast (Singla *et al.*, 2020). The findings of this study revealed that the *BAX* gene was expressed significantly differently between aneuploid and euploid embryos, respectively. Therefore, this may suggest that the *BAX* gene is functioning to activate the apoptotic pathway for the elimination of cells and eventually the whole embryo in euploid cases. Conversely, this mechanism is possibly hindered in aneuploidies. Conversely, there was no statistical difference in the expression levels of *BAK* and *MAD2L1* genes. Therefore, it seems the activation of apoptotic pathways is more complicated in preimplantation embryos.

One of the limitations of this study was that the fragmentation of the embryos was not investigated. Fragmentation can be associated with apoptosis. Apoptotic fragments of the embryos are expected to remain detectable if aneuploid cells are eliminated by apoptotic pathways. Therefore, in future studies, we are aiming to also include this assessment in our study. Another drawback of our study was the limited number of genes investigated. However, further studies are being performed to cover more genes in the apoptosis pathway as well as the spindle checkpoint. Conclusions of this preliminary study suggest that there may be a suppression of BAX-mediated apoptosis in aneuploid embryos however this intercepting mechanism is yet to be identified. This possible prevention of apoptosis might be the factor contributing to the persistence of aneuploidies in embryos that in turn may hinder success rates of pregnancies either by natural conception or via assisted reproductive technologies. Therefore, this study forms the basis of future studies. Further steps might also include the inclusion of translational studies to confirm that decreased BAX transcription is indeed able to exert influence on protein levels, as well as investigating possible suppressors of BAX/BAK-mediated apoptosis such as the Akt pathway and/or Bcl2 and other anti-apoptotic Bcl2 family members.

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