

Could Tissue-Specific Genes Be Silenced in Cattle Carrying the Rob(1;29) Robertsonian Translocation?

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1. Introduction

Robertsonian translocations (rob) are frequent chromosome rearrangements observed in human (Nielsen & Wohler, 1991), plants (Friebe et al., 2005), mice (Saferali et al., 2010) and domestic animals (Fries & Popescu, 1999), that are considered in arthropods classical mechanisms of chromosome evolution (Robertson, 1916). In cattle, this type of translocation is the most widespread chromosomal abnormality in European and American Creole cattle breeds. In Uruguayan Creole cattle the monocentric Robertsonian translocation rob(1;29) presents a frequency of 4%, whereas a frequency between 4 to 10% is described in beef and dairy cattle breeds (King, 1991; Postiglioni et al., 1996). In these populations there is a high incidence of heterozygous carriers and a very small number or even absence of homozygous individuals (Kastelic & Mapletoft, 2003). This situation has also been observed in the majority of cattle populations in which rob(1;29) has been detected (Ducos et al., 2008).

1.1 The finding of rob(1;29) when trying to solve a sub-fertility problem in an Artificial Insemination Center

In 1969, I. Gustavsson described for the first time the Robertsonian translocation rob(1;29) in cattle. This finding, together with the discoveries of pioneers in the field such as Ohno et al., (1962) and Herzog & Hohn (1968), have been important milestones in the development of domestic animal cytogenetics. Particularly, rob(1;29) was found in the Swedish Artificial Insemination Center of Black and White cattle, associated with decreased fertility. This aneuploid alteration causes a reduction in reproductive efficiency, increasing calving intervals, non-return to service and culling rates; thus leading to important economic losses in commercial cattle herds (Bonnet-Garnier et al., 2006, 2008).

1.2 Chromosome rearrangements in the bovine genome – The origin of rob(1;29)

Bovine presents a complex genome due to the presence of high number of repetitive sequences involved in macro and micro-rearrangements that are essential in the evolution of bovids. Schibler et al., (1998) presented a high resolution integrated bovine comparative map where the analysis of break point regions revealed specific repeated density patterns, suggesting that transposons (TEs) may have played a significant role in chromosome evolution and genome plasticity. However, bovines have a morphologically simple karyotype, corresponding to $2n=60, XX; XY$. All the autosomes pairs are acrocentrics and the sex chromosomes are the large submetacentric X and the tiny metacentric Y. In females the sex chromosomes correspond two large submetacentric X's.

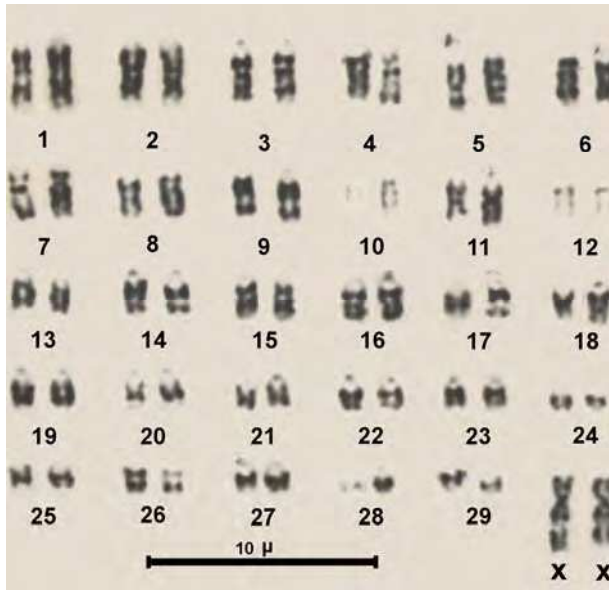


Fig. 1. Karyotype of cattle female, treated to G-banding. Observe all the acrocentric autosome pairs and the large submetacentric X's. Observe light G-banding in BTA1 and X's chromosomes (courtesy: Postiglioni, A., 1987).

Theoretically, all the acrocentric autosomes have the same probability to be involved in centric fusions, but the only known monocentric Robertsonian translocation corresponds to rob(1;29), being considered as a chromosome polymorphism (Di Meo et al., 2006). Lymphocyte culture in cattle carriers of rob(1;29) clearly present all the metaphases with this chromosome rearrangement.

Today, it is known that this chromosome translocation may have originated both from a centric fusion of chromosomes 1 and 29 and from chromosome rearrangements like pericentric inversions, in which a DNA probe (INRA143) normally mapping to BTA29, appeared proximally to rob(1;29)q-arm. Recently, Di Meo et al.,(2006) suggested a transposition as a complex rearrangement that could also be involved in the origin of this chromosome alteration.

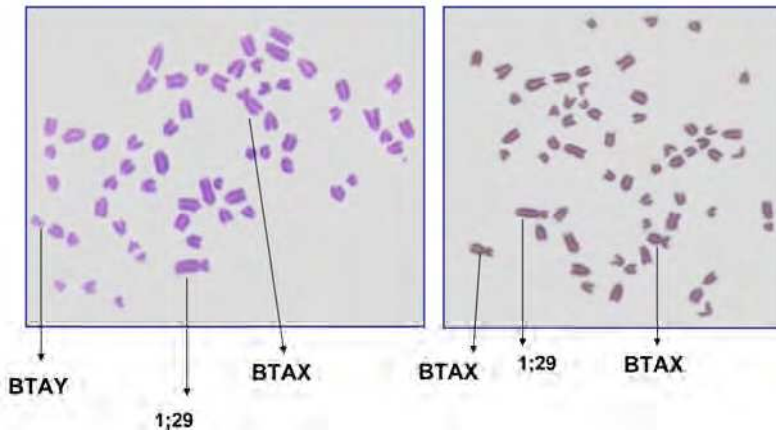


Fig. 2. Lymphocyte metaphases of male and females cattle carriers of rob(1;29) (courtesy: Iriarte,W., 2008).

Treatments on metaphase chromosomes obtained from lymphocyte culture have been done (C-banding, G banding, Restriction-banding, R-banding, FISH), where the chromatin showed differential expression in the trivalent: chromosome 1, chromosome 29 and the submetacentric rob(1;29). G-banding is obtained with Giemsa stain following digestion of chromosomes with trypsin. It shows clearly dark banding along the rob(1;29) (Fig. 3A).

When metaphases of cattle carriers of rob(1;29) were treated with restriction enzymes (MspI) and C-banding, and counter-staining with propidium yodide, all the autosome centromeres were brilliant in contrast to pale regions in rob(1;29) and X chromosomes (Fig. 3B) (Postiglioni et al., 2002).

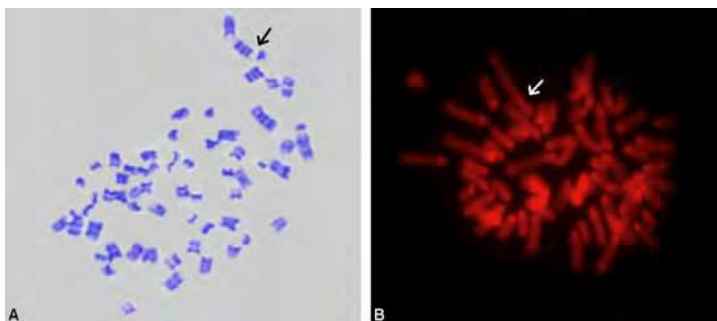


Fig. 3. Lymphocyte metaphases of cattle carriers of rob(1;29). A) treatment to G-banding. B) treatment to restriction-enzyme (MspI) and C-banding, counter staining with propidium yodide. Arrow signed the pale pericentromeric region in rob(1;29) and X chromosomes, in contrast all the autosome centromeres (courtesy: Postiglioni, A., 2002).

Changes in chromatin structure may occur during the establishment of rob(1;29), altering nucleotide sequences that could be involved in embryo development and enhancing epigenetic changes (Postiglioni et al., 2011). Furthermore, King (1991) suggested an

important role of gene mutation occurring on the early embryonic development of rob(1;29) carriers, probably involving collagen genes.

1.3 What happens in the pachytene synapsis of cattle carrier of rob(1;29)?

Synaptonemal complex analysis in bull heterozygous for rob(1;29) translocation revealed an absence of pairing (unsynapsing) between the proximal regions of chromosome 1 and 29 and the rob(1;29) at early pachytene indicating a lack of homology in these regions of the trivalent (Switonski et al., 1987). These authors also observed that the end-to-end association of segments of the X and Y chromosomes at early pachytene apparently resulted in a high incidence of dissociation. Recently, Raudsepp et al., (2011) demonstrated that the pseudoautosomal region (PAR) in cattle has moved to the end of the long arm (Xq) due to X chromosome rearrangements. The location of the PAR on the long or short arm of the sex chromosomes does not affect X-Y pairing.

However, these kind of transpositions might have genetic implications in the case that these structural chromosomal rearrangements were associated with the gene content of the involved regions, that may critically implicate embryonic survival. A similar situation could be involved in the chromosome rearrangements that generated the rob(1;29), where a gene of the collagen multifamily (COL8A1) is proximal to this affected region (Postiglioni et al., 2011).

Genetic causes of these effects are not yet well understood, but there are indications that the PAR and the proximal region of rob(1;29) have tissue specific genes that might be critically involved in placental and trophoblastic embryo membranes formation, and that could have a genomic imprinting effect during early embryo development (Postiglioni et al., 2009, 2011; Raudsepp et al., 2011). This statement implies that these functional regions are not only limited to morphological segregation in male meiosis, but encouraged silenced of expression of tissue specific genes involved in different stages of development in carriers of rob(1;29).

Similar events have also been shown in heterozygous individuals for Robertsonian translocations in human and mice. Moreover, these actions can cause infertility if unpaired autosomes or autosomal segments are paired with the X chromosome. A clear case was shown in a cross-breed, female Limousin-Jersey studied by Basrur et al., (2001) in which chromosome 1 was associated with the inactive X chromosome.

Recently a meiotic silencing of unsynapsed chromatin (MSUC) occurs in mice germ cells of rob(8;12) translocation carriers causing meiotic arrest and infertility. In normal mice spermatogenesis, an accumulation of DNMT3A protein was shown during the mid-pachytene stage and also a distinct association with the XY body. But in the carriers of a translocation, this protein was proportionally less abundant in unsynapsed pericentromeric regions of chromosomes 8 and 12. This event was associated to silencing of *Dnmt3a* gene, with the consequence of incomplete methylation of imprinted genes (Saferali et al., 2010). If *Dnmt3a* is silenced, it will lead to abnormal epigenetic marking of the spermatocyte genome, including those imprinted regions that gain methylation during spermatogenesis. In mice, heterozygous carriers of the rob(8;12) translocation are fertile but fail to completely establish methylation imprints in a proportion of their sperm. Saferali et al. (2010), proposed that this imprinting defect is due to meiotic silencing of a gene or genes located in the pericentric regions of chromosome 8 and 12 during pachytene.

So, the hypothesis tested in mice was that the carriers of the translocation had lost expression of tissue specific genes from the pericentromeric region of chromosome 12 in a proportion of pachytene spermatocytes due to lack of synapsis.

Considering that DNA methylation is a potent transcriptional repressor, these authors demonstrated that lack of synapsis of chromosomes 8 and 12 during the early pachytene stage of meiosis interfere with the proper establishment of gene methylation imprinting and suggests transient silencing of genes in the unsynapsed regions. These findings support the notion that imprinting establishment extends into the prophase I of meiosis and that a similar mechanism could take place in carriers of rob(1;29).

2. An approach to understand the causes of genetic sub-fertility in rob(1;29) carriers

Cattle chromosomes BTA1 and BTA29 have genes that could be involved in early embryonic mortality, the main reproductive cause of sub-fertility of rob(1;29) carriers. The tissue-specific gene referred to as collagen type VIII alpha 1 (Col8A1) is related to extracellular matrix proteins that could be associated with placental mammal-specific gene groups. This gene is located in rob(1q_{13/21};29), next to the microsatellite BMS4015. Chromosome 1 has also another member of the collagen family, Col6A1 gene located in rob(1q_{4.3};29). Besides, the well-known and imprinted gene insulin-like growth factor 2 (IGF2) is located in the short arm of rob(1;29).

As this is a gene that has an important role in embryonic and fetal gestation, as it promotes growth of early embryos, it could also be involved in early embryonic mortality (Postiglioni et al., 2009)

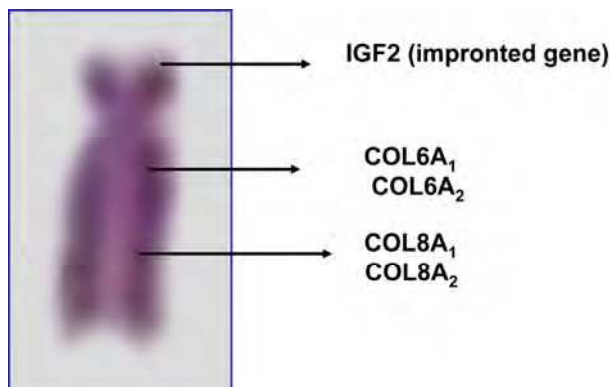


Fig. 4. The sub-metacentric chromosome rob(1;29), with the location of possible gene involved in genetic sub-fertility (courtesy: Postiglioni,A., 2009).

2.1 Chromosomal regions and clastogenic agents

In a recent study we analysed the pericentromeric region where chromosomes 1 and 29 might have suffered chromatin rearrangements due to the Robertsonian translocation polymorphism constitution. After applying different banding techniques, such as C, G and

RE (Fig. 3), clastogenic agents, like aphidicolin (APC), 5-azacytidine-C(5-AZA) and 5-bromodeoxyuridine (5-BrdU) were selected as tools to study chromatin structure alterations (Di Bernardino *et al*, 1983; Sutherland & Hecht, 1985; Verma & Babu, 1995). Particularly, aphidicolin (APC) inhibits DNA polymerase α during replication. This fact allows the identification of regions rich in dCTP due to competition and spreading of the enzyme. The methodology used was the incorporation of APC (0,3 μ M) in one cell cycle (24 hrs.) of lymphocyte cultures, as was previously done in humans (Glover *et al.*, 1984); cattle (Postiglioni *et al.*, 2001; Rodriguez *et al.*, 2002) and other domestic animals. The purpose was to find regions that are rich in cytosines and sensible to APC, and to control the response to its action in both normal and rearranged chromosomes. Other clastogenic agent used was the demethylant agent 5-azacytidine, which at high concentrations produces an undercondensation of late replicating X chromosome in G₂, due to DNA demethylation (Haaf & Schmid, 2000). A similar effect of decondensation in hypomethylated DNA was found in this important heterochromatin region of rob(1;29), after 2 hrs. of lymphocyte induction with high concentration of 5-aza-C (10mM). These findings contribute to support our hypothesis that proximal to rob(1;29) centromere exist dynamic heterochromatic regions where multiple microrearrangements could have occurred during chromosome evolution and that are possibly affecting gene expression. This hypothesis is also supported if we consider this chromosome rearrangements as possible defense mechanisms against transposons, which perpetuate in animal population as well as in microorganisms (Doolittle & Sapienza, 1980; Yoder *et al.*, 1997).

2.2 Aphidicolin action – The response of chromatin rearrangement rob(1;29)

Inducing one cell cycle of lymphocyte cultures with APC (0,3 μ M), we found a new break point: (relative distance: p2c/p1=0,45), proximal to the location of IGF2 gene, and two fragile sites (c-fra) in the long arm of rob(1q_{13/21};29) and rob(1q₄₃;29), corresponding to the location of the collagen genes mentioned above (Artigas *et al.*, 2008a).

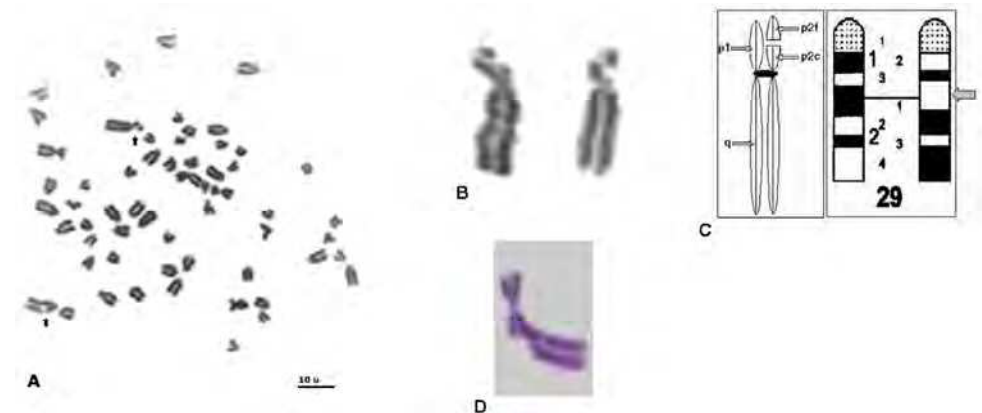


Fig. 5. Lymphocyte metaphases treated with aphidicolin (0,3 μ M). Observed the break point in rob(1;29p_{13/21}) and rob(1q_{13/21};29). The scheme illustrates the regions measured (courtesy: Artigas, R., 2008).

Chromosomes of Creole cows carrying rob(1;29) revealed heterozygosity in the expression of the fragile site rob(1q_{13/21};29), when processed with the clastogenic agent aphidicolin.

Comparing APC effects on BTA1q_{13/21} and rob(1q_{13/21};29), the high damage found in this specific region of the chromosome rearrangements, revealed an heterozygous expression behavior in the sensitive chromatin region rob(1q_{13/21};29) (Table 1).

| Creole cattle | (a) BTA 1;29 | (b) BTA 1 | (axb) | Total | Authors |
|---------------|-----------------|--------------|-------------|------------|---------------------------|
| Cow 1 | 55 80% | 13 20% | 0,16 16% | 68 100% | Tellechea et al., 2004 |
| Cow 2 | 44 72% | 17 28% | 0,20 20% | 61 100% | Tellechea et al., 2004 |
| Cow 3 | 28 57% | 21 43% | 0,25 25% | 49 100% | Artigas et al., 2008 |



Table 1. Comparing APC effects on BTA1q_{13/21} and rob(1q_{13/21};29). Simultaneous expression (a x b) corresponds to homozygosity for fragility expression.

It is important to connect this results to those that were discussed in: “*What happens in the pachytene synapsis of cattle carrier of rob(1;29)?*” (1.1.3). Two important facts, an unsynapsed region of the trivalent and the heterozygous expression of APC, indirectly suggest the incorporation of a nucleotide sequence rich in CTP in this region of rob(1;29), as the action of APC was higher than its “homologous” BTA1 chromosome. This particular APC effect could be related to epigenetic mechanisms (Wang, 2006). So, this particular region of late replication has been transformed to a heterozygous segregation region (Sutherland & Hecht, 1985).

In this particular region, we identified the microsatellite BMS4015 and the tissue-specific gene collagen type VIII alpha 1 (Col8A1) related to extracellular matrix proteins that could be associated with placental mammal-specific gene groups (Artigas et al., 2008a; Di Meo et al., 2006; Postiglioni et al., 2011).

To prove the existence of a foreign nucleotide sequence in this particular chromatin region we could incorporate the research of Joerg et al., (2001). The analysis of the microsatellite BMS4015 allowed them to find a specific allele in all rob(1;29) carriers, that clearly differentiated normal cattle that acted as a control of the experience.

2.3 5-azacytidine-C action – The response of chromatin rearrangement rob(1;29)

A pronounced chromatin despiralization of rob(1;29) similar to the inactive X chromosome of female mammals, was demonstrated when lymphocyte cultures were exposed to a DNA demethylating agent (5-azacytidine-C) (Artigas et al., 2008b; Artigas et al., 2010). This demethylating agent is considered a useful tool to study chromatin decondensation (Verma & Babu, 1995; Haaf, 1995; Haaf & Schmid, 2000).

This agent is a cytidine analog with a nitrogen atom replacing the carbon at the 5th position of the pyrimidine ring. When incorporated into DNA, the cytosine cannot be methylated and causes almost complete demethylation of genomic DNA, expressing a despiralization of the condensed chromatin of metaphase chromosomes. 5-aza-C has the ability to induce inhibition of chromatin condensation in autosomic heterochromatin, constitutive and facultative, and in the genetically inactive late replicating X chromosome (Haaf, 1995; Haaf & Schmid, 2000). The effects on chromatin structure are highly dependent on 5-aza-C concentration and treatment time. In females, a concentration of 5-aza-C of $1 \times 10^{-3}\text{M}$ for 2 h inhibits condensation of the inactive X chromosome, without affecting the active X chromosome and autosomes.

Our first experiment consisted on using this demethylant agent in lymphocyte cell cultures of normal and carriers of rob(1;29) female Creole. This inductor revealed decondensation of the region proximal to the centromere of rob(1;29) and complete despiralization of the inactive X chromosome (Artigas et al., 2008b). It has been demonstrated that DNA methylation is essential for the control of gene activity in a large number of normal and pathological cellular processes, including differentiation, genomic imprinting, X inactivation and silencing of intragenomic parasitic sequence elements (Jaenisch, 1997; Yoder et al., 1997; Ng & Bird, 1999). Besides, it is used as a drug against cancer that has already been approved by the US Food and Drug Administration (FDA) for the treatment of myelodysplastic syndromes (MDS) and chronic myelomonocytic leukemia (CML) (Kulis & Esteller, 2010).

An important despiralization of the inactive X chromosome resulted similar to the rob(1;29), while the other X chromosome and the autosome chromosomes 1 and 29, maintained their condensation (Fig. 6).

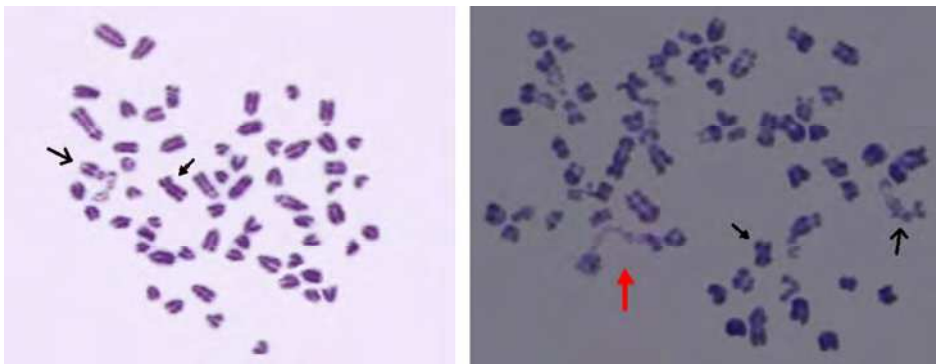


Fig. 6. Lymphocyte metaphases of cattle female normal and carriers of rob(1;29), treated to 5-aza-C ($1 \times 10^{-3}\text{M}$, 2 h). Red arrow shows the demethylate rob(1;29); black arrows signed the inactive and active X's chromosomes (cortesy: Artigas, R & Iriarte, W., 2009).

To demonstrate this observation, we measured 28 metaphases using the program UTHSCSA ImageTool Version 3.0 to account for the decondensing effects of 5-aza-C on chromatin structure. Both despiralized chromosomes presented an average value and standard error of 0.75 ± 0.11 and 0.75 ± 0.083 , respectively. Data from the active X chromosome clearly differed, with an average of 0.54 ± 0.09 and 1.07 ± 0.2 , respectively for centromeric and biarmed despiralization (Fig. 7).

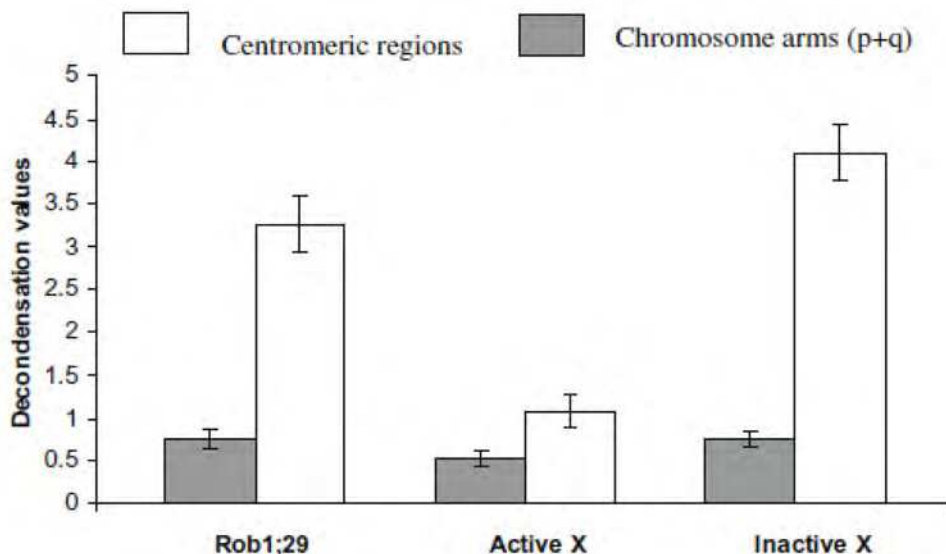


Fig. 7. Effects of 5-Aza-C on biamed chromosome of rob(1;29) carrier cow. Histograms depict average decondensation values and standard errors in centromeric regions and chromosome arms (p + q) for rob(1;29); active and inactive X chromosomes.

Using no-parametric tests (Kruskal-Wallis global test) we demonstrated our observations with level of significance ($H = 32.11$; $p 1.07 \times 10^{-7}$) and the Mann-Whitney U-test permitted to demonstrate differences taking in pairs, among the three samples (Artigas et al., 2010).

We also observed a single block of condensed chromatin in the inactive X chromosome which contrasted with the high level of condensation inhibition in the rest of the chromosome. This region (Xq₁₃) corresponds to the early-replication segment associated with the center of inactivation referred to as Xist (Haaf, 1995). A similar region of condensation was observed in the rob(1q₄₃;29). According to the International System for Cytogenetic Nomenclature of Domestic Animals (ISCNDB, 2001), both regions correspond to R+ bands. Recently, an important gene related to placenta structure, named TRO, has been studied at the molecular level (Llambi et al., 2010). This gene, that encodes the protein trophinine, has been mapped in region BTAXq₂₅₋₃₃, where a fragile site of APC was found and probably proximal to the PAR region (Rodriguez et al., 2002; Raudsepp et al., 2011).

The nature and pattern of expression of genes located in condensed regions that despiralize with the action of demethylating agents, are considered facultative heterochromatin and correspond to G-bands, which may involve tissue-specific genes (Holmquist & Ashley, 2006). The rob(1q₁₃/21;29) region was hypomethylated after treatment with 5-aza-C (1×10^{-3} M) for 2 h. This effect could reflect a dynamic process of the heterochromatin where multiple micro-rearrangements have occurred affecting the expression of genes co-habiting the same chromatin domain or “genomic neighborhood”. These regions provide a new model for studying epigenetic changes in the bovine genome (Artigas et al., 2010).

3. An approach to silencing tissue-specific genes proximal to the rearrangement region of rob(1;29)

Based on our previous results and keeping in mind that imprinted genes are essential in embryonic development, we decided to query genes located on BTA1 and BTA29 that could undergo genome imprinting.

We have just hypothesized that the expression of tissue-specific genes co-habiting the same chromatin domain or “genomic neighborhood” could be affected by multiple micro-rearrangements that have occurred in this regions. Sequences of the region proximal to BTA1 centromere are known to have abundant CpG islands that could suffer methylations. The heritable modification of cytosine residues within CpG dinucleotides represents an important epigenetic mark that affects gene expression in diverse species (Vrana, 2007; Biliya & Bulla, 2010).

Particularly on BTA1 we begun to study genes related to structural proteins of the extracellular matrix that could be associated to a placental mammal-specific orthologous group including genes collagen typeVI- α_1 (Col6A₁), collagen typeVI- α_2 (Col6A₂), collagen typeVIII- α_1 (Col8A₁) and collagen typeVIII- α_2 (Col8A₂) (Elsik et al., 2009). As it was mentioned above, on BTA29 there is the imprinted gene insulin like growth factor II (IGF₂), which plays a key role in mammalian growth and is located proximal to the sensible chromatin region to dCTP, expressed as a break point to APC-induction (Schmutz et al. 1996; Vrana, 2007; Artigas et al., 2008a).

So, our next experience was to perform an *in silico* screening for CpG islands in the collagen typeVIII- α_1 (Col8A₁) promoter located on BTA1q_{13/21}, to uncover putative targets of methylation. DNA bisulfite conversion and sequencing methods were used to compare differential methylation patterns in the bovine Col8A₁ promoter. For the bisulfite conversion, we used the protocol referred to as “Sodium Bisulfite Conversion of Unmethylated Cytosines in DNA from solutions with low concentration of DNA” (Qiagen, Alameda, CA, USA). Besides, we used the “MethPrimer” program to design the primers to amplify the rich CpG promoter region for methylated (M) and unmethylated (U) cytosines (Li & Dahiya, 2002) (Fig. 8).

The target sequence was amplified from genomic DNA of lymphocyte cells. The eight sequence traces were aligned with sequence NM_001101176, *Bos taurus* collagen, typeVIII- α_1 (Col8A₁), position from 2068 to 2255 (Table 2). The alignment of the Col8A₁ sequence revealed 37 converted cytosines (75,51%), 10 methylated cytosines (20,41%) that correspond to CpG islands, and 2 inespecific alignment (4,08%). Two positions (2165 and 2186 from NM_001101176) were inespecific since some individuals showed unconverted cytosines, but others showed two peaks corresponding to a converted cytosine and an unconverted cytosine.

BiQ-Analizer software was used to show the selective conversion of unmethylated cytosines to uracils obtaining the following results: unmethylated CpGs: 0.000 (0 cases), methylated CpGs: 0.802 (77 cases) and CpGs not present: 0.198 (19 cases). All CpG islands in the promoter region of the gen Col8A₁ were methylated in the eight bovine samples (Bock et al., 2005, Luedi et al., 2007; Postiglioni et al., 2011) (Fig. 10).

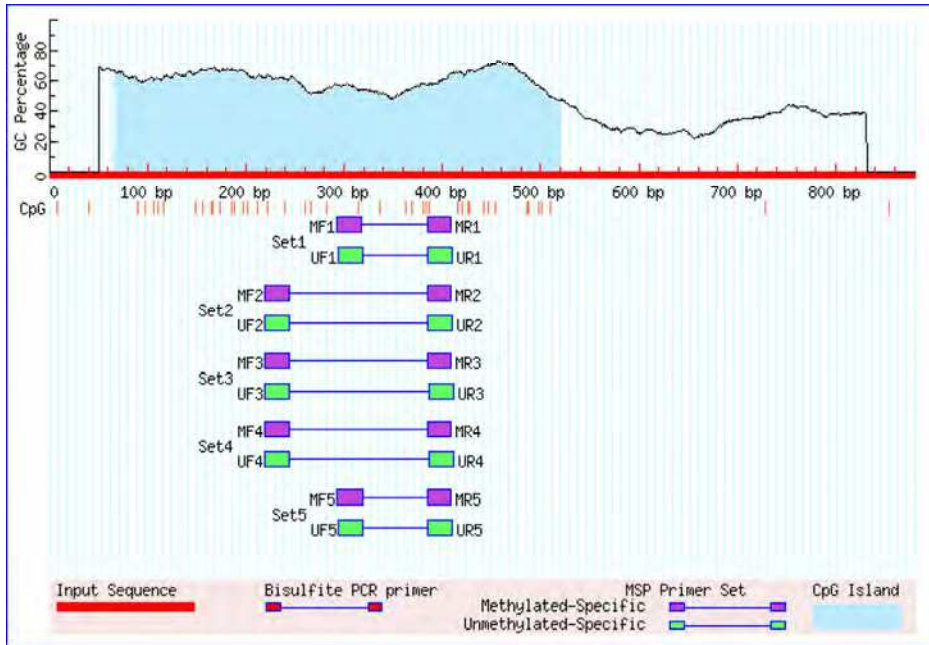


Fig. 8. Promotor region of Col8A1 gene reach in CpG island. The Methyl Primer Program permitted to select the pair primers for PCR.

| Types of cytosines | NM_001101176 positions |
|---|--|
| Unmethylated cytosines (converted by MSP into uracils) | 2075, 2079, 2082, 2084, 2093, 2097, 2102, 2105, 2108, 2109, 2112, 2120, 2123, 2126, 2128, 2129, 2138, 2139, 2140, 2147, 2150, 2153, 2162, 2163, 2169, 2171, 2174, 2183, 2197, 2199, 2210, 2217, 2218, 2228, 2243, 2252, 2255 |
| Methylated cytosines | 2072, 2090, 2110, 2116, 2132, 2213, 2219, 2230, 2234, 2237 |

Table 2. Position of methylated and unmethylated cytosines in a sample of Creole cattle traces with respect to sequence NM_001101176.

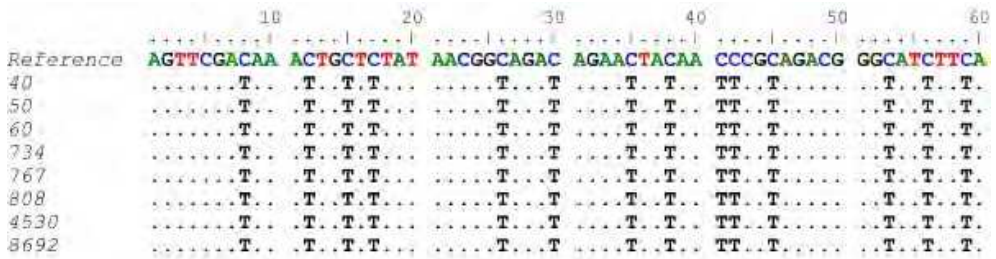


Fig. 10. Partial consense alignment of Col8A1 gene, after treatment with Sodium Bisulfite Conversion of Unmethylated Cytosines. Observe a total of eighteen *blue* cytosines; four of them (joined to guanine) are remained as 5-methylcytosine (methylated). The resting fourteen are recognized as thymine (unmethylated).

This technique was successfully applied in this study, showing that this is a straightforward methodology that can be used to evaluate gene expression in different tissues.

4. Conclusion

We presented a research approach to the problem of changes in tissue-specific gene expression related to genetic sub-fertility problems in cattle carriers of Robertsonian translocations (early embryo mortality, slow embryonic development). The most relevant results suggest that this chromosome rearrangement rob(1;29), in heterozygous conditions, changes the nucleotide sequences around the pericentromeric region, compared with its homologous chromosomes. As this is a region rich in CpG islands, probably foreign sequences like transposons could be involved in this particular region, which also showed a despiralization of its chromatin when exposed to a demethylating agent. The bisulfite methodology applied to CpG islands of collagen promoters should be done on tissue-specific genes, like fibroblasts, to have a consistent answer to our question. Taking into account that Robertsonian translocations are the most common chromosomal rearrangements in humans, specially the rob(13q14q) and rob(14q21q), these experiences could contribute to the advance in this area. Besides, our results could be taken in consideration as a model in human cases.

Future research will have to be done to demonstrate that methylation of tissue-specific genes CpG islands occur in animals carrying the rob(1;29) Robertsonian translocation. Cattle fibroblasts, semen, early embryos and trophoblastic membranes will have to be analyzed to determine the genetic causes of embryo mortality. Our cyto-molecular approach will allow the use of this knowledge as an animal biotechnology tool in reproduction programs. New methodologies, such as high throughput sequencing and transcriptome analysis, could be incorporated to the present work to improve the understanding of questions such as: which are the precise functions of collagen genes and other genes involved in embryonic implantation and development? Is it really the rob(1;29) acting as a defensive mechanism to regulate gametic selection? As said by many other researchers: "These are just some of the questions that arise from the above summarized observations. Yet, there are very few answers".

5. Acknowledgment

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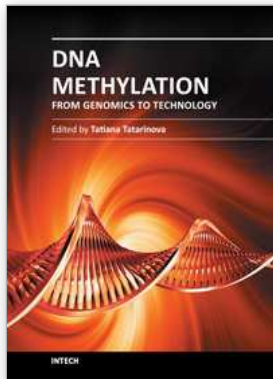
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Epigenetics is one of the most exciting and rapidly developing areas of modern genetics with applications in many disciplines from medicine to agriculture. The most common form of epigenetic modification is DNA methylation, which plays a key role in fundamental developmental processes such as embryogenesis and also in the response of organisms to a wide range of environmental stimuli. Indeed, epigenetics is increasingly regarded as one of the major mechanisms used by animals and plants to modulate their genome and its expression to adapt to a wide range of environmental factors. This book brings together a group of experts at the cutting edge of research into DNA methylation and highlights recent advances in methodology and knowledge of underlying mechanisms of this most important of genetic processes. The reader will gain an understanding of the impact, significance and recent advances within the field of epigenetics with a focus on DNA methylation.

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