

**Title: Epidemiology of disorders of sex development in Southern Mozambique and molecular characterization of cases of complete sex reversal (Acronym: Arose)**

**Background (250/200-250 palavras)**

During mammalian embryonic development, an important and defining event for an individual is the differentiation of the bipotential gonads into either testes or ovaries, whether the embryo inherits XY or XX chromosomes, respectively. The functional ovary or testis is generated by a cascade of events that need the activation of a network of genes that result in a female or male phenotype, respectively. Failures in one of these events can lead to disorders of sex development (DSD)<sup>1-3</sup>. DSDs are congenital conditions in which there are atypical chromosomal, gonadal, and anatomical sex development, including disorders with ambiguous genitalia as well as without ambiguous genitalia<sup>1,4-6</sup>. Phenotypes vary from mild forms of hypospadias (birth defects in which the opening of the urethra is not located at the tip of the penis) to a complete sex reversal. Total sex reversal occurs when a 46,XX individual has a male phenotype or a 46,XY individual has a female phenotype<sup>7-9</sup>. A surgery of correction can mitigate some conditions related to DSDs, however, some alterations and mutations are related to post-surgery complications<sup>10</sup>.

In Mozambique, the Genetics Service has documented approximately 100 individuals with ambiguous genitalia, out of 6000 patients observed since 2016 (unpublished data). However, no further characterization to unravel the genetic etiology of the disease have been carried out and the epidemiology of DSDs is unknown in the region. Furthermore, some of these patients have been submitted to surgery and had complications, highlighting the need of characterization to decide properly how to manage them.

**Aim (151/100-150 palavras)**

The project is an initial part of a doctoral project that aims to describe the epidemiology of disorders of sex development in Southern Mozambique and characterize the molecular mechanisms underlying the sex reversal phenomena in Mozambique. For that, there are specific objectives, namely:

1. To determine the incidence of disorders of sex development in Southern Mozambique from 2016 to 2025.
2. To determine the risk factors associated with failures in the sexual differences that lead to disorders of sex development
3. To evaluate the health and disease determinants associated with disorders of sex development
4. To identify the genes and alterations associated with the sex reversal phenomena in 46,XX male and 46,XY female.

The study will inform the epidemiology of disorders of sex development and will raise awareness of the existence of this disorders in Mozambique and give tools for the healthworkers and public health to diagnose and manage DSDs patients.

**Potential impact (182/150-200palavras)**

The outlined project has the potential to inform the most frequent DSDs among Mozambicans, risk factors and health determinants associated to the disorder, and ultimately inform the existence of these conditions among the African population. This project will build the capacity to detect and diagnose different DSDs, including the sex reversal cases, using not only classical cytogenetic techniques, but also, molecular and high throughput techniques. The results from this project will raise awareness of the existence of these disorders in Mozambique, and Africa, and better inform the clinicians to properly manage the patients who seek the hospitals with such problems.

In summary, this project's innovative design, use of advanced genomic techniques, and its potential to uncover the molecular mechanisms of sex reversal make it a valuable and impactful contribution to the field of developmental genetics, reproductive biology, and public health. The outcomes will have broader implications for understanding the epidemiology of disorders of sex development and alterations associated to sex reversal that will allow to decrease the cases of post-surgery complications after a surgery of correction in cases that it is needed.

### **Methods (251/200-250 palavras)**

The study will have a retrospective and prospective design. Will be retrospective because data collected from August 2016 to September 2024 in the Medical Service of Genetics in the Maputo Centre Hospital will be used, in addition to the data that will be prospectively collected from participants recruited during 6 months (October 2024 to March 2025). This will be passive hospital-based surveillance, where Mozambican national individuals with the following symptoms: ambiguous genitalia, amenorrhea, early or late menstruation, atypical development of the genitalia, and infertility will be recruited in the medical genetics consulting in the Maputo Central Hospital. After signing the informed consent, a questionnaire will be administered to collect clinical and demographic information in the REDCAP software (current version, Vanderbilt University, USA) to have information about risk factor, health and disease determinants. 5 ml of blood samples will be collected from the participants, that in the first stage, will be cultured to carry out karyotyping analysis by G-banding to detect the confirm the clinical diagnose. Sex reversal will be confirmed when the karyotypical sex and the genital sex do not match. In the second stage, DNA will be extracted from the blood samples from sex-reversal cases and will be shipped to the Centre de Biologie Intégrative (CBI), in France, lead by Rafael Galupa (the supervisor of the doctoral thesis) to determine the genetic aetiology of the sex reversal phenomena. The genomic analysis will be performed in collaboration with R. Galupa's research team using in situ hybridization (FISH) and high-throughput sequencing technologies.

### **Applicant's expertise (139/100-150 palavras)**

This will finance the beginning of the PhD project of the applicant, so she will be fully dedicated to developing and implementing the outlined project, and will dedicate at least 40 hours/week. The applicant has worked with characterization of mutations that cause Cystic fibrosis and devising methods to correct these mutations during her master's degree, what will help in methods to uncover the mechanisms underlying the sex reversal phenomena. Notwithstanding, she integrated a team that operationalized the first public cytogenetic laboratory in Mozambique, where the study will take place, and she knows the team. Since 2019, she has been actively involved in molecular epidemiology projects, which will help add a public health perspective to the study. The project will help her consolidate study methods from a clinical and laboratory point of view and translate them into public health language.

### **Research environment (XX/200-300 palavras)**

The applicant is affiliated with the Centro de Investigação em Saúde de Manhiça (CISM) and will work in collaboration with the Maputo Centre Hospital and the Centre of Integrative Biology at the University of Toulouse Paul Sabatier, France, where the applicant will be enrolled in the doctoral program. The recruitment of the participants and the first stage of the sample processing will take place in the Medical Genetics Service (SGM) of the Maputo Centre Hospital (HCM), in Southern Mozambique, 85Km from the institution where the applicant is affiliated. The HCM is the largest referral hospital, receiving all complex cases from the country. This Hospital has several departments and services, including the SGM which was created in 2016 to serve individuals who came with infertility, congenital malformations, and genetic disorders, for diagnosis and counseling. This service has three kinds of consulting, namely,

pediatric genetics (for children under 15 years old); genetic counseling (for individuals 15 years old or older), and infertility and prenatal consulting (for women of reproductive ages). Under this service, there is a cytogenetic laboratory, that has routinely diagnosed structural and numeric chromosomal mutations, since June 2018, and the Director of the Service is a medical geneticist and one of the supervisors of the PhD. The CISM and HCM have a Memorandum of understanding for research, and has a strong international collaboration. CSM has been running a demographic and morbidity surveillance since 1996, and has experienced researchers who will help the applicant to implement the study following all ethics standard, and one of the Senior researchers will be her supervisor too. The deep characterization of individuals with complete sex reversal will be done in Centre of Integrative Biology of the University of Toulouse Paul Sabatier, in France, in a team that are expert in disorders related to X chromosome and gene dosage, the team leader of this team is also her supervisor. So, she will have an interdisciplinary environment with researchers from different backgrounds interacting to have the project running in the best way.