UDC 636.27 (477):576.316

CHROMOSOMAL ANOMALIES IN DAIRY CATTLE AS REASONS OF IMPAIRED FERTILITY

V. V. Dzitsiuk, H. T. Tipilo

Institute of Animal Breeding and Genetics NAAS, named after M.V. Zubets
1, Pohrebniaka Str., Chubynske village, Boryspil District, Kyiv Region, Ukraine 08321

E-mail: valentynadzitsiuk@gmail.com

Received January 10, 2019 / Received February 15, 2019 / Accepted March 22, 2019

Aim. The evaluation of animals for the presence of chromosomal anomalies is one of the main tasks of practical selection, aimed at detecting undesired chromosomal anomalies in early age, which may have negative impact on the reproductive and productive capability of cows and lead to considerable economic losses. The aim of the work is a cytogenetic analysis of the chromosome set in cows of Ukrainian Red-and-Motley dairy cattle breed, which will allow assuming a decrease in reproductive functions with chromosomal aberrations. **Methods.** We examined 53 cows of the Ukrainian Red-and-Motley dairy cattle breed in SE Research Farm Khrystynivske, IABG named after M.V. Zubets, NAAS. The investigation of chromosomal anomalies involved 72-h cultivation of lymphocytes from the peripheral blood of animals using the common methods. During a routine analysis the preparations were stained with 2 % Giemsa staining solution. The induction of G-bands for differential staining of chromosomes was conducted using 0.25 % solution of trypsin. The processing of study results was performed with Microsoft Excel software package. **Results.** The investigations in the aberration spectrum detected aneuploid and polyploid cells, breaks and fragments of chromosomes, premature chromosome disjunction in mitosis and translocation. The total number of aberrant cells in cows with decreased fertility was 14.69 ± 0.56 %, the number of aberrations per one investigated cell was 0.144, which was almost twice reliably ($P < 0.999$) exceeding the values of similar features for cows which did not have problems with reproduction. GTG-banding method was used to detect a new RT 13/23 Robertsonian translocation. **Conclusions**. The cytogenetic analysis of chromosome set of Ukrainian Red-and-Motley dairy breed cows allows assuming the connection between a decrease in the fertility of cows and chromosomal instability. A routine screening of dairy cows allows both evaluating the karyotype saturation with undesired chromosomal aberrations and using the obtained results to forecast the reproductive ability of an animal in the early age.

Keywords: karyotype, aberrations, Ukrainian Red-and-Motley breed, reproductive ability.
DOI:

INTRODUCTION

Species, breed, linear and familial features of animals are passed from generation to generation due to material inheritance media – chromosomes. The diploid set of somatic cells contains a complete, stable and specific volume of information, remarkable for the species, and ensures a full-fledged functioning of the systems of the animal organism.

However, factors of different nature in the structure or in the number of karyotype chromosomes may trigger changes, leading to the disruption of gene functioning and formation of hereditary defects which compose undesired genetic burden of the population and lead to economic losses [1].

Over 50 years ago, Professor Ingemar Gustavsson, a pioneer of veterinary cytogenetics, presented evidences of the negative impact of karyotype anomalies on the development and functioning of organism systems in domestic animals, in a renowned scientific journal *Nature* [2]. Later studies allowed identifying chromosomal aberrations in animals, which lead to the decrease in fertility, structure and functions of the phenotype, and the stability of their gene fund in general [3–5].

Cytogenetic studies, conducted by different investigators in different countries using different equipment,

© V. V. DZITSIUK, H. T. TIPILO, 2019

confirm that the decrease in fertility of cows occurred after the start of instability of chromosomal apparatus [6, 7]. At present, over 10 different types of chromosomal anomalies have been detected in animals of different cattle breeds which result in up to 45 % loss of progeny (embryonic, fetal, perinatal) [8]. While chromosomal anomalies are in heterozygous state in the karyotype, they may not be manifested in the phenotype of a host animal, but may present the threat to spreading undesired genetic burden in the population [9–11].

Severe requirements have been put forward to hereditary features of breeding bulls for a long time, as it is related to the possibility of obtaining hundreds and thousands more calves from one bull via artificial fertilization, compared to one cow. Currently, the value of some cows, especially highly productive ones, increases considerably with the distribution of biotechnological methods in reproduction (transplantation of embryos, etc.). Thus, more attention is paid to thorough genetic evaluation of the breeding stock of pedigree herds.

Repeat breeding of animals, miscarriages, birth of deformities, dead, inviable litter are reasons for cytogenetic analysis. The evaluation of animals for the presence of chromosomal anomalies is one of the main tasks of practical selection with the purpose of detecting undesired chromosomal anomalies in early age, which may have negative impact on the reproductive and productive capability of cows.

The aim of our study was to analyze a spontaneous level of chromosomal aberrations in animals with different manifestations of the reproductive function.

MATERIALS AND METHODS

The object of the study was a breeding stock of Ukrainian Red-and-Motley dairy breed cows, bred in SE Research Farm Khrystynivske of the Institute of Animal Breeding and Genetics n.a. M.V. Zubets, NAAS. The materials of zootechnic registration of the computer information management system *Intesel Orsek* for dairy farming were used to select cows with reproductive impairments (23 animals) and cows with normal reproductive function (30 animals).

The material for investigation was peripheral blood, taken from the caudal vein of cows into sterile syringes with the heparin solution. The procedure of blood sampling was conducted with thorough compliance with sterility requirements, sterilizing the skin surface of animals with 70 % of ethyl alcohol at the place of injection prior to sampling. Blood samples were transported in the insulated container at 2–4 °C.

The cytogenetic analysis was conducted in the Laboratory for Genetics, the Institute of Animal Breeding and Genetics n.a. M.V. Zubets, NAAS.

Obtaining chromosome preparations was performed using the culture of lymphocytes (1 ml), cultivated in the cultural medium RPMI 1640 with L-glutamine (Sigma, USA) (5 ml) with the addition of phytohemagglutinin R (PHA-R, Sigma, USA) (0.015 ml) and embryonic calf serum (1 ml) for 72 h in the thermostat at 37 °C. Two hours prior to the end of cultivation, each vial was added colchicine to stop the division of cells at the metaphase stage (Sigma, USA) in the concentration of 0.05 μg/ml. It allowed analyzing the cells of the first mitosis mostly. After the cultivation, the content of vials was poured into centrifuge tubes and centrifuged for 15 min at 1.000 rpm. Hypotonic treatment of cells was conducted with the obtained *ex tempore* and heated to 38 °C 0.075 M potassium chloride solution for 20 min. After hypotony, the suspension of cells was centrifuged to remove the potassium chloride solution. The supernatant solution was carefully removed with the 1 ml measuring flask, the cell pellet was fixed with the freshly made cooled mixture of ethanol (or methanol) and icy acetic acid in the 3 : 1 ratio. The treatment with the fixative was conducted 3–4 times with intermediate resuspending and centrifuging until complete discoloration of the mixture. The obtained fixed cell pellets were kept in the freezer up to the moment of preparing metaphase chromosome preparations. To prepare the latter, a cell mixture was dissolved with a small amount of the fixative to achieve the desired density, resuspended carefully and several drops were applied to a cold wet specimen slide. Then the glass slide was quickly passed through the flame of alcohol burner to blaze away the fixative which promoted the improvement in preparation quality.

Giemsa staining involved the use of 2 % solution with phosphate buffer for 10–15 min. To achieve differential GTG-banding, the preparations of metaphase chromosomes were placed for 10–15 sec in 0.25 % trypsin solution, dissolved in 0.25 % Hanks' solution at room temperature and stained with the solution of azure eosin.

The slides with no overlapping layers of chromosomes were used for the analysis which allowed counting the total number and identifying them. The intermediate count of sex chromosomes, each of which

corresponded to one haploid set, was used to count polyploid cells.

Metaphase slides were photographed with a digital camera Olympus D-460 ZOOM.

The frequency of aberrant metaphases and the spectrum of chromosomal aberrations were defined as parameters of chromosomal instability in all the studies. The cells were deemed to be aberrant if they had at least one structural or quantitative impairment of karyotype. Chromosomal aberrations were counted in at least 30 metaphases. The analysis of preparations of metaphase cells included the following indices: frequency of aneuploid and polyploid cells, frequency of cells with structural aberrations of chromosomes (chromosome breaks, fragments of chromosomes, premature centromere division of mitotic chromosomes – (PCDMC)).

The organization of sampling considered typicality, objectivity, and homogeneity of primary materials of pedigree records. At least 30 metaphase slides were analyzed for each animal in both groups.

Student's t-test was used to estimate the results with three levels of reliability values ($P > 0.95$, $P > 0.99$, $P > 0.999$). The processing of study results was performed with Microsoft Excel software package.

RESULTS OF STUDIES

Two groups of cows were formed with the consideration of functional impairments of the reproduction system: group I – animals with frequent stillborn calves and spontaneous miscarriages during the usage period ($n = 23$); group II – cows with no decrease in the reproductive function ($n = 30$).

The frequency of aberrations in the karyotype of cows with different reproductive ability

Group	I	II
Animals	23	30
Investigated metaphases	678	882
Total aberrant cells, %	14.69 ± 0.56	7.10 ± 1.0
Frequency of genomic aberrations, %		
aneuploid cells	2.33 ± 0.64	1.39 ± 0.31
polyploid cells	1.92 ± 0.23	1.38 ± 0.31
Frequency of structural aberrations of chromosomes, %		
breaks	2.70 ± 0.47	0.75 ± 0.39
fragments	1.69 ± 0.41	1.27 ± 0.28
translocations	1.34 ± 0.03	
PCDMC	2.66 ± 0.71	0.37 ± 0.30

The analysis of chromosome preparations demonstrated that the spectrum of aberrant metaphases had aneuploid and polyploid cells, chromosomes with breaks, paired and single fragments of chromosomes, translocations and premature centromere division of mitotic chromosomes (PCDMC). Aberrant cells had one-three aberrations.

Cytogenetic analysis of 678 investigated metaphases in 23 cows with the decrease of the reproductive function demonstrated 98 aberrant cells (14.69 ± 0.56 %). Among them, there were 4.25 % heteroploid cells, the main share of which (2.33 %) consisted of aneuploidy and 1.92 % – polyploidy (Table). Structural aberrations of chromosomes had the frequency of 4.70 %, with the highest frequency for cells with chromosome breaks – 2.70 ± 0.47.

The analysis of 882 metaphase slides from 30 cows with normal reproductive ability revealed 63 aberrant cells, which demonstrated the frequency of 5.16 %. The cumulative mean level of heteroploidy was 2.77 %, including almost equal frequencies of aneuploid and polyploid cells of 1.39 % and 1.38 % respectively. The cells with chromosome breaks had the frequency of 1.75 %, the ones with fragments of chromosomes – 1.27 %, which formed the total for the group of structural aberrations of chromosomes of 2.02 %.

The number of aberrations per one investigated cell of cows with normal reproductive function was 0.071, and that of cows with impaired function – 0.144.

The cumulative frequency of aberrant cells in cows with impaired reproductive function was 14.69 ± ± 0.56 %, which was almost twice reliably ($P < 0.999$) exceeding the value of a similar feature in cows with normal reproduction.

In particular, the level of numerical aberrations (aneuploidy and polyploidy) in the cells of cow's blood with impaired reproductive ability was reliably exceeding ($P < 0.999$) similar indices for cows, which did not have miscarriages and stillborn calves. It was also determined that there was a reliably higher frequency of polyploid cells in cows with prolonged service-periods.

The comparison of metaphase frequencies and chromosome breaks allowed detecting statistically higher ($P > 0.99$) mean value of this feature in animals with impaired reproductive function.

PCDMC is a feature, demonstrating the instability of chromosome set of the animal. The frequency of cells with this feature was almost 7-fold statistically reliably

higher (P > 0.999) in animals with impaired reproductive function.

The analysis of the spectrum of karyotype aberrations in the examined animals demonstrated structural reconstructions of chromosomes, formed as a combination of two acrocentric non-homologous chromosomes with centromere areas with the formation of one metacentric chromosome, qualified as Robertsonian type (RT) translocations. When balanced, they do not have any visible impact on the organism, as the genetic material, present in the fusion area, is the same as in two separate chromosomes. However, other researchers [12] report that they are rather dangerous due to the fact that their carriers are phenotypically normal, but in breeding age they often have decrease in the fertility, and in some cases – sterility.

The analysis of routinely stained preparations demonstrated Robertsonian translocations in three animals with the frequency of 0.31±0.03 %. One of them was identified as RT 1/29 (Fig. 1). This translocation was detected with the frequency of 0.063 % in cows with impaired reproductive system.

RT 1/29 – the centric fusion of the largest and smallest chromosomes – is the most common for cattle. This translocation was registered in 30 bovine cattle breeds with the frequency from 0.1 to 32 %. If an animal karyotype has RT 1/29, the chromosome set consists of 59 chromosomes ($2n = 59$) and routine staining allows identifying a large submetacentric chromosome on the preparation, in addition to sex chromosomes. Different authors described over 50 types of Robertsonian translocations in bovine cattle with different autosomes, but with more frequent involvement of chromosomes from pairs 1, 9, 14, 16, 21, 27, and 29 into structural transformations [13–15].

It was demonstrated that Robertsonian translocations often occur spontaneously in the progeny of parents with normal karyotype. Although current mechanisms of forming Robertsonian translocations have not been determined completely, the study results of cytogenecists indicate that they may occur due to reparation errors, which conditions inaccurate coupling of breaks via non-homologous recombination [16].

Most types of centric fusion in dairy cattle, including RT 1/29, condition the decrease in fertility [17]. Regardless of the fact that the carriers do not have deviations in the phenotype, the translocations lead to their formation of chromosome-unbalanced gametes, which are a reason of early embryonic death after fertilization

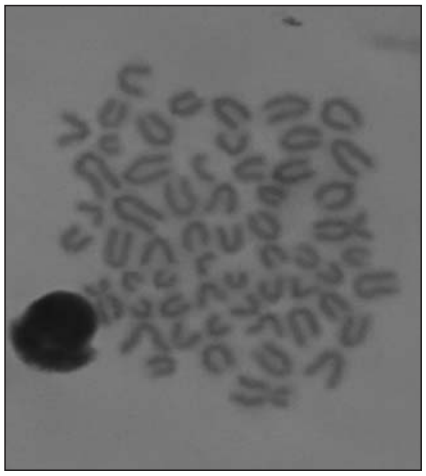
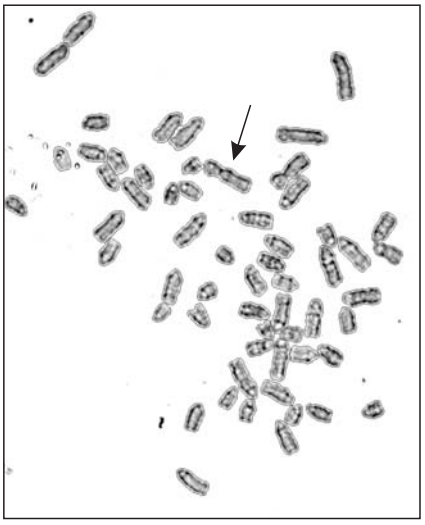


Fig. 1. Metaphase slide of a cow with translocation RT 1/29. Magnification: vol. ×100; cir. ×10



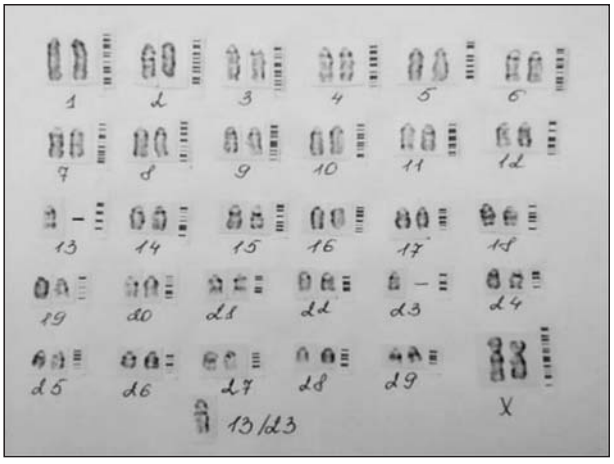


Fig. 2. Metaphase slide of Ukrainian Red-and-Motley dairy breed cows with RT 13/23 and the relevant karyogram

[18]. The karyotype of bovine cattle is notable for the presence of only autosomes of acrocentric type in their

set and it is almost impossible to distinguish their larger part using routine staining. Still, it is possible to identify RT 1/29 with high probability on the preparations with routine staining, as the translocation involves the largest and smallest chromosomes of the set. Limited possibilities of routine staining make it doubtful to identify translocations with the participation of other chromosomes. Only the application of the methods of differential staining, G-banding in particular, allows determining which chromosomes are involved in the translocation.

Our analysis of differentially stained preparations of chromosomes revealed a cow (inventory number 6040) with the set of chromosomes $2n = 59$ and the chromosome transformation – Robertsonian translocation. GTG-banding of preparations of the chromosome set of the animal demonstrated that this translocation was formed out of two non-homologous acrocentric chromosomes from pairs 13 and 23 and identified as Robertsonian translocation RT 13/23 (Fig. 2). The presence of large blocks of centromeric heterochromatin in the translocated chromosome allows for the assumption that the translocation was formed *de novo*.

During the period of staying at a holding, this cow gave a phenotypically healthy calf (heifer), the milk yield for the first lactation was 6.787 kg, service-period after the first calving was 188 days, and the calving interval was 470 days. The second calving was ended with a miscarriage.

It is known that animals with translocation in the heterozygous state form sex cells with excessive or insufficient chromosomes in the meiosis along with full-fledged gametes. The presence of a chain or a ring of quadrivalents in diakinesis of meiosis leads to uneven disjunction which results in the formation of sex cells with unbalanced set of chromosomes at the stage of metaphase II of meiosis. Thus, the phenotypic manifestation of this type of chromosomal aberrations is manifested in increased embryonic morbidity [19].

We have not found any communications in the scientific literature about any translocation with chromosome 13 and 23 in bovine cattle. Obviously, RT 13/23, detected by us, is a poorly investigated or not investigated translocation.

The data of cytogenetic studies, collected up till now, indicate that Robertsonian translocations of chromosomes are sporadic in many genetically unrelated breeds of bovine cattle, thus, it is probable that this translocation 13/23 could occur *de novo* due to spontaneous mutation processes.

CONCLUSIONS

The cytogenetic analysis of chromosome set of Ukrainian Red-and-Motley dairy breed cows allows assuming the connection between a decrease in the fertility of cows and chromosomal instability. It was determined that the frequency of chromosomal aberrations in animals which had miscarriages and stillborn calves were higher compared to cows with normal reproductive function. Translocation RT 1/29 was found to have the frequency of 0.063 %. A new Robertsonian translocation – RT 13/23 – was found and identified. It is reasonable to have regular cytogenetic monitoring to increase the productivity of dairy cattle and maintain its reproductive health.

This article *does not relate to any studies using humans and animals as investigation subjects.*

Conflict of interests. *The authors deny any conflict of interests.*

Financing. *This study did not receive any specific grant from the financing institutions in state, commercial or non-commercial sectors.*

Хромосомні аномалії у молочної худоби як причини порушення фертильності

В. В. Дзіцюк, Х. Т. Типило

Інститут розведення і генетики тварин
імені М. В. Зубця НААН,
вул. Погребняка, 1, Чубинське Бориспільського р-ну
Київської обл., Україна, 08321

e-mail: valentynadzitsiuk@gmail.com

Мета. Оцінка тварин на наявність у них хромосомних аномалій є однією із важливих задач практичної селекції з метою виявлення у ранньому віці небажаних хромосомних аномалій, які можуть негативно позначитися на відтворювальній і продуктивній здатності корів та призвести до значних економічних втрат. Метою роботи є цитогенетичний аналіз хромосомного набору у корів української червоно-рябої молочної породи, який дає змогу припустити наявність зв'язку порушень репродуктивної функції із аберраціями хромосом. **Методи.** Досліджені 53 корови української червоно-рябої молочної породи ДП «ДГ «Христинівське» ІРГТ ім. М. В. Зубця» НААН. Для дослідження хромосомних аномалій використовували 72-годинне культивування лімфоцитів з периферійної кров тварин за загальноприйнятими методиками. При рутинному аналізі препарати фарбували 2%-ним розчином Гімза. Індукування G-смуг для диференційного забарвлення хромосом виконували з використанням 0,25%-ного розчину трипсину. Обробку результатів досліджень проводили за допомогою пакету програм Microsoft Excel. **Результати.** В результаті до-

сліджень у спектрі аберацій виявили анеуплоїдні та поліплоїдні клітини, розриви і фрагменти хромосом, передчасне розходження хромосом у мітозі та транслокації). У корів з порушеннями репродуктивної функції загальна частота аберантних клітин склала 14,69 ± ± 0,56 %, число аберацій на одну досліджену клітину – 0,144, що майже вдвічі вірогідно (P < 0,999) переважає значення аналогічних ознаку у корів, які не мають проблем із репродукцією. З використанням методу GTG-banding виявлено нову робертсонівську транслокацію RT 13/23. **Висновки.** Цитогенетичний аналіз хромосомного набору корів української червоно-рябої молочної породи дає змогу припустити зв'язок порушень репродуктивної функції корів з хромосомною нестабільністю. Рутинний скринінг каріотипу корів молочного напряму продуктивності дозволяє не лише оцінити насиченість каріотипу небажаними аберраціями хромосом, а також дає змогу використати отримані результати для прогнозування в ранньому віці тварини її стан репродуктивної здатності.

Ключові слова: каріотип, аберації, українська червоно-ряба молочна порода, відтворна здатність.

Хромосомные аномалии молочного скота как причины нарушения фертильности

В. В. Дзицюк, К. Т. Типило

Институт разведения и генетики животных
имени М.В. Зубца НААН,
ул. Погребняка, 1, Чубинское Бориспольского р-на
Киевской обл., Украина, 08321

e-mail: valentynadzitsiuk@gmail.com

Цель. Оценка животных на наличие в них хромосомных аномалий является одной из важных задач практической селекции с целью выявления в раннем возрасте нежелательных хромосомных аномалий, которые могут негативно сказаться на воспроизводственный и продуктивной способности коров и привести к значительным экономическим потерям. Целью работы является цитогенетический анализ хромосомного набора у коров украинской красно-пестрой молочной породы, что позволяет предположить наличие связи нарушений репродуктивной функции с аберрациями хромосом. **Методы.** Исследованы 53 коровы украинской красно-пестрой молочной породы ГП «ОХ «Христиновское» ИРГЖ им. М. В. Зубца» НААН. Для исследования хромосомных аномалий использовали 72-часовое культивирования лимфоцитов из периферической крови животных по общепринятым методикам. При рутинном анализе препараты окрашивали 2%-ным раствором Гимза. Индуцирование G-полос для дифференциальной окраски хромосом выполняли с использованием 0,25%-ного раствора трипсина. Обработку результатов исследований проводили с помощью пакета программ Microsoft Excel. **Результаты.** В результате исследований в спектре аберраций обнаружили анеуплоидные и полиплоидных клетки, разрывы и фрагменты хромосом, преждевременное расхождения хромосом в митозе и транслокации. У коров с нарушениями репродуктивной функции общая частота аберрантных клеток составила 14,69 ± 0,56 %, число аберраций на одну исследованную клетку – 0,144, что почти в два раза достоверно (P < 0,999) преобладает значение аналогичных признаков у коров, не имеющих проблем с репродукцией. С использованием метода GTG-banding обнаружена новая робертсоновская транслокация RT 13/23. **Выводы.** Цитогенетический анализ хромосомного набора коров украинской красно-пестрой молочной породы позволяет предположить связь нарушений репродуктивной функции коров с хромосомной нестабильностью. Рутинный скрининг кариотипа коров молочного направления продуктивности позволяет не только оценить насыщенность кариотипа нежелательными аберрациями хромосом, а также позволяет использовать полученные результаты для прогнозирования в раннем возрасте животные ее состояние репродуктивной способности.

Ключевые слова: кариотип, аберрации, украинская красно-пестрая молочная порода, воспроизводительная способность.

REFERENCES

1. *Yimer N.* Chromosomal Anomalies and Infertility in Farm Animals: A review. Pertanika J. Tropical Agric. Sci. 2014;**37**(1):1–18.
2. *Gustavsson I.* Chromosome Abnormality in Cattle. Nature. 1966;**211**:865-6. doi: 10.1038/211865a0.
3. *Terje Raudsepp, Bhanu P Chowdhary.* Chromosome Aberrations and Fertility Disorders in Domestic Animals. Ann. Rev. Anim. Bioscie. 2016;**4**:15–43. doi:org/10.1146/annurev-animal-021815-111239.
4. *De Lorenzi L, Morando P, Planas J, Zannotti M, Molteni L, Parma P.* Reciprocal translocations in cattle: frequency estimation. J. Anim. Breed. Genet. 2012;**129**(5):409–6. doi: 10.1111/j.1439-0388.2011.00983.x.
5. *Khatun MR, Arifuzzaman M, Ashraf A.* Karyotype for Identification of Genetic Abnormalities in Cattle. Asian J. Anim. Veterin. Advan. 2011;**6**(2):117–25. doi: 10.3923/ajava.2011.117.125.
6. *Szczerbal I, Switonski M.* Chromosome Abnormalities in Domestic Animals as Causes of Disorders of Sex Development or Impaired Fertility. Insights Anim. Reprod. 2016;**9**. doi: 10.5772/62053.
7. *Trukhachev VI, Oleynik SA, Zlydnev NZ, Morozov VJu, Selionova MI, Chizhova LN, Skokova AV.* Chacteristics of the chromosome set of Holstein cows with reproductive disorders in the North-Caucasian cattle population. Cytol. Genet. 2017;**51**(4):272–7. doi: 10.3103/S0095452717040119.

8. *EL-Bayomi KM, EL-Araby IE.-S, Zaglool AW.* Cytogenetic analysis related to some infertility problems in Cattle. Global Veterinaria. 2011;**7**(4):323–9.
9. *Switonski M.* 50 years of studies on bovine 1/29 Robertsonian translocation–from Giemsa staining to genomic analysis. Conference Paper in Chromosome Research, Ischia, Naples, Italy. 2014;**22**:395.
10. *Anaya G, Valera M, Moreno-Millán M, Azor P, Peral-García P, Demyda-Peyrás S.* Sex chromosomal abnormalities associated with equine infertility: validation of a simple molecular screening tool in the Purebred Spanish Horse. Anim. Genet. 2017;**48**(4):404–11. doi: 10.1111/age.12543.
11 *Udroiu I, Sgura A.* Cytogenetic tests for animal production: state of the art and perspectives. Anim. Genet. 2017;**48**(5):505–15. doi: 10.1111/age.12581.
12. *De Lorenzi L, Genualdo V, Gimelli S, Rossi E, Perucatti A, Iannuzzi A, Zannotti M, Malagutti L, Molteni L, Iannuzzi L, Parma P.* Genomic analysis of cattle rob(1;29). Chromosome Res. 2012:**20**(7):815–23. doi: 10.1007/s10577-012-9315-y.
13. *Yimer N, Rosnina Y.* Chromosomal anomalies and infertility in farm animals: a review. Pertanika J. Trop. Agric. Sci. 2014;**37**(1):1–18.
14. *De Lorenzi L, Rossi E, Gimelli S, Parma P.* De novo reciprocal translocation t(5;6)(q13;q34) in cattle: cytogenetic and molecular characterization. Cytogenet Genome Res. 2014;**142**(2):95–100. doi.org/10.1159/000356209.
15. *Ciptadi Gatot, Nur Ihsan, Muhammad-Rahayu, Sri-Nurgiartiningsih V-Mudawamah, Mudawamah-Putri Irsanti*. The Comparison of Chromosome Analysis Result by Manual and Software Cytovision Image Analysis Using Simple G-Banding Research. J. Life Sci. 2017;**4**(2);106–10. doi: 10.21776/ub.rjls.2017.004.02.3.
16. *Dumont BL.* Variation and evolution of the meiotic requirement for crossing over in mammals. Genetics. 2017;**205**(1):155–68. doi: 10.1534/genetics.116.192690.
17. *Rodero-Serrano E, Demyda-Peyrás S, González-Martineza A, Rodero-Franganillo A, Moreno-Millán V.* The rob(1;29) chromosome translocation in endangered Andalusian cattle breeds. Livestock Sci. 2013;**158**(1–3): 32–9. doi.org/10.1016/j.livsci.2013.10.001
18. *Omaima H Ezzo, Farghaly AA, Amal M Abo El-Maaty, Karima Gh MM.* Hormonal and Cytogenetic Investigations in Mares with Early Embryonic Death. Global Veterin. 2011;**7**(3):211–8.
19. *O'Connor RE, Fonseka G, Frodsham R, Archibald AL, Lawrie M, Walling GA, Griffin DK.* Isolation of subtelomeric sequences of porcine chromosomes for translocation screening reveals errors in the pig genome assembly. Anim. Genet. 2017;**48**(4):395–403. doi: 10.1111/age.12548.