CHROMOSOME ABERRATIONS AS A BIOMARKER FOR GENOMIC INSTABILITY IN CELL CULTURES ORIGINATED FROM BOVINES, CANINES AND EQUINES INFECTED WITH PAPILLOMAVIRUS

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Abstract
Chromosomal abnormalities or aberrations were investigated in ten bovines, three canines and three equines presenting cutaneous papillomatosis. The aim of the present study was to evaluate possible chromosomal abnormalities as a biomarker of genomic instability induced by the interaction of the viral genome with the chromatin of the host cell in peripheral blood lymphocytes and skin warts lesion cultures. Genomic DNA was extracted from blood samples and epithelial biopsies. Amplification products from PCR using degenerated primer pair FAP59/FAP64 were sequenced. Peripheral blood lymphocytes and tumor cell cultures were performed. Structural chromosomal aberrations were verified as associations, chromosomal fragments, centric fusions, open chromatids, gaps, rearrangements and rings. Several levels of premature chromosome condensation were observed in both cultures. Numeric abnormalities as aneuploidy were also detected and hypoploid cells were found in a larger number than the hyperploid cells. This cytogenetic study in detecting premature chromosome condensation in the three species investigated describes by the first time the structural and numerical abnormalities in canines and equines presenting papillomatosis lesions. These abnormalities are similar of the three groups of animals, but virus specific.

Key words: papillomavirus; peripheral blood lymphocytes; tumor cell cultures; chromosome aberrations; genomic instability

Introduction
The papillomaviruses belong to the Papillomaviridae family and new genera have been described on the basis of the interspecies phylogenetic similarity. The papillomavirus is a DNA virus that induces the formation of benign and malignant tumors in a vast range of hosts.

Previous studies have reported the occurrence of chromosomal aberrations in peripheral blood lymphocytes cultured from cattle infected by Bovine papillomavirus (BPV) after consumption or not of the carcinogenic substances of the fern Pteridium aquilinum, regardless of the development of bovine enzootic hematuria (Stocco dos Santos et al., 1998). Presence of DNA sequences of the Bovine papillomavirus 2 (BPV-2) was detected in the cattle with bladder cancer. The correlation data suggested that the BPV-2, together with Pteridium aquilinum, acts synergistically to produce the chromosomal instability (Lioi et al., 2004).

Experimentally, BPV DNA sequences have been detected in peripheral blood of non-clinically affected cattle by papillomatosis, presenting high levels of chromosomal aberrations or abnormalities (Campos et al., 1994; Stocco dos Santos et al., 1998). Cytogenetic abnormalities and numerical and structural chromosome aberrations, such as endoreduplications, gaps, break chromatids, centric rings and dicentric chromosomes were observed in human keratinocytes and mouse cells transfected with E7 oncogene HPV-16. Chromosomal fragility has been detected also in lymphocytes of women with lesions in the cervix after submitted to a treatment against HPV (Paz-y-mino et al., 1992).

Campo et al., (1994) and Campo (2002) suggested that the viral latency site occurred in lymphocytes when studying BPV DNA sequences detected in lymphocytes of bovines with or without papillomatosis, as well as HPV DNA in blood cells of women with HPV urogenital and cervical cancer infection.
These HPV infected cells express viral oncoproteins (E6 and E7) that inactivate the function of the tumor suppressor genes and induce the formation of chromosomal aberrations, interfering in checkpoint control mechanism G1 and G2, and braking cell division (Chang et al., 1997; Courses et al., 1997). In the case of the BPV, the mechanism is different. Experimental data showed that the BPV is unable to associate with mitotic chromosomes by E2 protein, being this mechanism less studied. The mitotic activity of epithelial cells in mucosa and skin tissue of persistent infections is able to maintain the viral genome with a low number of copies (Mc Phillips et al., 2006).

Papillomavirus oncoproteins interfered in DNA mitotic checkpoints which are normally activated when an event occurs, such as breaks in DNA, triggering cells to enter the S phase or mitosis, to repair cell damage (Thompson et al., 1997). The first observation of chromosomes condensed prematurely or premature chromosome condensation (PCC) was recorded in virus-infected cells, mediating cell fusion in the interphase of mitotic cells (Kato and Sandberg, 1967; Gotoh and Durante, 2006).

The mechanisms of chromosome condensation have been intensively investigated. The appearance of the spreaded chromatin has been associated with chromosomal fragments induced by viruses (Kato and Sandberg, 1967 in Gotoh and Durante, 2006). From the mid-70s, the PCC has been recognized as a tool for the investigation of chromosomal dynamics in radioactive cytogenetic and chromosome replication studies (Gotoh and Durante, 2006). Condensinas, histone H1, kinase and topoisomerase II (TOPO II) are some molecules occurring in cell induction mechanism of PCC (Lee et al., 2007).

Cyclin-dependent kinases (cdks) govern cell cycle progression in eukaryotes. In mammalian cells, the transition from the G1 / S and G2 / M phase are regulated by complex distinct cdk / cyclin. In conjunction with cdc2, cyclin A and B regulate the entry into cell mitosis (Pines, 1996 cited Thompson et al., 1997). The activation of cdc2 is mediated by a complex starting with the increase of cyclin B1 in the S and G2 phase in the presence of Epstein-Barr virus (EBV) (Lee et al., 2007). The cyclin-dependent kinase activity in the S phase increases during viral replication, while the cell DNA replication is inhibited. Genetic and biochemical evidence suggests that the condensed complex of protein subunits and DNA topoisomerase II, essential for mitotic condensation, both developed important role in the assembly and organization of mitotic chromosomes (Lee et al., 2007).

The increase of cdc2 kinase activity associated with histone H1 (Thompson et al., 1997) has been detected in cell populations expressing E6 of HPV-16. The ATR enzyme belongs to the kinase family and is able to prevent cell induction by checkpoint regulation of G1. However, cancer cells and the loss of p53 function causes the inhibition of ATR and thus interfere in the checkpoint G1, sensitizing the cells to the formation of PCC (Nghiem et al., 2001).

The aim of the present study is to evaluate possible chromosomal abnormalities induced by the interaction of the viral genome with the chromatin of the host cell in peripheral blood lymphocytes and skin wart lesion cultures of bovines, canines and equines infected with papillomavirus.

Material and Methods

Clinical and histopathology examination

Skin warts lesions of cutaneous papillomatosis were detected in biopsy fragments of ten bovines, three canines and three equines samples, and kept in RPMI medium to tumors cell cultures for cytogenetic studies. Tumor samples were fixed in 10% phosphate-buffered formalin solution, embedded in paraffin and stained with haematoxylin and eosin (H&E) described by Marins et al. (2010).

Cytogenetic analysis

Blood collection was carried out using syringes with heparin or heparinized vacutainer tubes for each species. Peripheral blood lymphocyte cultures were realized at 37°C for 72 hours and 5% CO2. Cytogenetic preparation of the tumor cell cultures obtained from skin biopsy was reported by Marins et al., (2012). The karyotypes were assembled using the Photoshop CS; version 9 software. After selecting the metaphases, the karyotypes constructed were classified about the number of chromosomes in complete, incomplete and overlaps (not considered appropriate for analysis).

Molecular screening

DNA extraction was carried out from blood samples and epithelial tissues of cattle, canines, and equines using QIAamp DNA blood and tissue mini kit following the manufacturer’s protocol (Qiagen). Polymerase chain reaction (PCR) was performed with β-globin gene PCR and generic primer pairs PCR FAP59 (forward: 5´- TTA CWC TIG GIC AYC CWT ATT – 3´) and FAP64 (reverse: 5´- CCW ATH TCW VHC ATI TCI CCA TC – 3´), performed according to Marins et al., (2012). In sequence, 5µl of the purified PCR product using the GFX PCR DNA purification kit (GE Healthcare), were used. Subsequently, 2µl of the purified PCR product was quantified. The sequences were analyzed using the Sequence Analyser software of Cimarron Base Caller 3.12. The quality of the sequences obtained was evaluated by Chromas program version 2.33 and / or the software Biological Sequence Editor Alignment (BioEdit). The identities of the products were searched using the Basic Local Alignment Search Tool program (Blast).

Statistical Analysis

Chromosomal analysis was performed by the program Statistical Package for the Social Sciences (SPSS), version
13. Wilcoxon test was applied to compare two variables of a same animal and Friedman test was computed to analysis the parameters of a same group of animals.

**Results**

The microscopical patterns were characteristic of carcinoma and papilloma infection presenting hyperproliferation in epidermal layer showing inflammatory cells specially lymphocytes. Hyperkeratosis, hypergranulosis and basophilic intranuclear inclusion bodies were detected by histopathology.

L1 DNA sequences were detected using degenerate primers FAP59/FAP64. Samples sequenced of the *Bovine papillomavirus* (BPV) and *canine oral papillomavirus* (COPV) showed similarity with the sequences of the HPV by molecular analysis.

Chromosome aberrations were detected in the peripheral blood lymphocyte and tumor cell cultures of the bovines (Fig. 1 - 2), canines (Fig. 3) and equines (Fig. 4) infected with papillomavirus. Viral DNA amplification was detected in the samples of skin biopsy and blood of animals with cutaneous papillomatosis. In this study, canines carrying adenocarcinomatosa lesions and bovines with papilloma infection showed genetic similarity with the HPV DNA sequence.

Structural chromosome aberrations were verified as associations, chromosomal fragments, centric fusions, open chromatids, gaps, rearrangements and rings. In both types of cell cultures several levels of premature chromosome condensation was observed.

![Fig. 1: Chromosome rearrangements observed in bovine peripheral blood lymphocytes culture. The suggestive lesions are (arrows): A) chromosome association; B) chromosomes with rearrangements and open chromatids; C) Fusion of telomeres with centromeres and telomeres fusion; D) metaphase with chromosome associations and possible centric fusion.](image-url)
Fig. 2: Chromosome structural aberrations of bovine cultured peripheral blood lymphocytes. The arrows indicate possible chromosomal damage: A) metaphase with female sex chromosome (X) presenting chromosomal fragility and chromosomal rearrangement (x); B) Female sex chromosome (X) presenting possibly gaps, rearrangements, centric fusion and fragments (x); C) open chromatid; D) lesion indicative of centric association.

Fig. 3: Chromosome abnormalities in peripheral blood lymphocytes from canines. The arrows indicate lesions suggestive of: A) chromosome fragments (Long & short arrows); B) Cell in the G1 phase with only one chromatid (long arrows) and G2 phase with PCC chromosomes showing double chromatids (short arrows).
Fig. 4: Chromosome damage in peripheral blood lymphocytes from equines. The arrows indicate chromosomal lesions suggestive of: A) open chromatids during metaphase with chromosomal fusion; B) centric fusion.

Table 1. Percentage of cells with numerical-type aberrations in the animal groups investigated

| Species | Completed metaphases (%) | Hypoploid cells (%) | Hyperploid cells (%) | Numerical changes (%) |
|---------|--------------------------|---------------------|----------------------|-----------------------|
| Bovines | 28.68 ± 16.12            | 67.20 ± 16.06       | 4.01 ± 4.04          | 71.31 ± 16.12         |
| Canines | 15.28 ± 13.25            | 48.46 ± 42.05       | 36.22 ± 55.24        | 84.71 ± 13.25         |
| Equines | 45.24 ± 26.33            | 50.89 ± 22.95       | 3.86 ± 3.38          | 54.76 ± 26.32         |

Among the metaphase in bovines, the average proportion of whole cells with diploid number of chromosomes was 28.68% ± 16.12% (CV = 0.56). Averaged 71.31% of the cells showed numerical changes with a standard deviation of 16.12% (CV = 0.23). It was concluded that the differences between the whole cell percentage and the numeral changes are not significantly different by Wilcoxon test (p-value = 0.007). There are significant differences between the percentages of whole cells, hypoploid (67.29% ± 16.06) and hyperploid cells (4.02% ± 4.04) in cattle by the Friedman test (p-value = 0.000). The differences between the mean proportion of metaphases and PCC are not significant, with a p-value of 0.173 (Wilcoxon test).

In the canines, it was found that an average of 15.28% ± 13.31% (CV = 0.87) corresponded to complete metaphases and 84.72% ± 13.31% (CV = 0.45) to numerical changes. Among the cells with numerical changes, hypoploid cells presented an average of 48.46% and hyperploid cells of 36.26%. Comparing the percentage of metaphases of total cells analyzed with the PCC percentage, an average of 54.06% ± 50.28% (CV = 0.93) corresponded to metaphases and 45.94% ± 50.28% (CV = 1.09) to PCC cells.

In the equines, an average of 45.24% ± 26.33% (CV = 0.58) was found in complete metaphases and 54.76% ± 26.33% (CV = 0.48) of numerical changes. Among the numerical changes, hypoploid cells showed an average of 50.89% ± 22.96 (CV = 0.45), and hyperploid cells of 3.87 ± 3.38 (CV = 0.87). An average percentage of metaphases of 62.22% ± 7.70% (CV = 0.12) was found when total cells were evaluated, and 37.78% ± 7.70% (CV = 0.20) of PCC cells (Table 1).

Discussion and Conclusion

Chromosomal instability

In general, viruses have the ability to enter cells by different mechanisms, leading the cell chromosomal instability, and some of them contribute to the development of cancer cells (Duelli and Lazebnik, 2007). The interaction with the host cell chromatin, which occurs when the circular DNA is associated with histones of the cell and being able to express the ORF genes is the most accepted mechanism to justify the chromosomal instability of HPV. The viral genome is integrated into mitotic chromosomes through the action of the E2 gene, allowing segregation of the episomal genome inside the host daughter cells. Thus, the replication and maintenance of the viral genome in the host cell and during cellular proliferation is maintained (Mc Phillips et al., 2006). In this context, the present study reports the occurrence of chromosome aberrations as a biomarker for...
Chromosome numerical and structural aberrations

The present data show many variations in the diploid number, smaller or larger than normal number of chromosomes (hypoploid and hyperploid), in peripheral blood lymphocytes of bovines, canines and equines investigated. Numerical abnormalities in immortalized epithelial cells of the HPV 16 have been described as triploidias (3n) and tetraploidias (4n) by Yasumoto and Hashida (1991). High incidence of hypoploid cells and hyperploid was also described in studies in bovines by Leal et al. (2003).

According to Reis et al. (1984) cited Mansur and Androphy (1993) the aneuploid (hyperploid and hipoploid) cells percentage ranged from 33% to 82% in cases of cervical intraepithelial neoplasia in women infected with HPV. Similar findings were found in cattle with an average percentage of 67.29% in cells with hypoploid and 4.01% in the cells with hyperploid. According to Leal et al., (2003), the average percentage was also higher with hypoploid in cells experimentally infected with BPV-4 E7 oncogene. The number of hypoploid cells of equines and canines were also higher than in hyperploid cells in this present study.

Chromosomal aberrations such as: associations, fusions, fragments and gaps described in bovines lymphocytes, according to previous reports are consistent with our results. In the karyotypes of bovines, equines and canines, in this study, it was found that not all karyotypes showed a number of chromosomes exceeding the normal diploid cells (2n). According to Nicholas (1997), abnormal karyotypes arise from errors in their chromosomal replication.

Structural aberrations detected with chromosome markers have been found also in bovine and human populations (Recouso et al., 2003). These markers identify abnormal and morphologically different chromosomes. They have a centromere and are generally derived from a chromosome breakdown with loss of an acentric fragment (Richardson, 1991). In the present study, changes were found suggestive of chromosome fragments in metaphases of lymphocytes from cattle with cutaneous papillomatosis. Breaks and gaps which occur at various stages of cell division have been found also. The chromatid breaks or gaps occur only on one chromatid, for the other hand breakage or chromosomal gap refers to structural abnormality that occurs in both chromatids at the same point in the chromosome (Richardson, 1991).

Numerous chromosomal overlaps and a large variation in the number of chromosomes in metaphase, higher than structural abnormalities were observed when analyzing lymphocyte cultures and neoplastic lesions of the studied groups of animals. Due to the large number of cattle and...
Premature chromosome condensation (PCC)

There was a variation in the number of cells with PCC in our study, particularly in lesion cultures, causing a possible chromosomal instability as a result of fusion between cells in the mitotic or interphase with pre-mitotic cells. The PCC findings, mostly performed in the S phase, and according to studies of Duelli and Lazebnik (2007), refer to multiple DNA breaks resulting in multiple chromosomal fragments attesting the pulverized appearance of the cell. According to reports of Lee et al., (2007), the chromatin of nasopharyngeal carcinoma cells became highly condensed when positive Epstein–Barr Virus (EBV). Similarly, the chromatin of adenocarcinoma cells in canine and bovine papilloma infected cells showed high levels of PCC. Similar findings were reported also by Marins et al., 2010 in peripheral blood lymphocytes and skin warts lesions cultures of the bovines and by Ito et al., (2002), after analysis by confocal microscopy of condensed chromatin in interphase in EBV infected cells.

The high PCC index observed in the present study was similar to the observation of Lee et al., (2004), treating the lymphocytes with calyculin A, experimentally inducing the formation of PCC, and in accordance also with Lee et al., (2004) to verify that the number of PCC cells ranged from 160 to 1,040 the each sample of patients with lung cancer treated chemically with calyculin A.

Cultures of lesions and lymphocytes from cattle, equines and canines showed cellular phenotype similar to prophase, in agreement with the study of the Marins et al., (2010), Marins et al., (2012) and Trimborn et al., (2004), referring to the PCC aberrations found as genetic mutations.

Many cells showed also highly fragmented chromosomes, indicating breaking of lymphocytes of the investigated animals. In experiments with lymphocytes treated with methyl methanesulphonate for chemical induction of PCC, chromosomal breakage with the appearance of highly fragmented chromosomes was found, and detected only in high chemical doses and related to the dose-dependent chromosomal breaks (Garcia et al., 2001). These findings from cultures of skin lesions of cattle suggest different stages of chromatin condensation, corresponding to the specific nomenclature of premature chromosome condensation (PCC). Rao et al., (1981) described the chromosomal interphase morphology consistent with studies of human leukemia (Hittelman et al., 1981). In the present study, the PCC found in the S phase with chromosomal pulverizations indicates failure in cell cycle subject to genomic instability and are according to Thompson et al., (1997) as the uncontrolled cellular mechanisms.

The resulting PCC of the fusion of interphase cells and mitotic cells presented here in was firstly described in the 70s by Rao and Johnson. To date, the PCC had not been registered with the papillomavirus infection but with Sendai Virus (SV) infections (Rao et al., 1981) and more recently with EBV (Ito et al., 2002; Lee et al., 2007). Thus, for the first time the presence of PCC in cells infected with PV in bovines, canines and equines were demonstrated.

In the tissue of skin lesions in bovines and canines, we detected the presence of viral DNA nucleotide sequence by PCR. Although the phenomenon of PCC can be induced by chemical processes (Thompson et al., 1997; Garcia et al., 2001; Trimborn et al., 2004; Gotoh and Durante, 2006; Lee et al., 2004), other studies have shown the occurrence of PCC resulting of SV (Rao et al., 1981) and EBV infection (Ito et al., 2002; Lee et al., 2007).

PCC was found in bovine lymphocyte cultures in equines and canines infected with PV without intervention or chemical therapeutic process, which can likely be attributed to the presence of the virus. The PCC found in this study probably resulted from an exacerbated PV action inducing cell proliferation and promoting the fusion of pre-mitotic cells, as formerly described by Rao et al., (1981), Ito et al., (2002) and Lee et al., (2007).

This finding for cattle was similar to those described by Leal et al., (2003) in cell lines of the palate male fetal bovine transfected with E7 gene BPV4, specific oncogene activation and after treatment with quercetin (5,7,3’,4’-tetrahydroxyflavone), cofactor that acts in synergy with viral action.

However, these tests do not showed a correlation between the amount of PCC observed in the culture of lymphocytes and bovine lesions, demonstrating that this were probably independent factors. By statistical analysis it was demonstrated only that the occurrence of cells with hypoploid was higher in all species examined, as found by Leal et al., (2003) in the case of bovine cells experimentally infected E7 oncogene BPV-4.

This cytogenetic study is pioneer in the detection of premature chromosome condensation in the three species investigated. In addition, it described at the first time the structural and numerical chromosome abnormalities in canines and equines with papillomatosis lesions. Therefore, chromosome condensation, numerical and structural
aberrations are similar in bovines, canines and equines infected with papillomavirus, but are virus species dependent.

Acknowledgments
The authors express their gratitude at Dra. Denise Monnerat Nogueira and Dra. Keila Mara Cassiano for technical contributions. A research fellowship was obtained by the first author from the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes) and by the second author from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

References
Campo MS (2002) Animal models of papillomavirus pathogenesis. Virus Research 89 (2): 249-261. DOI: 10.1016/S0168-1702(02)00193-4
Campo MS, Jarret WFH, O’Neil W and Barron RJ (1994) Latent papillomavirus infection in cattle. Research in Veterinary Science 56 (2): 151-157. DOI: 10.1016/0034-5288(94)90097-3
Chan SY, Bernard HU, Ratterree M, Birkebak TA, Faras AJ and Ostrow R (1997) Genomic diversity and evolution of papillomaviruses in Rhesus Monkeys. Journal of Virology 71 (7): 4938-4943.
Coursen JD, Bennet WP, Gollahan L, Shay JW and Harris CC (1997) Genomic instability and telomerase activity in human bronchial epithelial cells during immortalization by human papillomavirus -16 E6 and E7 genes. Experiential Cell Research 235 (1): 245-313. DOI: 10.1006/excr.1997.3670
Duelli D and Lazebnik Y (2007) Cell-to-cell fusion as a link between viruses and cancer. Nature Reviews - Cancer 7: 968-976. DOI: 10.1038/nrc2272
Fillipin C, Chistoforetti LD, Ribeiro MCM and Vituri CL (2006) Determinacao do numero de regioes organizadoras de nucleolo (Ag NOR) em lesoes do epitelio cervical uterino. Revista Brasileira de Análises Clínicas 38 (3): 133-139.
Garcia CL, Carloni M, Palma de la Peña N, Fontin E and Palitti F (2001) Detection of DNA primary damage by premature chromosome condensation in human peripheral blood lymphocytes treated with methyl methanesulfonate. Mutagenesis 16 (2): 121-131. DOI: 10.1093/mutage/16.2.121
Gotoh, E and Durante M. (2006) Chromosome condensation outside of mitosis: mechanisms and new tools. Journal of cellular physiology 209: 297-304. DOI: 10.1002/jcp.20720
Hashida T and Yasumoto S (1991) Induction of chromosome abnormalities in mouse and human epidermal keratinocytes by the human papillomavirus type 16 E7 oncogene. Journal of General Virology 72: 1569-1577. DOI: 10.1099/0022-1317-72-7-1569
Hittelman WN, Rao PN and Mc Credie KB (1981) Premature Chromosome condensation studies in human leukemia. In: Genes, Chromosomes and Neoplasia. Francis Arrighi, Potu Rao e Elton Stubblefield (Eds.). Raven Press: New York. p. 379-403.
Ito S, Gotoh E, Ozawa S and Yanagi K (2002) Epstein-Barr virus nuclear antigen-1 is highly colocalized with interphase cromatin and its newly replicated regions in particular. Journal of General Virology 83: 2377-2383. DOI: 10.1099/0022-1317-83-10-2377
Leal AM, Ferraz OP, Carvalho C, Freitas AC, Beniston RG, Beçak W, Campo MS and Stocco dos Santos RC (2003) Quercetin induces structural chromosome aberrations and uncommon rearrangements in bovine cells transformed by the E7 protein of bovine papillomavirus type 4. Veterinary and Comparative Oncology 1 (1): 15-21. DOI: 10.1046/j.1476-5829.2003.00008.x
Lee CP, Chen JY, Wang JT, Kimura K, Takemoto A, Lu CC and Chen MR (2007) Epstein-Barr virus BGLF4 kinase induces premature chromosome condensation through activation of Condensin and Topoisomerase II. Journal of Virology 81 (10): 5166-5180. DOI: 10.1128/JVI.00120-07
Lee R, Yamada S, Yamamoto N, Miyamoto T, Ando K, Durante M and Tsujii H (2004) Chromosomal aberrations in lymphocytes of lung cancer patients treated with carbon ions. Journal Radiation Research 45: 195-199. DOI: 10.1269/jrr.45.195
Lioi MB, Barbieri R, Borzacchielo G, Dezzi S, Roperto S, Santoro A, Russo V and Roperto F (2004) Chromosome aberrations in cattle with chronic enzootic haematuria. Journal Comparative Patholog 131: 233-236. DOI: 10.1016/j.jcpa.2004.01.011
Mansur CP and Androphy EJ (1993) Cellular transformation by papillomavirus oncoproteins. Biochimic Biophysic Acta. 1155: 323-345.
Marins RSQS, Cassiano KM, Pereira SRFG, Nogueira DM and Carvalho EQ (2012) Canine latent papillomavirus infection and chromosomal instability studies in peripheral blood lymphocytes and tumors cells cultures from lesions biopsy. International Research Journal of Biochemistry and Bioinformatics 2 (3): 62-68.
Marins RSQS, Nogueira DM, Pereira SRFG, Cassiano KM, Maues T, Dias VG, Moreira L and Carvalho EQ (2010) Cytogenetic studies: Premature chromosome condensation (PCC) in peripheral blood lymphocytes cultures and skin lesions cultures induced by Papillomavirus spontaneous infection in bovines. Revista de Ciências Médicas e Biológicas 9 (1): 3-9.
McPhillips MG, Oliveira JG, Spindler JE, Mitra R and Mc Bride AA (2006) Brd4 is required for E2-mediated transcriptional activation but not genome partitioning of all papillomaviruses. Journal of Virology 80 (19): 9530-9543. DOI: 10.1128/JVI.01105-06
Nghiem P, Park PK, Kim Y-S, Vaziri C and Schreiber SL (2001) ATR inhibition selectively sensitizes G1 checkpoint-deficient cells to lethal premature chromatin condensation. Proceedings of the National Academy of Sciences 98 (16): 9092-9097. DOI: 10.1073/pnas.161281798
Nicholas FW (1997) Cromosomas y aberraciones cromosómicas. In: Genética Veterinaria, Nicholas (Ed.) Acribia: Zaragoza: España, p.103-159.

Paz-y-mino C, Ocampo L, Navvaez R and Navvaez L (1992) Chromosome fragility in lymphocytes of women with cervical uterine lesions produced by human papillomavirus. Cancer Genetics and Cytogenetics 39: 173-176. DOI: 10.1016/0165-4608(92)90211-P

Rao PN, Sunkara PS and Wright DA (1981) Chromosome condensation factors of mammalian cells. In: Frances, E. Arright, Potu, N Rao, Elton Stubblefield (eds.). Genes, chromosomes and neoplasia, New York: Raven Press, p. 49-60.

Recouso RC, Santos RCS, Freitas R, Santos RC, Freitas AC, Brunner O, Beçak W and Lindsey CJ (2003) Clastogenic effect of bracken fern (Pteridium aquilinum v. arachnoideum) diet in peripheral lymphocytes of human consumers: preliminary data. Veterinary and Comparative Oncology 1: 1-9. DOI: 10.1046/j.1476-5829.2003.00006.x

Richardson A (1991) Chromosome analysis. In: The ACT - Cytogenetics Laboratory Manual, Barch, M.J (Ed.), 2.ed., Raven Press, New York.

Stocco dos Santos RC, Lindsey CJ, Ferraz OP, Pinto JR, Mirandola RS, Benesi FJ, Birgel EH, Pereira CAB and Beçak W (1998) Bovine papillomavirus transmission and chromosomal aberrations: an experimental model. Journal of General Virology 79: 2127-2135. DOI: 10.1099/0022-1317-79-9-2127

Thompson DA, Belinsky G, Chang THT, Jones DL, Schlegel R and Munger K (1997) The human papillomavirus -16 E6 oncoprotein decreases the vigilance of mitotic checkpoints. Oncogene 15: 3031-3035. DOI: 10.1038/sj.onc.1201495

Trimborn M, Bell SM, Felix C, Rashid Y, Jafri H, Griffiths PD, Neumann LM, Krebs A, Reis A, Sperling K, Neitzel H and Jackson AP (2004) Mutations in Microcephalin cause aberrant regulation of chromosome condensation. American Journal of Human Genetics 75: 261-266. DOI: 10.1086/422855