THE IMPACT OF THE SELECTED TYPES OF SERUM ON THE DIAGNOSTIC VALUE OF CYTOGENETIC TESTING

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S u m m a r y. The development of classical and molecular cytogenetics has opened new possibilities to investigate correlations between diseases of genetic origin or congenital malformations and chromosome aberrations. Cytogenetics has been used in the diagnosis of hematologic malignancies and as a diagnostic and prognostic tool in oncology. It has been applied in prenatal diagnosis and diagnosis of reproductive failures. Both classical and molecular cytogenetic study is based on slide analysis which contains the prophase, prometaphase and metaphase chromosomes derived from a cell culture.

Composition of a growth medium is an important factor affecting the morphology of a slide. Therefore an adequate serum should be used.

The aim of this study was to evaluate the impact of different sera on the diagnostic value of cytogenetic testing.

The material used in the study was venous blood collected into test tubes with lithium heparin from 10 healthy adults aged 18-50 years. We analyzed five types of sera: sheep, bovine, rabbit and horse sera compared to fetal bovine serum, which is routinely used in the cytogenetic diagnosis. The cell cultures have been grown under standard conditions (37°C, 72 hours, 5% CO_2 atmosphere). They were stopped by applying the Colcemid and then fixated with methanol and glacial acetic acid (3:1) solution. After the automatic preparation of 100 slides by *ZenDropper* (ZenTech) they were stained by GTG and evaluated using an Olympus light microscope (magnification 400x) and cytogenetic Applied Spectral Imaging system coupled with the Olympus BX61 microscope.

The correlation between the used serum and the quality of the chromosomes was found. The type of serum affects the morphology and spread of chromosomes, but has no impact on the mitotic index, resolution banding, cellularity or the purity of the slide.

The type of serum affects the diagnostic value in cytogenetic testing.

K e y w o r d s: cell culture, cytogenetics, diagnostics, serum.

INTRODUCTION

Classical cytogenetics is a branch of genetics which is based on the analysis of a number and structure of metaphase, prometaphase and prophase chromosomes. Chromosomes are obtained from cell cultures. By applying differential staining each pair of chromosomes obtains a characteristic pattern of alternating bands that differ in size and staining intensity (Szczałuba et al. 2010; Bal 2001; Rooney 2001; Srebrniak et al. 2008; Małuszyńska 2007). This technique allows a laboratory diagnostician not only to recognize a particular examined chromosome, but also to discover many chromosomal aberrations being the cause of genetically based diseases. One of the characteristics of a preparation is its resolution. The greater the resolution, the more chromosome bands can be

observed and therefore more subtle disorder in the chromosomal structure can be detected. Sensitivity of a standard karyotype testing does not exceed 5 million base pairs (5 Mbp), which corresponds to 450-550 chromosome bands. Better results can be obtained using high resolution techniques (HRT). They allow to identify changes in up to 3 Mbp. Classical cytogenetics has limitations related to identification of submicroscopic changes. The development of molecular biology techniques gave rise to a new branch of cytogenetics - molecular cytogenetics which is a fusion of classical cell culture methods with methods of molecular biology. It allows to detect slighter chromosomal abnormalities, even in 0.001 Mbp (Szczałuba et al. 2010). These aberrations may prove to be the cause of known syndromes or certain rare diseases.

CYTOGENETICS IN THE DIAGNOSIS

POSTNATAL DIAGNOSIS

Classical and molecular cytogenetics in hematology are used in the diagnosis of hematologic malignancies, including chronic myeloid leukemia (CML), myelodysplastic syndrome (MDS), acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) (Mrózek et al. 2004; Chase et al 2001; Le Beau 1999; Haas 1999; Sacha et al. 2010; Jędrzejczak et al. 2011).

In 85% patients CML is due to typical Robertsonian translocation t(9;22)(q34;11) leading to the formation of the fusion gene BCR-ABL called the Philadelphia chromosome (Ph'). Standard karyotype testing can detect this translocation. The remaining 15% patients have a completely different chromosomal structural abnormality. In those cases molecular methods are helpful in diagnosis, including the technique of fluorescence in situ hybridization (FISH) (Chase et al. 2001; Sacha et al. 2010).

Standard cytogenetic methods are also commonly used in the diagnosis of myelodysplastic syndromes. Myelodysplastic syndromes are characterized by the presence of a few short-living clones of hematopoiesis-inhibiting cancer cells in the bloodstream. Moreover, those syndromes are often accompanied by anemia (Jędrzejczak et al. 2011). Commonly (in up to 70% of cases of primary MDS) myelodysplastic syndromes coexist with clonal chromosomal aberrations (Le Beau 1999; Haas 1999).

Moreover, cytogenetics is used in the diagnosis of acute leukemia. In acute myeloid leukemia, karyotype abnormalities are observed more frequently in pediatric patients than adults. Approximately 200 chromosomal abnormalities, both with regard to number and structure, are known to be associated with AML. Many of them are rare aberrations, detected in a small number of patients. Aberrations are detected by classical cytogenetic techniques. A study by Brown et all. found subtelomer chromosomal rearrangements in patients with normal karyotype and proved that FISH may be a useful technique in the diagnosis of chromosomal abnormalities in AML. Karyotype changes in acute lymphoblastic leukemia, contrary to AML, are more frequent in adults than children. This could be of prognostic value in therapy (Mrózek et al. 2004).

Cytogenetics is an important diagnostic and prognostic tool in oncology (Pandey et al. 2007). Cytogenetic techniques are frequently used in cancer diagnosis, e. g. cancer of the head and neck (including retinoblastoma), lung cancer, bladder cancer and many more (Pandey et al. 2007; Bartova et al. 2003; Veiga et al. 2003).

Thanks to the GTG banding method it is possible to locate the sites of frequent chromosomal deletions (those are probably the regions of tumor suppressor genes) as well as sites involved in genetic rearrangements (activation of oncogenes) (Pandey et al. 2007).

Research conducted by Luciana CS Veiga et al., showed that monosomy of chromosome 17 in a group of patients with head and neck squamous cell carcinoma (HNSCC) is more likely to be detected by FISH (with the probe specific to the centromere of chromosome 17) than classical cytogenetic methods. Other studies have shown that the loss of 17p and simultaneous doubling of 17q can lead to the development of lung cancer (Veiga et al. 2003).

Structural abnormalities of chromosome 13 may lead to the development of retinoblastoma. Retinoblastoma is a childhood intraocular cancer, occurring sporadically or as a family form. The hereditary character develops at an earlier age than the sporadic form. Retinoblastoma is caused by mutations in the Rb1 gene located on the long arm of chromosome 13 (13q14). Comparative genomic hybridization (CGH) is used in cytogenetic diagnosis of this tumor. It can also detect other changes in genes, e.g. duplication 1q31, 2p24-p25, 6p22, 13q32-q34 and 16q22 loss (Bartova et al. 2003).

PRENATAL DIGNOSIS

Cytogenetics is used not only in the postnatal, but also prenatal diagnostics and the diagnosis of reproductive failure (Szczałuba et al. 2010; Kwinecka- Dmitriew et al. 2010).

It is considered that ca. 3 - 6% of recurrent miscarriages are due to abnormalities in the structure of chromosomes from one parent (Kwinecka-Dmitriew et al. 2010, Egozcue et al. 1997). These are balanced disorders, generally a translocation, inversion or mosaicism, occasionally there are changes in the number of sex chromosomes. They do not result in any changes in the look or health of the carrier (parent). Disorders are generally diagnosed by cytogenetic testing of the couples experiencing reproductive failures (Kwinecka-Dmitriew et al. 2010, Egozcue et al. 1997; Carp et al. 2004). Chromosomal abnormalities and single gene defects underlie the majority of congenital anomalies. Congenital malformations are predominantly lethal, but they also occur in approximately 2-3% live births.

It is estimated that chromosomal changes occur in about 0.9 % of newborns, producing significant physical and mental disorders (Szczałuba et al. 2010; Pokrzywnicka et al. 2010). Using available diagnostic methods it is possible to detect them at an early stage of fetal life. Invasive prenatal diagnosis is only offered to pregnant women at risk. It carries a danger of causing complications, including fetal death (approximately 1-2%) (Stembalska et al. 2012). The most common syndromes detected by routine cytogenetic tests are: Klinefelter, Down, Edwards, Patau and Turner syndromes, which are a consequence of numerical aberrations. They are due to nondisjunction chromosomes during meiosis. Molecular cytogenetics offers such techniques as FISH, MLPA, aCGH and their high-resolution variations which complement classical cytogenetic analysis in prenatal diagnosis. The use of FISH enables diagnostician to detect submicroscopic changes, which are the cause of numerous developmental defects characteristic to numerous syndromes, e.g. Williams, Beckwith-Wiedemann, Miller-Dieker, DiGeorge, Cri-du-chat, Wolf-Hirschhorn, Prader-Willi and Angelman. Multiplex Ligation-dependent Probe Amplification method (MLPA) makes locating a subtelomer changes possible in addition to diagnosing microdeletions. The latest technique - CGH array – provides a means to evaluate the entire genome, which is very important in confirming or excluding the presence of disease. It should be remembered, however, that classical cytogenetics is still of major importance in prenatal diagnosis Szczałuba et al. 2010; Srebrniak et al. 2008).

CELL CULTURES

Obtaining reliable diagnostic results depends on a number of conditions, including preparation of a slide containing well-dispersed, non-overlapping chromosomes with a good banding resolution. Material used in the postnatal testing is mostly venous blood collected to a test tube containing lithium or sodium heparin (Bal 2001; Rooney 2001; Srebrniak et al. 2008; Lim et al. 2004; Gstraunthaler 2003). It is important to avoid contamination of the established culture, so material should be collected aseptically and cell cultures should be set in sterile conditions, preferably under a laminar chamber (Bal 2001; Srebrniak et al. 2008; Stokłosowa 2006). Whole blood or lymphocytes cultures are grown "in suspension" in a liquid medium in specially designed disposable sterile Falcon tubes in an incubator with CO_2 flow.

In some cases substances synchronizing cell division (ametophterin or thymidine) or preventing chromosome condensation may be added (ethidium bromide, actinomycin D, 5-Bromo-2´-Deoxyuridine (BrdU), 5-azacytidine) (Bal 2001; Rooney et al. 2001; Lim et al. 2004). To obtain metaphase chromosomes, cell cultures are incubated for over 72 hours at 37°C in 5% CO_2. After this time, colchicine, known as Colcemid, is added to arrest the cell cycle at the metaphase stage of mitosis (Bal 2001; Rooney 2001; Srebrniak et al. 2008; Lim et al. 2004; Stokłosowa 2006). The concentration and the amount of Colcemid significantly affects the mitotic index, spreading and length of the chromosomes (Lim et al 2004). To release chromosomes from cells it is necessary to induce a hypotonic shock (Srebrniak et al 2008; Stokłosowa 2006). In most cases this is done by applying 75 mM KCl. It has been proven that using a hypotonic solution of 0.8% sodium citrate instead of potassium chloride allows for better banding resolution of chromosomes (Lim et al. 2004). One of the final stages of culturing is fixation, done by using fresh mixture of methanol and glacial acetic acid at the 3:1 ratio. Fixed cells are necessary to make slides. Preparing slides manually

can cause a lot of problems (Bal 2001; Srebrniak et al 2008; Stokłosowa 2006). It is better to apply an automated method using ZenDropper (ZenTech). Slides are stained mostly by GTG technique, which uses trypsin and Giemsa dye (Szczałuba et al. 2010; Bal 2001; Srebrniak et al. 2008; Małuszyńska 2007; Stokłosowa 2006).

THE COMPOSITION AND ROLE OF SERUM IN A CELL CULTURE

It is necessary to add serum to culture media, thanks to which cells can grow and divide properly without any signs of degeneration. Sera added to the cultures are intended to "bring" cells into in vivo conditions (Stokłosowa 2006). They also provide nutrients such as fatty acids, lipids, carbohydrates and amino acids. They are rich in vitamins A, B, C and E, and elements important for enzymatic pathways (cobalt, nickel, zinc, copper, selenium and iron). Sera facilitate the exchange of these compounds between cells and fluid. They contain protein carriers for water-insoluble substances necessary for a cell, such as lipids, minerals and hormones. They contain numerous hormones and essential growth factors which stimulate the growth, differentiation and functioning of cells. It is known that growth factors, hormones, transport proteins, cofactors and trace elements are important factors in regulating gene expression, cell cycle and cell division. Epidermal growth factor (EGF) and platelet-derived growth factor (PDGF) are involved in the activation of protein kinases necessary for the growth and differentiation of cells. It was found that that cell proliferation is less efficient in a serum-free protein (Gstraunthaler 2003; Brunner et al. 2010). Sera also help to optimize culture conditions by neutralizing toxic substances, inhibit trypsin (α-antitrypsin and α2-macroglobulin) and maintain optimal pH range 7.2-7.4 (Rooney 2001; Gstraunthaler 2003; Stokłosowa 2006). To obtain good proliferation and division of cells in a culture the amount of serum should be about 5-20% of the culture medium, depending on the type of culture and the type of cultured cells or tissues (Gstraunthaler 2003; Stokłosowa 2006).

SERA USED IN CELL CULTURES

Fetal bovine serum (FBS) is used routinely in cell cultures. It is estimated that the world annual production amounts to about 500,000 liters. This quantity is derived from approximately 1 million fetal calves. FBS is obtained from blood collected from fetal bovine by beating heart puncture, umbilical vein puncture and jugular vein puncture (Gstraunthaler 2003; Brunner et al. 2010; Van der Walk et al. 2004). In cell and tissue cultures other sera have been used, e.g. neonatal bovine, bovine, cow, human and horse serum (Stokłosowa 2006).

AIM

The aim of this study was to evaluate the impact of different sera on diagnostic value of cytogenetic testing.

MATERIAL AND METHODS

MATERIAL

The study used venous blood collected aseptically into test tubes containing lithium heparin. The blood samples of 9 ml were collected from 10 healthy volunteers, aged 18-50, who were not chronically ill and who did not take drugs. The research material was collected with prior approval of the head of the clinic, after obtaining informed consent from the patients - in compliance with the Bioethics Committee protocol.

CELL CULTURE

Five cell cultures were grown from each blood sample, differing in the composition of a culture medium. All of the cultures grown on individual sera were effective. The variable factor was the type of serum applied.

Besides fetal bovine serum, which was used as the reference material, other sera collected from sheep, bovine, horse and rabbit were used (Biomed S.A).

Mmitogen-PHA and antibiotic - penicillin / streptomycin was added to each culture. Cultures were grown at 37^{o}C for 72 hours in 5 % atmosphere of CO_2. After this time the cultures were stopped using Colcemid and 0.075 M KCl hypotonic solution was added. Thus obtained cell culture was preserved by methanol and glacial acetic acid (3:1).

PREPARATION OF SLIDES

Cytogenetic slides, two for each culture, were made automatically using ZenDropper (ZenTech). Then they were left for a day at 50^{o}C to age and then stained with GTG technique.

EVALUATION OF CYTOGENETIC PREPARATION

Cytogenetic preparation was evaluated under a microscope:

- 400x zoom (Olympus BX41) – to rate the mitotic index, the cellularity and purity of the slides, the morphology and spread of the chromosomes,
- 1000x magnification under immersion oil (Olympus optical microscope coupled with Applied Spectral Imaging System) – to assess the resolution bands.

The study was carried out at the Department of Clinical Genetics of Medical University of Lublin.

RESULTS

100 slides stained using GTG technique were analyzed, 20 from each kind of serum. The evaluated parameters included mitotic index (Fig. 1.), the number of cells per field and the purity of the slide, the morphology and spread of the chromosomes as well as the resolution banding of chromosomes.

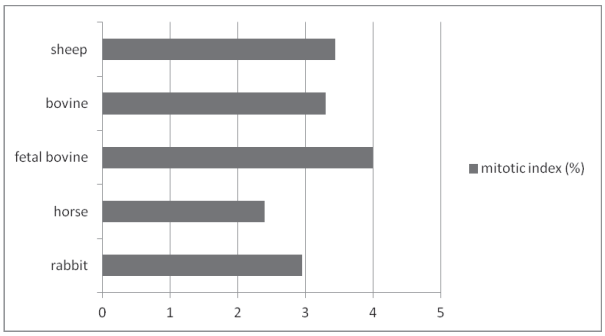

Fig. 1. Average mitotic index calculated from 100 slides (20 slides from each type of serum).

At the beginning, the mitotic indexes were compared and the results were similar for all five sera: from 2.20% for horse serum (the lowest score) to 4.22% for fetal bovine serum (the highest result). The next criterion was to assess the cellularity of the slides. The number of cells in the visual field for all five types of serum was greater than 20.

The next step was to evaluate the impact of the applied serum on the purity of the slides. Slides made from sheep and bovine serum were slightly cleaner than the others. Then, the morphology and the spread of chromosomes were analyzed. Similar results were observed for sheep, bovine and fetal bovine serum– chromosomes were well spread, the vast majority of chromosomes had long or medium length, only a few of them were crossed. The appearance and distribution of the metaphase chromosomes was comparable to the slides received from cultures containing horse or rabbit serum. In terms of banding resolution, no significant differences between cultures was noted (Tab. 1). In practice, all of the slides can be used to arrange karyotype (Fig. 2).

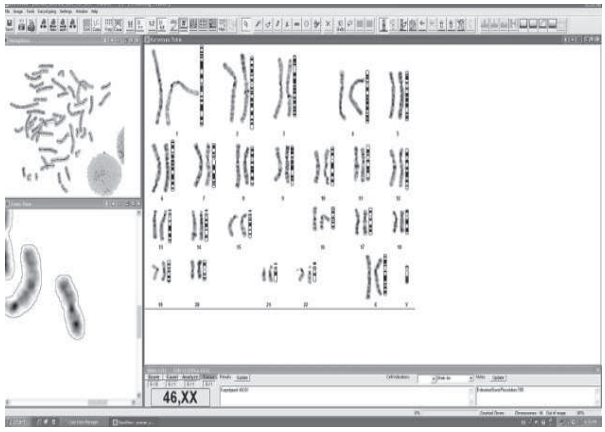

Fig. 2. The karyogram obtained from the culture grown on the rabbit serum using a computer system for cytogenetics: Applied Spectral Imaging

Table1. Serum parameters - the mean values obtained from the analysis of 100 slides

Type of serum	Sheep	Bovine	Fetal bovine	Horse	Rabbit
Mitotic index (%)*	3,44	3,30	4,00	2,40	2,95
Cellularity**	15-20	15-20	15-20	15-20	15-20
Slide purity***	4	4	3	3	3
Spreading of chromosomes	Chromosomes are well dispersed, few chromosomes folded, few overlap	Chromosomes are well dispersed, few chromosomes folded, few overlap	Chromosomes are well dispersed, few chromosomes folded, few overlap	Chromosomes are poorly distributed, folded and overlap	Chromosomes are poorly distributed, folded and overlap
Appearance of chromosomes	Advantages of chromosomes medium, long and short sparse	Advantages of chromosomes long and medium, short and thick few	Advantages of chromosomes long, thick and short sparse	Advantages of chromosomes medium and short, long few	Advantages of chromosomes medium and short, long few
Banding resolution of chromosomes ****	>450	>450	>450	>450	>450

** Mitotic index - the percent of metaphases in 200 cells; ** Cellularity - number of cells in the visual field; *** Slide purity scale 1-5 , 1 – dirty, 5 - cleanest; ***** Banding *resolution of chromosomes – number of bands*

DISCUSSION

Our study has shown that the type of serum has the influence on the morphology of chromosomes and the quality of slides. It appears that the use of sheep or bovine serum creates a similar culture conditions as the use of typical fetal bovine serum. Chromosomes obtained from these cultures are long, well banded and without overlaps. They can be used for karyotype analysis. It is easy to find good quality chromosomes in those slides.

In the cases of horse and rabbit sera, karyotype analysis is also possible but finding an appropriate metaphase plate poses many difficulties (Fig. 3). This is the result of the predominance of shortened, thickened chromosomes. This is likely due to similar composition of fetal bovine, sheep and bovine serum. Horse and rabbit sera most likely differ in the composition from the aforementioned three.

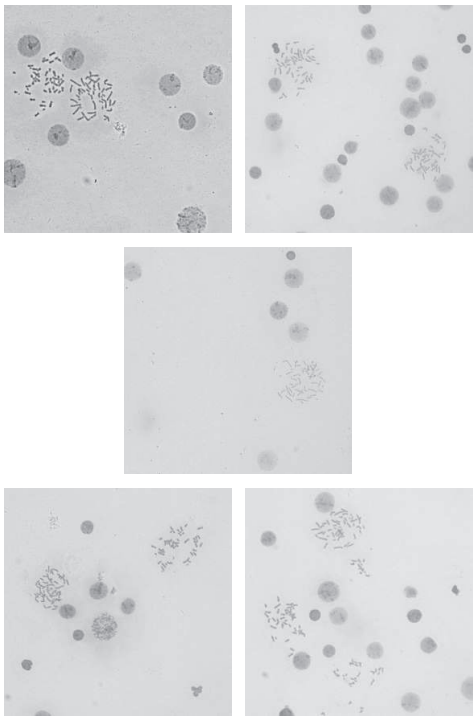

Fig. 3. Chromosomes stained by GTG technique. Mag 400x. From top left: sheep, bovine, fetal bovine, horse and rabbit serum.

The study proved that the type of serum used has no effect on the cellularity or mitotic index. Results of the former are comparable between the sera and show 1% differences. This appears to be obvious as the main role in the stimulation of cell divisions is played by a mitogen (PHA). It stimulates the specific cell populations, i.e. T lymphocytes to divide (Srebrniak et al. 2008; Stokłosowa 2006). Another factor having influence on the mitotic index is the concentration of Colcemid.

Surprisingly, despite the difficulties with finding metaphase plates suitable for the evaluation, the banding resolution in obtained chromosomes was good (Fig. 4). It can be concluded that the origin of sera is not the factor influencing the banding resolution.

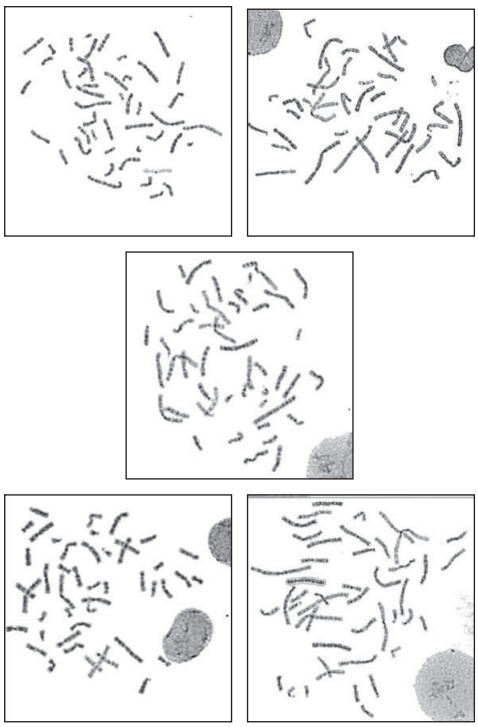

Fig. 4. Spreading chromosomes with visible banded patterns. Mag 1000x. From top left: sheep, bovine, fetal bovine, horse and rabbit serum.

CONCLUSION

The studies have found correlation between the type of serum applied and the morphology of the chromosomes (and thus the value of cytogenetic testing). The use of improper type of serum can cause difficulties in assessing a cytogenetic preparation. This is due to the impact of used serum on the morphology of chromosomes and metaphase plate.

Replacing fetal bovine serum with bovine or sheep serum is promising, however it needs further analysis.

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REFERENCES

1. Bal J (2010) Biologia molekularna w medycynie. Elementy genetyki klinicznej. Wydawnictwo Naukowe PWN, Warszawa, pp 38-40, 95-107.
2. Bartova E, Kozubek S, Gajova H, Jirsova P, Zluvova J, Taslerova R, Koutna I, Kozubek M (2003) Cytogenetics and cytology of retinoblastoma. Journal of Cancer Research and Clinical Oncology 129: 89-99.
3. Brunner D, Frank J, Appl H, Schoffl H, Pfaller W, Gstraunthaler G (2010) Serum free cell culture: The Serum-free Media Interactive Online Database. ALTEX 27: 53-62.
4. Carp HJA, Feldman B, Oelsner G, Schiff E (2004) Prenatal Karyotype and subsequent live births in recurrent miscarriage. Fertility and Strility 81: 1296-1301.
5. Chase A, Huntly BJP, Cross NCP (2001) Cytogenetics of chronic myeloid leukemia. Best Practice and Research Clinical Hematology 14: 553-719.
6. Egozcue J, Blanco J, Vidal F (1997) Chromosome studies in human sperm nuclei using fluorescence in situ hybridization (FISH). Human Reproduction Update 3: 441-52.
7. Gstraunthaler G (2003) Alternatives to the Use of Fetal Bovine Serum: Serum-free Cell Culture. ALTEX 20:275-81.
8. Haas OA (1999) Cytogenetic classification of MDS in Children. Leukemia Research 23: 6.
9. Jędrzejczak WW, Kuliczkowski K, Podolak-Dawidziak M, Urbaniak-Kujda D (2011) Zespoły mielodysplastyczne. Zalecenia postępowania diagnostyczno-terapeutycznego w nowotworach złośliwych.
10. Kwinecka-Dmitriew B, Latos-Bieleńska A, Skrzypczak J (2010) Przebieg ciąż u par nosicieli aberracji chromosomowych. Perinatologia, Neonatologia i Ginekologia 3: 192- 98.
11. Le Beau MM (1999) Advances in cytogenetic analysis of myelodysplastic syndromes (MDS). Leukemia Research 23: 5.
12. Lim AST, Chia P, Kee SK, Raman S, Tien SL (2004) The Importane of High Resolution Chromosome Analysis in the Diagnosos of Birth Defects: Case Reports of Holoproscencephaly and Cystic Hygroma. Annals Academy of Medicine. Singapore 33: 537-40.
13. Małuszyńska J (2007) Zobaczyć gen, chromosom i genom – czyli badania cytogenetyki molekularnej. Nauka 4:107-15.
14. Mrózek K, Heerema NA, Bloomfield CD (2004) Cytogenetics in acute leukemia. Blood Reviews 18:115-36.
15. Pandey A, Mishra A (2007) Cytogenetics in head and neck cancer. Indian Journal of Otolaryngoly and Head and Neck Surgery 59: 317-21.
16. Pokrzywnicka M, Krajewski K, Chudzik A (2010) Żywo urodzone dziecko z triploidią jako problem kliniczny zobrazowany opisem przypadku. Perinatologia, Neonatologia i Ginekologia 3: 153-55.
17. Rooney DE (2001) Human Cytigenetics: constitutional analysis a practical approach. Oxford University Press, USA, pp 33-50.
18. Sacha T, Foryciarz K (2010) Diagnostyka i ocena skuteczności leczenia przewlekłej białaczki szpikowej. Hematologia 1: 219-28.
19. Srebrniak M. I., Tomaszewska A (2008) Badania cytogenetyczne w praktyce klinicznej. Wydawnictwo lekarskie PZWL, Warszawa, pp 35-59, 81-92.
20. Stembalska A, Nomejko A, Pesz N (2012) Poradnictwo prenatalne- diagnostyka prenatalna inwazyjna. Perinatologia, Neonatologia i Ginekologia 5: 100-04.
21. Stokłosowa S (2006) Hodowla komórek i tkanek. Wydawnictwo Naukowe PWN, Warszawa, pp 53-60.
22. Szczałuba K, Obersztyn E, Mazurczak T (2010) Zastosowanie nowoczesnych technik cytogenetyki molekularnej w diagnostyce wrodzonych wad rozwojowych. Perinatologia, Neonatologia i Ginekologia 3: 106-08.
23. Van der Walk J, Mellor D, Brands R, Fischer R, Gruber F, Gstraunthaler G, Hellebrekers L, Hyllner J, Jonker FH, Prieto P, Thalen M, Baumans V (2004) The humane collection of fetal bovine serum and possibilities for serum free cell and tissue culture. Toxicology in Vitro 18: 1-12.
24. Veiga LCS, Bergamo NA, Kowalski LP, Rogatto SR (2003) Classical and molecular cytogenetic analysis in head and Neck squamous cell carcinomas. Genetics and Molecular Biology 26: 212-128.