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Cytogenetics and Molecular Genetic Analysis of Chimerism in Marmosets (*Callithrix*: Primates)

MONIQUE O.M. SILVA[1], JORGE LUÍS A. DE ARMADA[2], CARLOS EDUARDO S. VERONA[3], GABRIELA HELIODORO[4] and DENISE M. NOGUEIRA[2]

[1]Programa de Pós-Graduação em Biologia Animal, Instituto de Ciências Biológicas e da Saúde, Universidade Federal Rural do Rio de Janeiro/UFRRJ, Rodovia BR 465, Km 7, 23890-000 Seropédica, RJ, Brazil
[2]Departamento de Genética, Instituto de Ciências Biológicas e da Saúde, Universidade Federal Rural do Rio de Janeiro/UFRRJ, Rodovia BR 465, Km 7, 23890-000 Seropédica, RJ, Brazil
[3]Instituto Brasileiro para Medicina da Conservação/Instituto Tríade, Rua Silveira Lobo, 32, Caixa Postal 48, Casa Forte, 52061-030 Recife, PE, Brazil
[4]Instituto de Pesquisas Jardim Botânico do Rio de Janeiro/JBRJ, Rua Jardim Botânico, 1008, 22470-180 Jardim Botânico, RJ, Brazil

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ABSTRACT

The birth of fraternal twins is a characteristic frequently observed in callitrichids. Cytogenetic studies have demonstrated hematopoietic chimerism in marmosets with the occurrence of two cell lines 2n=46,XX/46,XY in females and males co-twins, without phenotypic changes. Amplification by PCR have also been used to verify the presence of the *SRY* gene in female chimaeras. Our aim was to verify the occurrence of chimerism in *Callithrix* sp. individuals considered as hybrids according to their intermediate phenotypes between *C. jacchus* and *C. penicillata.* Blood samples from 37 *Callithrix* sp. individuals were collected. Hematopoietic chimerism 2n=46,XX/46,XY was detected by cytogenetic analysis in five individuals, three males and two females. A fragment of approximately 200bp of the *SRY* gene was amplified in seven females with normal external genitalia. The percentage of 32% of chimeric individuals detected in the present study is similar to that observed for pure specimens of *Callithrix*. These data suggests that hybridization probably does not interfere with the occurrence of twin gestation, nor of chimerism. Although cytogenetics is the main tool to identify the two cell lineages present in cases of chimerism, the amplification of the *SRY* gene by PCR has proved to be more efficient to identify the Y chromosome in cases of chimeric female marmoset.

Key words: karyotype, *SRY*, chimaeras, hybridization.

INTRODUCTION

The callitrichids are primates of the New World, from the order Primates, Platyrrhini infraorder and Callitrichidae family, which has seven genera: *Leontopithecus*, *Saguinus*, *Cebuella*, *Mico*, *Callithrix*, *Callimico* and *Callibella* (Rylands et al. 2000), being the first two commonly called Tamarins and the others, Marmosets. They are characterized by their small size and by twin pregnancy, except in the genus *Callimico* (Miranda Ribeiro 1912) and *Callibella* (Van Roosmalen and

Correspondence to: Denise Monnerat Nogueira
E-mail: denisemn@ufrrj.br

Van Roosmalen 2003) that have only a cub by pregnancy (Hershkovitz 1977, Haig 1999, Reis et al. 2015).

The species that comprise the genus *Callithrix* (Erxleben 1777), namely: *C. jacchus*, *C. penicillata*, *C. aurita*, *C. geoffroyi*, *C. flaviceps* and *C. kuhlii* have pregnancy in an average of 145 days, with deliveries twice a year (Hill 1926, Oliveira et al. 2015). In addition to twins, there have been reports of birth of single, triple and even quadruple cubs, being the last two more common in captivity and with lower rates of individual survival (Tardif et al. 1984, Sweeney et al. 2012, Ward et al. 2014). When investigating the twin pregnancy in Marmosets, Hill (1926) found that the twins were from two oocytes and that they shared a single chorion. Later, Wislocki (1932, 1939) analyzing the placentas of several female marmosets with twin pregnancy, confirmed the anastomosis of the blood vessels. This anastomosis is responsible for the formation of chimaera individuals, which have chromosomally different cell lines derived from embryos of different sexes (Tarkowski 1970).

In humans, the occurrence of chimerism among monochorionic dizygotic twins is rare (Dunsford et al. 1953, Nicholas et al. 1957, Redline 2003, Assaf et al. 2010, Chen et al. 2013). It is found more often in cattle (Lillie 1916, 1917, Owen 1945, Dunn et al. 1968, Padula 2005), as well as in marmosets and tamarins (Wislocki 1932, 1939, Abbott 1984, Haig 1999, Wedi et al. 2011). In cattle, the whole gestation taken is approximately 290 days and the anastomosis of the placenta blood vessels occurs around the 30th to 40th day of gestation (Almeida and Resende 2012). In this case, the birth of chimeric females results in the freemartinism syndrome, (Lillie 1916, Padula 2005) classified as a disorder of sexual development (DSD) (Meyers-Wallen 2012) responsible for intersexuality of the female co-twin with a male with consequent masculinization of her reproductive tract. Most of freemartin are hermaphrodites, having ambiguous genitalia being sterile or infertile (Herschler and Fechheimer 1967, Mcfeely et al. 1967, Dunn et al. 1968, Padula 2005). This is because, in addition to the cellular exchange via anastomosis, the passage of androgenic hormones also occurs (Dominguez et al. 1990).

Bovine chimaeras presents the karyotype 2n=60,XX/60,XY. These two cell lines can be detected by culture of the testicular tissue, bone marrow and peripheral blood lymphocytes (Ohno et al. 1962, Herschler and Fechheimer 1967, Eldridge and Blazak 1976). Furthermore, it is also possible to amplify *SRY* gene sequences by Polymerase Chain Reaction (PCR) (Mullis et al. 1986) to confirm the chimerism (Padula 2005).

In mammals, the *SRY* gene is located on the short arm of the Y chromosome, and it is responsible for encoding a protein that, when expressed, initiates a cascade of events that lead to gonadal differentiation. This protein induces the formation of testes that are responsible for releasing androgenic hormones that lead to phenotypic sexual differentiation in males (Sinclair et al. 1990).

In callitrichids, the placental anastomosis initiates around the 19th day and it is complete around the 29th (Moore et al. 1985, Haig 1999), with cases of chimerism always coming from two or more concepts (Haig 1999). Chimaera unique cubs have been found, but this result possibly comes from the placental anastomosis with a co-twin that came to death followed by reabsorption of their fetal structures by the maternal organism (Jaquish et al. 1996). This characteristic is common in female marmosets and acts as a positive feedback in response to reproductive biology of the species to ensure the survival of the other twin (Tardif et al. 1984).

The hematopoietic chimerism in marmosets was firstly detected by conventional cytogenetic studies through the lymphocyte culture from peripheral blood and tissue culture of bone marrow, spleen and thymus. Two cell lines 2n=46,XX/46,XY

were found in marmosets (Benirschke et al. 1962, Benirschke and Brownhill 1963, Gengozian et al. 1964, 1969).

According to the karyotype, chromosome homogeneity is observed in five species of *Callithrix*, except for the size and morphology of the Y chromosome (Nagamachi et al. 1997). In the White-tufted-ear marmoset, *C. jacchus*, this chromosome can be metacentric, submetacentric or acrocentric, but in the Black-tufted-ear marmoset, *C. penicillata* it is metacentric or submetacentric (Nagamachi et al. 1997).

By PCR analyzes the *SRY* gene on the Y chromosome of males and females marmosets were amplified followed by DNA sequencing that proved to be identical for both males and females (Moreira 2002, Sanchez-Morgado et al. 2003, Takabayashi and Katoh 2011). Most cases of marmoset chimerism were studied in *C. jacchus*, a world-wide species used in biomedical research, being the first primate of the New World to have its genome sequenced (Worley et al. 2014). Specific XY chromosome probes have been developed as specific markers of chimerism in marmosets, allowing the precise identification of the cell type involved (Wedi et al. 2016).

In Brazil, *C. jacchus* is original from the Caatinga and Atlantic Forest biomes of Northeast and *C. penicillata* is native from the Cerrado in the Brazilian Central-Western (Hershkovitz 1977). According to the phenotype, *C. jacchus* exhibit white auricular tufts, gray coats in face, black crown and a white spot in the forehead. The back is light gray with dark streaks and a grayish brown tail with white rings around it. In *C. penicillata*, the auricular tufts are black with blackened face with a shiny white spot in the forehead. The back is light gray with dark streaks and a black tail with white rings around it (Oliveira et al. 2015). Both species were introduced in the Southeastern Brazil where they settled and have been mating, generating hybrid descendants (Mittermeier et al. 1988, Rylands 1993, 2000, Malukiewicz et al. 2015) including with the native marmoset species of the Southeast, *C. aurita* (Nogueira et al. 2011).

Our goal was to use the cytogenetic and the molecular genetic analyzes by means of PCR technique to verify the occurrence of hematopoietic chimerism in captive and free-living individuals of *Callithrix* sp. in the State of Rio de Janeiro, Brazil.

MATERIALS AND METHODS

Blood samples were collected by puncture of the femoral vein of 37 *Callithrix* sp. individuals (24 males and 13 females). The species to which the individuals belong could not be confirmed due to the occurrence of intermediate phenotypes between *C. jacchus* and *C. penicillata*, probably resulting from hybridization. For this reason, we used the nomenclature *Callithrix* sp.

The individuals were from three localities in the State of Rio de Janeiro. Eighteen free-living individuals were captured at the Botanical Garden of Rio de Janeiro/JBRJ (22º57' 22º59' S and 43º13.43º14' W); nine were from The Wild Animals Screening Center/CETAS-RJ/IBAMA (22°43'73"S and 43°42'28"W) and ten from the municipality of Campos dos Goytacazes, kept in the vivarium of the Carlos Chagas Filho Institute of Biophysics, Federal University of Rio de Janeiro/ UFRJ.

In each animal, the coloration of the coat of the back and the head was examined, with an emphasis on the ear tufts and the characteristics of the external genitalia.

The free-living individuals were captured with *Tomahawk* trap (18 x 18 x 60 cm) and subsequently sedated with Ketamine and Midazolam (10 mg/kg, intramuscular) for biological sample collection, identification and microchip marking (Microchip-partners®; 12 mm x 2.1 mm; 0.06g) which was inserted into the interscapular region. After the full

recovery, the free-living individuals were returned to their proper habitat.

All the procedures performed are in accordance with the System of Authorization and Information on Biodiversity/SISBIO (Number: 20435-2) and in the case of the animals of the UFRJ Vivarium, they were all registered in the National Council of Control of Animal Experimentation/CONCEA (Number: 01200.001568/2013-87).

CYTOGENETIC ANALYZES

To obtain metaphasic chromosomes, peripheral blood lymphocyte culture was performed (Moorhead et al. 1960). The chromosomes were stained with 3% Giemsa solution and the metaphases were analyzed under an Olympus® model CH30RF100 optical microscope, with a 100x objective.

The best metaphases were selected, photographed and the karyotype of each individual was assembled in the *Adobe Photoshop® CS3* program, following the organization according to Nagamachi et al. (1997).

MOLECULAR ANALYZES

To DNA extraction from the whole blood samples we used the saline precipitation method (Miller 1988). Genomic DNA was quantified in spectrophotometry (NanoDrop ND-2000, Thermo Scientific®).

For PCR, Forward (5 '-TAC AGG CCA TGC ACA GAG AG-3') and Reverse (5 '- CTA GCG GGT GTT CCA TTG TT-3') primers were used for amplification of a fragment of approximately 200bp of the *SRY* gene (Oliveira et al. 2010). The primer set was designed based in the *SRY* gene sequence of 838 bp generated by Moreira (2002) to amplify the region which includes the deletion of 9 bp in *C. aurita*. The PCR product was sequenced and then aligned with the Basic Local Alignement Search Tool (BLAST- blast.ncbi.nlm.nih.gov/Blast.cgi) to confirm the *SRY* origin of the amplified fragment considering that there are other genes as Sox 3 found on the X-chromosome of eutherian mammals (Graves 1998) that can present 50% of aminoacid similarity with the HMG box in *SRY*. With at least 100 sequences aligned there were significant similarity only with *SRY* gene sequence in many Primate species, confirming the *SRY* origin of the sequences amplified from the samples analyzed here. Each PCR reaction of 10 µL consisted of: 1X Buffer solution; 3 mM MgCl 2; 0.2 mM dNTPs; 1 µM of each primer; 1.25U of *Taq* DNA Polymerase and 10 to 20 ng/µl of genomic DNA. The reactions were amplified in the thermocycler (*ProFlex™ PCR System–Appllied Biosystems® ThermoFisher Inc*), with the following temperature cycles: initial denaturation at 94°C for 5 min, followed by 30 cycles at 94ºC for 1 min, 55ºC for 1 min and 72ºC during 1 min, ending with a period of extension at 72ºC for 10 min.

PCR products were separated by electrophoresis in 5% polyacrylamide gel at 100V / 1h. In each gel besides the PCR product of the samples analyzed we also included a molecular weight marker (100 pb DNA ladder), a male and female positive control and a negative control reaction with ultra-pure water instead of DNA to verify occurrence of contamination of the reagents. The gel result was evaluated after staining with 0.2% silver nitrate solution and the gel image was captured using the transilluminator apparatus software (*L.Pix Loccus Biotechnology ©)*.

RESULTS

The analyzes of morphological traits such as the presence of the white spot on the forehead characteristic of *C. jacchus* and *C. penicillata*, was present in all individuals analyzed. The coloration of the head coat and auricular tufts presented a gradation from black to white. On the back of the individuals a variation in color from light gray to

deep brown with transverse streaks were observed. On the tail, lighter colored rings around it was present. These characteristics contributed to classify as hybrids, *Callithrix* sp., the individuals studied. The analyzes of the external genitalia showed to be normal for both females, with the normal labia and vulva, as for the males, with scrotum and penis.

From the 37 individuals of *Callithrix* sp. studied cytogenetic analyzes was successful in 20 individuals, being 5 females and 15 males (Table I). In the remaining 17, it was not possible to obtain a result by this methodology. The analyzes of the karyotypes revealed a diploid number 2n=46,XX or XY, NF=74, being the X chromosome submetacentric and the Y metacentric, corresponding to the morphology described for *C. jacchus* and *C. penicillata*. The hematopoietic chimerism 2n=46,XX/46,XY was detected in five of the 20 analyzed individuals of *Callithrix* sp., being three males and two females (Figure 1).

Amplification of the 200 bp *SRY* gene fragment occurred in samples from 22 males of *Callithrix* sp. In only one sample, there was no amplification, probably due to the poor DNA quality (Table II). In the females, from the 13 studied, amplification of *SRY* gene fragment occurred in seven, including a female in which hematopoietic chimerism had already been detected in the cytogenetic analyzes. With respect to the other chimeric female detected by cytogenetics, we suppose that there was no amplification of the *SRY* gene, because of the low quality of the DNA extracted (Table II).

DISCUSSION

Hybridization in *Callithrix* was primarily investigated in captivity (Coimbra-Filho 1970, Coimbra-Filho and Maia 1976, Coimbra-Filho et al. 1976, Rylands 1993) being also reported in hybridization zones where *Callithrix* species originally occurs (Hershkovitz 1975, Rylands et al. 2000, Arnold and Meyer 2006). Since the 1950's, human intervention has been associated with the range expansion of both species (Vieira 1995, Avila-Pires 1969) and recently interbreeding of *C. jacchus* and *C. penicillata* in Southeastern Brazil, have been also confirmed in anthopogenic hybrid zones (Malukiewicz et al. 2015, Cezar et al. 2017).

The presence of two cell lineages 2n=46,XX/46,XY in *Callithrix*, comes from the cellular exchange via placental anastomosis between heterosexual twins (Benirschke et al. 1962, Gengozian et al. 1964). The frequent occurrence of chimerism (Ardito et al. 1995, Gengozian et al. 1980) in callitrichids may be related to evolutionary aspects about their social systems (Haig 1999). In these animals, parental care depends on the father and other members of the group other than the mother, explained by the high-energy costs that the female would have for the creation of twins with two births per year (Santos and Martins 2000). According to Ross et al. (2007), the chimeric lineages could generate signs of recognition of kinship between the members of the group through the genomic and phenotypic homogeneity between the individuals. This may be one of the reasons that could explain the unusual attraction of the father by the cubs, which would favor the paternal and alloparental care in the callitrichids.

Anomalies in the external genitalia of chimaera individuals have not been reported in the cases studied so far in *Callithrix*. However, in a female chimaera of *Leontopithecus chrysomelas* (2n=46,XX/46,XY) clitoris enlargement and constriction of the vulvar opening were described (Goldschmidt et al. 2005). In the present study, the phenotype of the external genitalia was normal in males and females with chimerism, which corroborates previous studies that described that *Callithrix* chimaeras are normal and fertile (Benirschke et al. 1962, Ardito et al.1995).

The detection of the chimerism occurred in 32% of the total number of sampled individuals of *Callithrix* sp., free-living and captive in the State of

TABLE I
Cellular lineages of the 20 individuals of *Callithrix* sp., with the diploid number, the identification code, sex, site and percentage of the chimerism observed for each individual. [ ] Number of metaphases found for each cell lineage.

Individual	Sex	Age	Site	Cellular Lineages	% Chimerism
Csp001	M	Juvenile	CETAS-RJ/IBAMA	2n=46,XY[19]	0
Csp002	F	Adult	CETAS-RJ/IBAMA	2n=46,XX[5]/46,XY[2]	28%
Csp003	M	Adult	CETAS-RJ/IBAMA	2n=46,XY[10]	0
Csp005	F	Adult	CETAS-RJ/IBAMA	2n=26,XX[14]	0
Csp006	F	Adult	CETAS-RJ/IBAMA	2n=46,XX[5]/46,XY[5]	50%
Csp007	M	Adult	CETAS-RJ/IBAMA	2n=46,XX[5]	0
Csp008	M	Adult	CETAS-RJ/IBAMA	2n=46,XY[7]	0
Csp009	M	Juvenile	CETAS-RJ/IBAMA	2n=46,XX[20]/46,XY[5]	80%
Csp010	M	Adult	JBRJ	2n=46,XY[50]	0
Csp011	M	Adult	JBRJ	2n=46,XY[30]	0
Csp012	M	Adult	JBRJ	2n=46,XY[15]	0
Csp013	M	Adult	JBRJ	2n=46,XX[5]/46,XY[6]	45%
Csp014	F	Adult	JBRJ	2n=26,XX[30]	0
Csp016	M	Juvenile	JBRJ	2n=46,XX[3]/46,XY[1]	75%
Csp017	F	Adult	JBRJ	2n=26,XX[11]	0
Csp021	M	Juvenile	JBRJ	2n=46,XY[6]	0
Csp022	M	Adult	JBRJ	2n=46,XY[10]	0
Csp024	M	Juvenile	JBRJ	2n=46,XY[6]	0
Csp025	M	Adult	JBRJ	2n=46,XY[10]	0
Csp040	M	Adult	JBRJ	2n=46,XY[6]	0

Csp= *Callithrix* sp.; M= Male; F= Female.

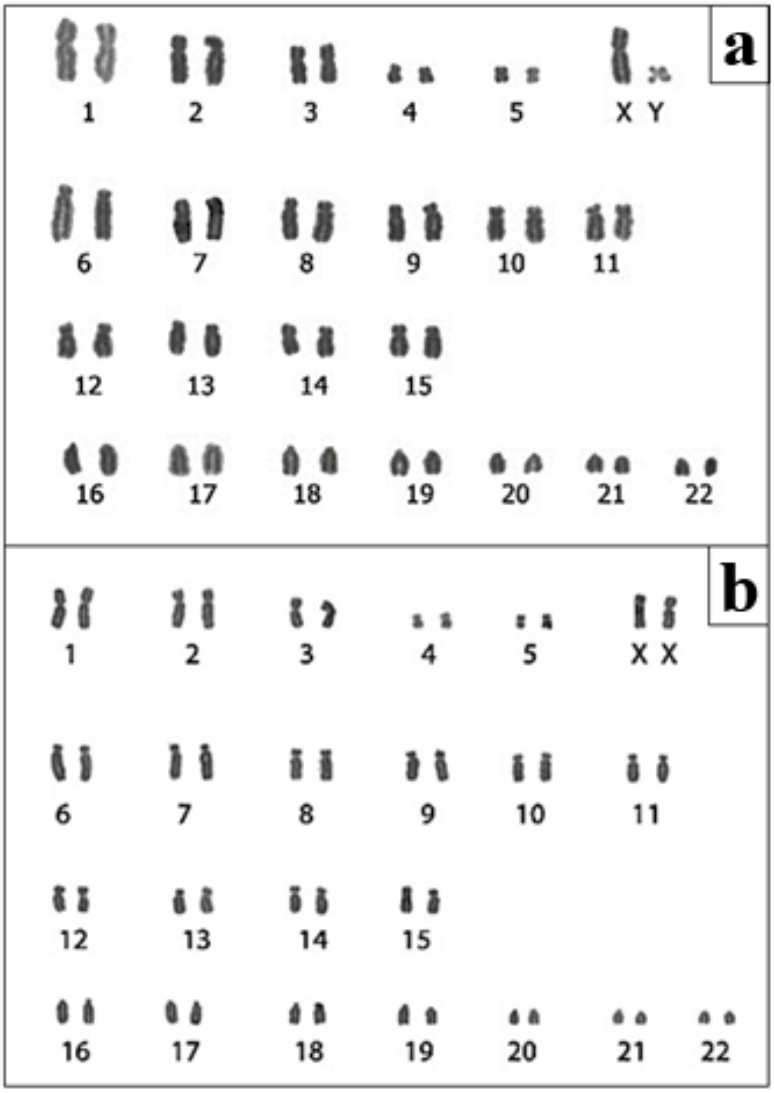

Figure 1 - Conventionally stained karyotype of a male *Callithrix* sp. with hematopoietic chimerism, evidencing the two cell lines (**a** and **b**) 2n = 46,XX[5]/46, XY[25]. [ ] Number of metaphases found for each cell lineage.

Rio de Janeiro, Brazil, by means of the cytogenetic analyzes and molecular amplification of the *SRY* gene by PCR. The PCR technique made it possible to detect the presence of the *SRY* gene in a larger number of females compared to cytogenetic analyzes.

Through cytogenetic analyzes, it was possible to identify chimerism in 13.5% of *Callithrix* sp. sampled. The low percentage of success in cell culture by this technique may be related to the fact that the marmosets are animals weighing on average 350g (Oliveira et al. 2015), which reduces the total blood volume that can be collected. In addition, considering that obtaining metaphase chromosomes depends on approximately 30% of circulating nucleated white cells, the low number of metaphases analyzed in this study may have come from the small volume of blood collected from each individual also shared with molecular analyzes.

TABLE II
***Callithrix* sp. (23 males and 7 females) samples where a 200 bp fragment of the *SRY* gene was amplified by PCR, including identification code, sex, age and site.**

Indivíduo	Sexo	Idade	Local	Gene *SRY*
Csp003	M	Adulto	CETAS-RJ/ IBAMA	+
Csp004	M	Juvenil	CETAS-RJ/ IBAMA	+
Csp006	F	Adulto	CETAS-RJ/ IBAMA	+
Csp007	M	Adulto	CETAS-RJ/ IBAMA	+
Csp008	M	Adulto	CETAS-RJ/ IBAMA	+
Csp009	M	Juvenil	CETAS-RJ/ IBAMA	+
Csp010	M	Adulto	JBRJ	+
Csp011	M	Adulto	JBRJ	+
Csp012	M	Adulto	JBRJ	+
Csp013	M	Adulto	JBRJ	+
Csp015	F	Adulto	JBRJ	+
Csp016	M	Juvenil	JBRJ	+
Csp017	F	Adulto	JBRJ	+
Csp018	M	Adulto	JBRJ	+
Csp019	M	Adulto	JBRJ	+
Csp021	M	Juvenil	JBRJ	+
Csp022	M	Adulto	JBRJ	+
Csp024	M	Adulto	UFRJ	+
Csp025	M	Adulto	UFRJ	+
Csp028	M	Adulto	UFRJ	+
Csp029	F	Adulto	UFRJ	+
Csp030	F	Adulto	UFRJ	+
Csp031	M	Adulto	UFRJ	+
Csp033	M	Adulto	UFRJ	+
Csp035	M	Adulto	UFRJ	+
Csp036	F	Adulto	UFRJ	+
Csp037	F	Adulto	UFRJ	+
Csp039	M	Adulto	UFRJ	+
Csp040	M	Adulto	JBRJ	+
Csp041	M	Adulto	JBRJ	+

Csp= *Callithrix* sp.; M= Male; F= Female; += presence *SRY* gene.

In molecular genetic analyzes, the amplification of 200 bp of the *SRY* gene by the PCR technique was successful in approximately 78% of the total sampled (23 males and 7 females). In six females (46.15%) where the 2n=46,XY cell lineage had not been detected by cytogenetic analyzes, the PCR technique proved to be effective in demonstrating the presence of the *SRY* gene that is probably a consequence of the exchange of cells in these phenotypically normal individuals without sexual anomalies.

The causes of non-phenotypic alteration may be related to the existence of protection mechanisms that minimize the effects of the male twinning of the co-twin. Some of these mechanisms were assumed by French et al. (2016), which compare the reproductive and survival potential of at least one male co-twin callitrichids females with non-co-twin males and also analyzed the coding regions of genes linked to sexual differentiation. In the first case, no significant differences were observed between male and non-female co-twin females, showing that the presence of males in gestation does not alter the reproductive behavior nor influence on the survival of these females. Already in the second case, amino acid substitutions of the proteins expressed from the candidate genes have been observed to be responsible for the effects of endocrine regulation of the anti-Müllerian (AMH) hormones and steroids that are associated with sexual differentiation in mammals. These substitutions predicted in programs that assess the impact on the biological function of proteins, were mainly significant for the AMH system which, according to the authors reduce the channeling of early reproductive development toward the male phenotype.

Although there has been more success in *SRY* gene amplification by PCR, the use of this methodology alone is not enough to prove the occurrence of chimerism. In normal males, this gene is normally located on their Y chromosome,

and in the females, the presence of the *SRY* gene may also be from translocation or unequal crossing-over during Meiosis. A similar case was analyzed by Sanchez-Morgado et al. (2003), who studied a female *Callithrix jacchus*, with atypical external genitalia exhibiting only one opening to the urethra, without the presence of vulva or testicles. By PCR, the *SRY* gene were amplified and the sequencing did not reveal mutation leading the authors to diagnose as an XY female, probably considering the abnormalities of the external genitalia. Nevertheless, in our view, due to the presence of a normal *SRY* gene, it was expected that the individual presented testes instead of ovaries. However, the karyotype analyzes was not performed to ascertain whether there was only one cell line, 2n=46,XY, or the chimeric line 2n=46,XX/46,XY.

A methodology developed by Wedi et al. (2016) seems to be a suitable identification of the cell line with chimerism. The authors developed probes for Fluorescent *in situ* hybridization (FISH) of the X and Y chromosomes of marmosets that can be used for any nucleated cell, allowing to identify precisely in which cellular types the chimerism occurs. In the absence of cells in metaphases originated from peripheral blood lymphocyte culture, interphase and metaphase cells obtained from other cultures such as the medulla, liver and spleen may be used. In addition, as a single cell-based cytological sexing technique it would not be questioned about contamination of various types of biological sample with blood, differently from other techniques that could produce false positives in relation to blood nucleated cell contamination.

Thus, we emphasize the importance of performing cytogenetic and molecular analyzes to diagnose chimerism in mammals, taking into account the limitations of both techniques and the possibility of errors in the interpretation of the result obtained only by PCR amplification of the *SRY* gene or based on the characteristics of the external genitalia.

The percentage of 32% of chimeric individuals detected in the present study is close to that observed by other authors in marmosets who analyzed a sample number similar to that reported here (Ardito et al. 1995, Gengozian et al. 1964) in the callitrichids. These data demonstrate that hybridization probably does not interfere with the occurrence of twin gestation, nor of chimerism. This is corroborated by the studies with *Callithrix* sp. (*C. jacchus* x *C. penicillata*) fertile hybrids in captivity (Coimbra-Filho and Maia 1976, Coimbra-Filho et al. 1976) and free-living in hybridization zones (Hershkovitz 1975). High levels of fertility in chimeric individuals was demonstrated by Ardito et al. (1995), where females 2n=46,XX/46,XY generated more concepts than females 2n=46,XX.

Although cytogenetics is the main tool to identify the two cell lineages present in cases of chimerism, the amplification of the *SRY* gene by PCR must be used to identify the Y chromosome mainly in females. A multidisciplinary approach using the confirmation of chimerism by cytogenetics and molecular genetics together with the clinical examination, histological analyzes of gonads, hormonal dosage, and behavioral assessment with a focus on paternal and alloparental care can provide a more comprehensive framework of the implications of chimerism in marmosets.

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