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Chromosomal alterations associated with infertility in Ecuador, found in 2021

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CHAPTER 1

INTRODUCTION

INTRODUCTION

A common definition of sub- and infertility is very important for the appropriate anagement of infertility.

Subfertility generally describes any form of reduced fertility with prolonged time of unwanted non-conception. Infertility may be used synonymously with sterility with only sporadically occurring spontaneous pregnancies.

The major factor affecting the individual spontaneous pregnancy prospect is the time of unwanted non-conception which determines the grading of subfertility. Most of the pregnancies occur in the first six cycles with intercourse in the fertile phase (80%). After that, serious subfertility must be assumed in every second couple (10%) although - after 12 unsuccessful cycles - untreated live birth rates among them will reach nearly 55% in the next 36 months.

Thereafter (48 months), approximately 5% of the couples are definitive infertile with a nearly zero chance of becoming spontaneously pregnant in the future. With age, cumulative probabilities of conception decline because heterogeneity in fecundity increases due to a higher proportion of infertile couples. In truly fertile couples cumulative probabilities of conception are probably age independent.

Under appropriate circumstances a basic infertility work-up after six unsuccessful cycles with fertility-focused intercourse will identify couples with significant infertility problems to avoid both infertility under- and over-treatment, regardless of age: Couples with a reasonably good prognosis (eg unexplained infertility) may be encouraged to wait because even with treatment they do not have a better chance of conceiving. The others may benefit from an early resort to assisted reproduction treatment[1].

Infertility is a prevalent condition affecting an estimated 70 million people globally. The World Health Organization estimates that 9% of couples worldwide struggle with fertility issues and that male factor contributes to 50% of the issues.

Male infertility has a variety of causes, ranging from genetic mutations to lifestyle choices to medical illnesses or medications.

Recent studies examining DNA fragmentation, capacitation, and advanced paternal age have shed light on previously unknown topics.

The role of conventional male reproductive surgeries aimed at improving or addressing male factor infertility, such as varicocelectomy and testicular sperm extraction, have recently been studied in an attempt to expand their narrow indications.

Despite advances in the understanding of male infertility, idiopathic sperm abnormalities still account for about 30% of male infertility.

With current and future efforts examining the molecular and genetic factors responsible for spermatogenesis and fertilization, we may be better able to understand etiologies of male factor infertility and thus improve outcomes for our patients[2].

Male infertility is a multifactorial pathological condition affecting approximately 7% of the male population. The genetic landscape of male infertility is highly complex as semen and testis histological phenotypes are extremely heterogeneous, and at least 2,000 genes are involved in spermatogenesis.

The highest frequency of known genetic factors contributing to male infertility (25%) is in azoospermia, but the number of identified genetic anomalies in other semen and aetiological categories is constantly growing.

Genetic screening is relevant for its diagnostic value, clinical decision making, and appropriate genetic counselling.

Anomalies in sex chromosomes have major roles in severe spermatogenic impairment. Autosome-linked gene mutations are mainly involved in central hypogonadism, monomorphic teratozoospermia or asthenozoospermia, congenital obstructive azoospermia, and familial cases of quantitative spermatogenic disturbances[3].

Secondary infertility is the most common form of female infertility around the globe, often due to reproductive tract infections. The three major factors influencing the spontaneous probability of conception are the time of unwanted non-conception, the age of the female partner and the disease-related infertility.

The chance of becoming spontaneously pregnant declines with the duration before conception. The fertility decline in female already starts around 25-30 years of age and the median age at last birth is 40-41 years in most studied populations experiencing natural fertility[4].

Infertility is estimated to affect as many as 186 million people worldwide. Although male infertility contributes to more than half of all cases of global childlessness, infertility remains a woman's social burden.

Unfortunately, areas of the world with the highest rates of infertility are often those with poor access to assisted reproductive techniques (ARTs). In such settings, women may be abandoned to their childless destinies. However, emerging data suggest that making ART accessible and affordable is an important gender intervention[5].

INFERTILITY CAUSES

Infertility is a potentially life-changing diagnosis for couples who are trying to conceive. A diagnosis of infertility and the associated management plan can lead to psychological stress, anxiety, and depression for one or both partners.

Once a couple is determined to be infertile, prompt referral to a specialist is indicated. Treatment varies according to the cause and may include medication, surgical intervention, or assisted reproductive technology[7].

Infertility is a complex pathophysiological condition. It may cause by specific or multiple physical and physiological factors, including abnormalities in homeostasis, hormonal imbalances and genetic alterations[6].

Fertility rates are affected by multiple factors: age, acute or chronic conditions, environmental toxins, occupational exposures, general lifestyle issues, infectious diseases, genetic conditions, and specific reproductive disorders that can affect either the man or woman attempting to conceive.

According to a World Health Organization (WHO) study of 8,500 infertile couples in developed countries, the following causes were found: female factor infertility, 37%; male factor infertility, 8%; and both female and male factor infertility, 35%.

The remaining couples were considered to have unexplained infertility or became pregnant during the study.

Another study reported combined factors, 40%; male factor infertility, 26% to 30%; ovulatory dysfunction, 21% to 25%; and tubal factors, 14% to 20%. These findings may be interpreted as incongruent but they actually are a reflection of the uncertain causal relationship between abnormalities on infertility testing and the actual cause of infertility[7].

Subfertility affects one in seven couples and is defined as the inability to conceive after 1 year of regular unprotected intercourse.

This article describes the initial clinical evaluation and investigation to guide diagnosis and management. The primary assessment of subfertility is to establish the presence of ovulation, normal uterine cavity and patent fallopian tubes in women, and normal semen parameters in men.

Ovulation is supported by a history of regular menstrual cycles (21-35 days) and confirmed by a serum progesterone >30 nmol/L during the luteal phase of the menstrual cycle. Common causes of anovulation include polycystic ovary syndrome (PCOS), hypothalamic amenorrhoea (HA) and premature ovarian insufficiency (POI).

Tubal patency is assessed by hysterosalpingography, hystero-contrast sonography, or more invasively by laparoscopy and dye test. The presence of clinical or biochemical hyperandrogenism, serum gonadotrophins (luteinising hormone/follicle stimulating

hormone)/oestradiol, pelvic ultrasound to assess ovarian morphology/antral follicle count, can help establish the cause of anovulation[8].

The spermatogenic process relies on the concerted actions of various hormones, local secretory factors and testis-specific genes. Defects at any of these levels can lead to accumulation of errors resulting in impaired spermatogenesis leading to male infertility.

Genetic defects commonly observed in infertile males include karyotypic abnormalities, gene copy number variations [CNVs], single gene mutations/polymorphisms and deletions on the long arm of the Y chromosome [Yq microdeletions].

These genetic defects impede the development of the male gonads or urogenital tract during development, cause arrest of germ cell production and/or maturation or produce non-functional spermatozoa. Amongst the various factors, karyotypic abnormalities and Yq microdeletions are the leading genetic causes of male infertility[9].

Association of the causes of infertility that govern regulation of gene expression with genetic factors and altered epigenetic mechanisms can help in better understanding of this complex and chronic physiological conditions.

Sequence analysis of human genome provide brief molecular genetics of complex disorders and elucidates physical structure of DNA, in addition to significant details of the major part of the non-coding human genome.

Discovery of the significant role of various molecular mechanisms intricate in the expression of coding and noncoding part of human genome at different time points of cell cycle in tissue specific manner during development and in normal or pathological condition may further help in understanding the complexity of diseases like infertility. This is because complete human genome is transcribed at some point of cell cycle.

Molecular mechanisms involved in regulation of genomic and chromosomal variations associated with infertility phenotype, with consecutive pregnancy losses or recurrent miscarriage and idiopathic cases are still to be reported[6].

CHROMOSOMAL CAUSES

Chromosomal abnormalities, mainly balanced rearrangements, are common in couples with reproductive disorders including recurrent abortions[13].

Chromosomal aneuploidy is the leading cause of pregnancy loss and developmental disabilities in humans. Aneuploidy is predominantly maternal in origin, but concerns have been raised regarding the safety of intracytoplasmic sperm injection as infertile men have significantly higher levels of sperm aneuploidy compared to their fertile counterparts.

Chromosomal aneuploidy refers to an alteration in chromosomal number from the normal diploid chromosomal complement in somatic cells or haploid complement in gametes. Chromosomal aneuploidy is the leading cause of pregnancy loss and developmental disabilities in humans.

Aneuploidy can be either numerical or partial in nature involving either gain or loss of an entire chromosomal; or structural involving a gain or loss of a segment of a chromosome.

Chromosomal aneuploidy is, for the most part, catastrophic for development and has been reported for all chromosomes in spontaneous abortions. In humans, aneuploidy is surprisingly common occurring in around 4% of clinically recognized pregnancies.

However, it is estimated that up to 60% of conceptions are aneuploid but are spontaneously aborted, often even before a pregnancy is clinically recognized. It is also evident that loss (monosomy) of a chromosome is much more detrimental than gain (trisomy) of a chromosome.

Monosomy X is the only non-mosaic monosomic condition that is compatible with life, and is largely attributed to X chromosome inactivation (Lyonization).

In contrast, there are a handful of chromosomes (13, 18, 21, X and Y) that in a trisomic state can survive to term. Nevertheless, it should be noted that while aneuploidy for these chromosomes is compatible with live birth, the vast majority will be spontaneously aborted early on during development[11].

Genetic causes account for 10-15% of severe male infertility, including chromosomal aberrations and single gene mutations.

Natural selection prevents the transmission of mutations causing infertility, while this protective mechanism may be overcome by assisted reproduction techniques. Consequently, the identification of genetic factors has become good practice for appropriate management of the infertile couple.

Furthermore, patients affected by some forms of genetic alterations produce a higher frequency of sperm with aneuploidies. Sperm aneuploidies are the direct result of the constitutional genetic abnormality or are caused by meiotic errors induced by the altered testicular environment that these men present.

Sex chromosome aneuploidies, such as 47,XXY (Klinefelter's syndrome), 47,XYY and 46,XX males are the most common chromosome anomalies occurring at birth and in the population of infertile males. Klinefelter's syndrome is a form of primary testicular failure with a high prevalence in infertile men, up to 5.0% in severe oligozoospermia and 10.0% in azoospermia[10].

Table 1 Chromosomal Causes

Chromosomal Causes
• Chromosomal translocations and infertility • Chromosomal inversions • Y chromosomal and infertility

Chromosomal translocations and infertility

Balanced chromosomal translocations involve breaks in two chromosomes and abnormal repair of the chromosomal fragments resulting in the transposition of genetic material from one chromosome to another without the loss of any genetic material. In the vast majority of cases, carriers of balanced translocations are themselves phenotypically normal, unless one of the translocation breakpoints interrupts an important gene or via position effects.

Should a gene be translocated into a region in which expression is either up- or downregulated, it can result in an increased risk of cancer, for example, the translocation could inactivate a tumor suppressor gene or activate an oncogene. Nevertheless, carriers of balanced chromosomal translocations, while normal phenotypically, may experience reduced fertility, spontaneous abortions or birth defects. Normal meiotic segregation of these translocations in the gametes can lead to duplication or deletion of the chromosomal regions involved in the translocation. Reduced fertility in translocation carriers may in part be the result of the requirement during meiosis for chromosomal translocations to form a quadrivalent or trivalent structure (reciprocal and Robertsonian translocations, respectively) to enable homologous chromosomes to pair[11].

The 46,XX sex differentiation disorder in males represents the most common condition in which testicular development occurs in the absence of a Y chromosome. This disorder occurs at a frequency of 1/25.000 newborns.

Phenotypically the adults are similar to patients with Klinefelter syndrome, with normal male external genitalia, microrchidia and sterility.

Molecular analyses have shown that in approximately two thirds of these cases there are Y-chromosome sequences present in the genome, including the SRY gene[12].

Chromosomal inversions

Chromosome inversions are defined as the rearrangement produced by two break-points within the same chromosome, with the subsequent inversion and reinsertion of this fragment.

Chromosome inversions may be:

- Pericentric: if the inverted fragment includes the chromosome's centromere;
- Paracentric: if the inverted fragment does not include the chromosome's centromere.

The frequency in the general population of chromosome inversions is 1–5 in 10,000 for paracentric inversions and 1–7 in 10,000 for pericentric inversions.

Some inversions are chromosomal heteromorphisms include:

- inv(1)(p11q12);
- inv(2)(p11.2q13);
- inv(3)(p11q11);
- inv(3)(p11q12);
- inv(3)(p13q12);
- inv(5)(p13q13);
- inv(9)(p11q12);
- inv(9)(p11q13);
- inv(10)(p11.2q21.2);
- inv(16)(p11q12);
- inv(16)(p11q13);
- inv(Y)(p11q11).

In most cases, being a carrier of a chromosome inversion has no direct health implications. However, carriers of a balanced Robertsonian translocation have some reproduction implications, due to imbalanced gametes[27]:

- Reproduction issues:
- Sterility/infertility;
- Recurrent pregnancy losses (in 3%–9% of couples with recurrent pregnancy losses);
- Offspring with an unbalanced chromosome rearrangement:
- In unbalanced pericentric inversions: formation of recombinants with deletion and duplication of the inverted segment;
- In unbalanced paracentric inversions: inverted duplications of the segments.

As with chromosomal translocations, inversions can cause infertility, spontaneous abortions and birth defects. During meiosis, chromosomes are forced to form specialized structures (inversion loops) to enable homologous chromosomes to pair.

The formation of these loops can impact fertility due to the mechanics and time constraints associated with the formation of the inversion loop. Single-sperm PCR has also demonstrated that recombination within these loops is reduced which can lead to a breakdown in meiosis and hence, may lead to apoptosis of the cell leading to a reduced sperm count[11].

Y chromosomal and infertility

Cytogenetically, the human Y is an acrocentric chromosome composed of two pseudoautosomal regions (PARs), a short arm (Yp) and the long arm (Yq) that are separated by a centromere. While the PARs and the short arm are euchromatic, a large portion of the long arm is heterochromatic with the exception of the proximal portion juxtaposed to the centromere which is euchromatic in nature.

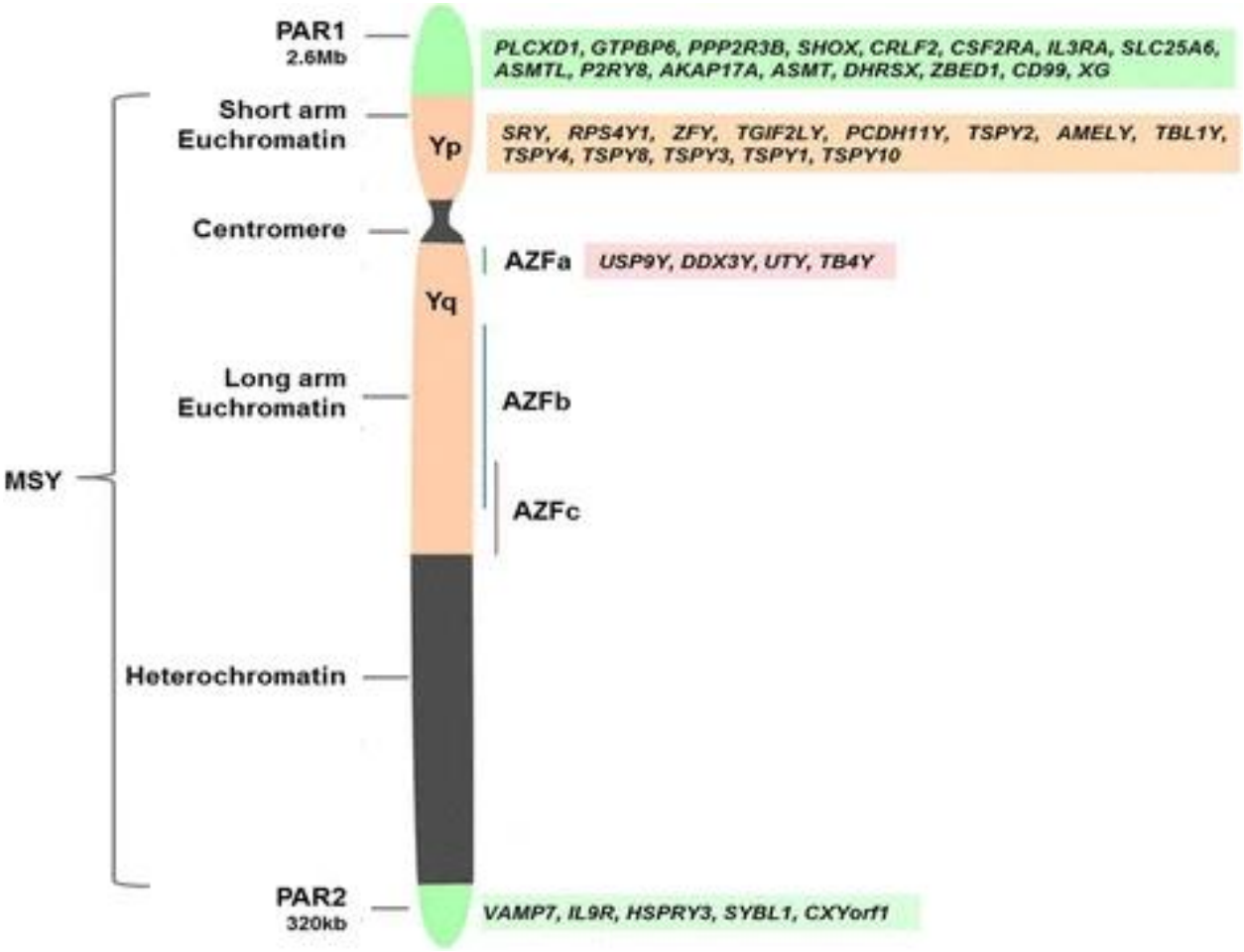


Figure 1. Structure of the human Y chromosome. By: https://doi.org/10.1016/j.reprotox.2006.04.016

Microdeletion

Y chromosome microdeletions represent the etiological factor of 10.0-15.0% of idiopathic azoospermia and severe oligozoospermia. The frequency of AZF deletions in infertile men ranges from 5.0 to 20.0% in worldwide surveys[10].

Microdeletions of the Y chromosome are also important causes of male infertility, and there are studies showing that deletions of azoospermia factor (AZF) loci on the long arm of the Y chromosome are associated with either reduced sperm count (oligozoospermia) or complete absence of spermatozoa (azoospermia).

Microdeletions in these regions are a cause of severe testiculopathy and are present in 2% of infertile men. In selected males affected by severe oligozoospermia or non-obstructive azoospermia, the frequency of Y microdeletions can be increased to as much as 15-20%[12].

Euchromatic region of Y

The euchromatic region of the Y lies distal to the PAR1 and consists of the short arm para-centromeric region, the centromere and the long arm para-centromeric region. This region contains sequences that are subdivided into three discrete classes: X-transposed, X-degenerate and ampliconic. The X-transposed sequences are so named, because of a massive X to Y transposition that occurred about 3–4 million years ago.

Most of these sequences are composed of repeat elements such as Alu, retroviral and Long interspersed nuclear elements. Some of the genes belonging to this region have ubiquitous tissue expression; the ampliconic sequences contain genes and transcription units that are expressed solely in the testes.

The protein products of the MSY genes, contribute to gonad formation, regulation of spermatogenesis, brain, heart, and kidney development suggesting its critical functions in tissue development and its adult functions. Approximately 70 genes have been identified on the Y chromosome and described below are some of these genes.

Genes on the short arm of the Y chromosome [Yp]

In the year 1959 two scientific reports on the Klinefelter syndrome and on the Turner syndrome, described for the very first time that the human Y chromosome contained at least one sex-determining gene that was responsible for the maleness of the embryo. A large numbers of sex reversed patients were subsequently identified to have deletions in portions of the Yp (XY sex reversal) or had additional portions of the Yp (XX males).

These patients immensely contributed to the discovery of the SRY gene which was responsible for testis determination during embryogenesis. In 1990, the gene responsible for testicular determination, SRY (Sex-determining Region on the Y chromosome), was identified and was found to be located on the short arm of the Y chromosome close to the pseudoautosomal boundary.

This gene is thought to have been evolved by a mutation in the SOX3 gene.

The human SRY is a single exon that encodes a protein of 204 amino acids which contains a conserved DNA-binding domain. SRY is essential for initiating testis development and differentiation of the bi-potential gonad into Sertoli cells, which then support differentiation and development of the male germline. Hence this gene has been proposed to be the master gene regulating the cascade of testis determination.

Mutations in the SRY gene are identified in approximately 15% 46, XY females (Swyer syndrome); translocation of the SRY gene to the X chromosome is reported in a subset of 46, XX males.

Beyond its expression in developing testis, SRY is reportedly expressed in adult testis and even ejaculated spermatozoa the functional significance of which is yet unclear. In addition SRY is also expressed in other somatic tissues such as adipose, oesophagus, thymus, adrenal glands, brain kidneys and also in some cancer cell lines suggesting its functions beyond sex determination[9].

Human gene mutations causing infertility

The identification of gene mutations causing infertility in humans remains noticeably deficient at present. Although most males and females with infertility display normal pubertal development, nearly all of the gene mutations in humans have been characterised in people with deficient puberty and subsequent infertility.

Gene mutations are arbitrarily categorised into four different compartments (I, hypothalamic; II, pituitary; III, gonadal; and IV, outflow tract). Diagnoses of infertility include hypogonadotrophic hypogonadism, hypergonadotrophic hypogonadism (III), and obstructive disorders. Most gene mutations identified to date affect gonadal function, but it is also apparent that a large number of important genes in normal fertility have yet to be realised[19].

INFERTILITY DATA IN THE WORLD

Infertility is estimated to affect as many as 186 million people worldwide. Although male infertility contributes to more than half of all cases of global childlessness, infertility remains a woman's social burden.

Unfortunately, areas of the world with the highest rates of infertility are often those with poor access to assisted reproductive techniques (ARTs). In such settings, women may be abandoned to their childless destinies[14].

Infertility affects an estimated 15% of couples globally, amounting to 48.5 million couples. Males are found to be solely responsible for 20-30% of infertility cases and contribute to 50% of cases overall. However, this number does not accurately represent all regions of the world. Indeed, on a global level, there is a lack of accurate statistics on rates of male infertility[15].

Infertility in resource-poor settings is an overlooked global health problem. Although scarce health care resources must be deployed thoughtfully, prioritization of resources may be different for recipient and donor countries, the latter of whom focus on maternal health care, prevention, and family planning.

For women and couples with involuntary childlessness, the negative psychosocial, sociocultural, and economic consequences in low-income countries are severe, possibly more so than in most Western societies.

Despite the local importance of infertility, few resources are committed to help advance infertility care in regions like sub-Saharan Africa. The worldwide prevalence of infertility is remarkably similar across low-, middle-, and high-income countries.

The World Health Organization (WHO) recognizes infertility as a global health problem and established universal access to reproductive health care as one of the United Nation's Millennium Developmental Goals for 2015. Currently, access to infertility care is varied and is usually only attainable by the very wealthy in low-income countries[17].

Infertility is a global epidemic that is estimated to affect one in four couples in developing countries. Although it is difficult to estimate due to inconsistent use of definitions and a lack of standardization in demographic surveys, the prevalence of infertility has not significantly changed in the past two decades.

As fertility care continues to expand worldwide due to Cross-Border Reproductive Care (CBRC) and medical tourism, it is important to understand how this impacts local communities. It is also important to determine if this expansion of services translates to more access for those suffering from infertility in the developing world.

Fertility care is steadily expanding in the Caribbean and other island nations. At this time, only a few fertility clinics in the Caribbean offer the full spectrum of infertility care, including diagnostic procedures and treatment options such as oral agents, intrauterine insemination (IUI), in vitro fertilization (IVF), and intracytoplasmic sperm injection (ICSI).

Given the inherent diversity of the Caribbean population, this region serves as an ideal model to assess how the phenomenon of CBRC intersects with race and ethnicity, both of which are known to affect ART outcomes in the United States.

Understanding the impact race has on infertility care has also become increasingly important in the United States (US). As the nation's multiracial and minority sub-populations continue to grow, a deeper dialog on health equity has developed. Race is known to affect timely access to infertility care, diagnosis, and treatment cycle characteristics.

US registry-based studies demonstrate that Black, Hispanic, and Asian women have lower clinical pregnancy rates and live birth rates after IVF, compared with White women. Particularly, the Society for Assisted Reproductive Technologies (SART) database studies have shown that African-American women have a significantly lower live birth rate (LBR) per fresh IVF/ICSI cycle than White American women.

One possible explanation for the aforementioned racial disparity within the US is the "Weathering Theory". This phenomenon suggests that racism in the US leads to chronic exposure to a stress milieu of inflammatory hormones and markers, which contributes to poorer health outcomes. This theory can be further appreciated in a discussion of race verses ethnicity.

A person's race (i.e., white, black, brown) can render a significantly different association to socio-economical experiences when further categorized based on ethnicity (i.e., Afro-Caribbean, African American, Afro European). It is likely that those of African ancestry living in the African diaspora have generalized experiences of racism and other social determinants of health measurable on a wide spectrum.

When it comes to infertility and the Weathering Theory, IVF outcomes of Afro-Caribbean women should be comparable to their White counterparts within and outside of their country. Studies have shown that Caribbean societies find themselves devoid of the type or degree of racial conflict found in the US.

Furthermore, a study specifically comparing the construct and impact of racism experienced by African Americans and Afro-Caribbeans provides additional support to the Weathering Theory.

In an observational study which utilized the United Kingdom (UK) national ART database, White British women, Black British women and Black-Caribbean women had comparable live birth rates per fresh IVF/ICSI cycle.

Based on these studies, we suspect that there is less weathering in the Caribbean than in the US. Consequently, we hypothesize that there will be less disparate IVF outcomes for Afro-Caribbean women, leading to superior outcomes for Afro-Caribbean compared to African-American women.

Although the US and the UK have national databases to track ART outcomes, this has not yet been established in the Caribbean.

Therefore, in this study, we surveyed general obstetrician gynecologists (Ob/Gyns) who provide infertility care, as well as Reproductive Endocrinology and Infertility (REI) specialists in the Caribbean and Bermuda.

We decided to incorporate practices that do not perform IVF in order to generalize our results to the Afro-Caribbean population as a whole. Our objective was to obtain an approximation of the ART outcomes of Afro-Caribbean women living in the Caribbean.

Also sought to characterize the experiences of women receiving infertility treatment in the Caribbean and to gather information on the current state of infertility care in the Caribbean from the provider perspective[18].

In 1992, ICSI was introduced in Latin America; in the last two decades, its use has increased substantially, even among patients without male factor infertility.

Despite its increasing use in non-male factor infertility cases, an advantage of ICSI over IVF has not yet been demonstrated in these cases. In a RCT that included 415 couples with non-male factor infertility, conventional IVF was associated with better fertilization rates and implantation rates than ICSI, although live birth rates were comparable. In a retrospective

analysis of 745 women over 40 years undergoing ART, no advantage in terms of delivery rate was demonstrated. Furthermore, a retrospective analysis of 350 women with a low response to stimulation did not find any improvement in fertilization or delivery rates.

Nonetheless, many centers in Latin America perform only ICSI. This preference might reflect a desire of both physicians and infertile couples to optimize the outcome of ART cycles since treatments are often paid out-of-pocket by patients.

ICSI is also pursued with the hope that it might diminish the risk of total fertilization failure and increase the number of embryos available[16].

DIAGNOSTICS OF INFERTILITY

Infertility is defined as the inability to become pregnant after 12 months of regular, unprotected intercourse. In a survey from 2006 to 2010, more than 1.5 million women, or 6% of the married population 15 to 44 years of age, reported infertility, and 6.7 million women reported impaired ability to get pregnant or carry a baby to term. Among couples 15 to 44 years of age, nearly 7 million have used infertility services at some point.

This encompasses couples with infertility and impaired ability to get pregnant, but it does not capture those who are not married, so actual numbers may be underestimated. These numbers are comparable to those of other industrialized nations. Infertility may arise from male factors, female factors, or a combination of these[20].

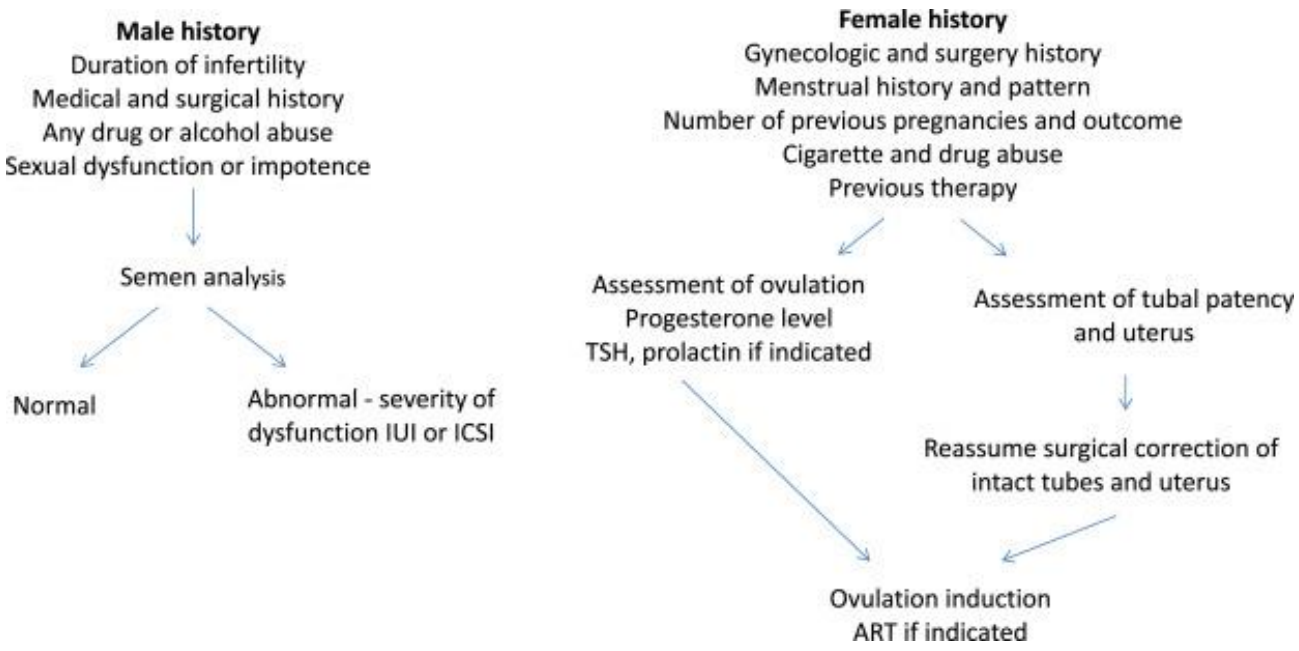


Figure 2 Infertility evaluation

SORT: KEY RECOMMENDATIONS FOR PRACTICE

Clinical recommendation	*Evidence rating*	*References*
Confirmation of ovulation should be obtained with a serum progesterone level on day 21 of a 28-day cycle or one week before presumed onset of menses.	C	6, 8
Hysterosalpingography should be offered to screen for uterine and tubal abnormalities in women with infertility who have no history of pelvic infections, endometriosis, or ectopic pregnancy.	C	8, 26, 27
Women with unexplained infertility should not be offered ovulation induction or intrauterine insemination because these have not been shown to increase pregnancy rates.	C	8, 45
Women with a body mass index greater than 30 kg per m^2 should be counseled to lose weight because this may restore ovulation.	B	46

A = consistent, good-quality patient-oriented evidence; B = inconsistent or limited-quality patient-oriented evidence; C = consensus, disease-oriented evidence, usual practice, expert opinion, or case series. For information about the SORT evidence rating system, go to http://www.aafp.org/afpsort.

Figure 3 KEY RECOMMENDATIONS FOR PRACTICE[20]

Table 2 Etiology of infertility[20]

Factors	Percentage
Combined factors	40
Male factors	26 to 30
Ovulatory disfunction	21 to 25
Tubal factors	14 to 20
Other (a.g., cervical factors, peritoneal factors, uterine abnornmalities)	10 to 13
Unexplained	25 to 28

Because 85% of couples conceive spontaneously within 12 months if having intercourse regularly, it is important to identify those who will benefit from infertility evaluation. Generally, evaluation should be offered to couples who have not conceived after one year of unprotected vaginal intercourse.

Counseling about options should be offered to couples who are not physically able to conceive (i.e., same-sex couples or persons lacking reproductive organs). Women older than 35 years or couples with known risk factors for infertility may warrant evaluation at six months.

It is important for primary care physicians to be familiar with the workup and prognosis for infertile couples.

A British study found that patients valued primary care physicians who were well informed about infertility and the treatment process.

Because anxiety over infertility may cause increased stress and decreased libido, further compounding the problem, formal counseling is encouraged for couples experiencing infertility[20].

Evaluation of men

Causes of male infertility include infection, injury, toxin exposures, anatomic variances, chromosomal abnormalities, systemic diseases, and sperm antibodies.

Additional risk factors may include smoking, alcohol use, obesity, and older age; however, the data are hampered by a lack of pregnancy-related outcomes.

One retrospective case-control study of 650 men with infertility and 698 control participants questioned the role of environmental risk; no association could be determined after assessing for multiple factors including shift work, stress, and pesticides.

Evaluation of male infertility starts with a history and physical examination focusing on previous fertility, pelvic or inguinal surgeries, systemic diseases, and exposures.

The laboratory evaluation begins with a semen analysis. Instructions for collecting the sample should include abstinence from ejaculation for 48 to 72 hours.

Because sperm generation time is just over two months, it is recommended to wait three months before repeat sampling. A normal sample according to the 2010 World Health Organization (WHO) guidelines.

If the semen analysis result is abnormal, further evaluation is indicated. If oligospermia or azoospermia is noted, hypogonadism should be suspected. Obtaining morning levels of total testosterone (normal range = 240 to 950 ng per dL [8.3 to 33.0 nmol per L]) and follicle-stimulating hormone (FSH; normal range = 1.5 to 12.4 mIU per mL [1.5 to 12.4 IU per L]) can help differentiate between primary and secondary disorders.

A decreased testosterone level with an increased FSH level points to primary hypogonadism. A low testosterone level with a low FSH level signals a secondary cause. Some causes, such as hyperprolactinemia, are reversible with proper treatment.

Other testing may be needed based on circumstances, including testicular biopsy, genetic testing, and imaging.

Postcoital testing and antisperm antibody testing are no longer considered useful in this evaluation[20].

Characteristic	*Normal reference*
Morphologically normal	4%
Motility (progressive)	32%
Motility (total)	40%
Sperm count	39 million per ejaculate; 15 million per mL
Vitality	58%
Volume	At least 1.5 mL

NOTE: *oligospermia = sperm count < 15 million per mL; asthenozoospermia = < 40% of the sperm are motile; teratozoospermia = normal morphology < 4%. If an individual has all three low sperm conditions, it is known as OAT syndrome, which is typically associated with an increased likelihood of genetic etiology of the infertility. Total motility differs from progressive motility only in the notation of forward movement.*

Information from reference 18.

Figure 4. 2010 WORLD HEALTH ORGANIZATION (WHO) GUIDELINES[20]

Evaluation of women

The etiology of female infertility can be broken down into ovulation disorders, uterine abnormalities, tubal obstruction, and peritoneal factors.

Cervical factors are also thought to play a minor role, although they are rarely the sole cause. Evaluation of cervical mucus is unreliable; therefore, investigation is not helpful with the management of infertility.

The initial history should cover menstrual history, timing and frequency of intercourse, previous use of contraception, previous pregnancies and outcomes, pelvic infections, medication use, occupational exposures, substance abuse, alcohol intake, tobacco use, and previous surgery on reproductive organs.

A review of systems and physical examination of the endocrine and gynecologic systems should be performed.

Other considerations include preconception screening and vaccination for preventable diseases such as rubella and varicella, sexually transmitted infections, and cervical cancer, based on appropriate guidelines and risk.

WHO categorizes ovulatory disorders into three groups: group I is caused by hypothalamic pituitary failure (10%), group II results from dysfunction of hypothalamic-pituitary-ovarian axis (85%), and group III is caused by ovarian failure (5%). Women in group I typically present with amenorrhea and low gonadotropin levels, most commonly from low body weight or excessive exercise.

Women in group II include those with polycystic ovary syndrome and hyperprolactinemia. Women in group III can conceive only with oocyte donation and in vitro fertilization[20].

Condition	*History and physical examination*	*Laboratory and radiologic testing*
Female		
Endometriosis or pelvic adhesions	History of abdominal or pelvic surgery; history consistent with endometriosis	Rarely helpful
Hypothalamic amenorrhea	Amenorrhea or oligomenorrhea; low body mass index	Low to normal FSH level; low estradiol level
Ovarian failure/insufficiency	Amenorrhea or oligomenorrhea; menopausal symptoms; family history of early menopause; single ovary; chemotherapy or radiation therapy; previous ovarian surgery; history of autoimmune disease	Elevated FSH level; low estradiol level
Ovulatory disorder	Irregular menses; hirsutism; obesity (polycystic ovary syndrome); galactorrhea (hyperprolactinemia); fatigue; hair loss (hypothyroidism)	Progesterone level < 5 ng per mL (15.9 nmol per L); elevated prolactin level; low TSH level
Tubal blockage	History of pelvic infections or endometriosis	Abnormal hysterosalpingography result
Uterine abnormalities	Dyspareunia; dysmenorrhea; history of anatomic developmental abnormalities; family history of uterine fibroids; abnormal palpation and inspection	Abnormal hysterosalpingography or ultrasonography result
Male		
Genetic etiology: Y deletions XXY (Klinefelter syndrome)	Y deletions: small testes Klinefelter phenotype: small testes, tall, gynecomastia, learning disabilities	Both syndromes result in normal semen volume but low sperm count Y deletions may present as normal hormone levels or have an elevated FSH level Klinefelter syndrome typically results in low testosterone level and an elevated FSH level
Other genetics: *CFTR* gene (cystic fibrosis) 5T allele (cystic fibrosis)	Absence of the vas deferens	Low volume semen analysis
Obstruction of the vas deferens or epididymis Ejaculatory dysfunction	History of infection, trauma, or vasectomy; normal testicular examination	Low volume semen analysis; transrectal ultrasonography can identify obstruction
Systemic disease (not all-inclusive): Hemochromatosis Kallmann syndrome Pituitary tumor Sarcoidosis	—	Low FSH level; low testosterone level; check prolactin level and, if elevated, perform imaging for pituitary tumor
Unclear etiology	Normal testicular examination	Normal FSH level; normal semen volume; low sperm count

FSH = follicle-stimulating hormone; TSH = thyroid-stimulating hormone.

Information from references 6 through 8, 10, 19, and 20.

Figure 5. EVALUATION AND ETIOLOGY OF INFERTILITY[20]

Chromosomal studies

The X chromosome is a key player in germ cell development, as has been highlighted for males in previous studies revealing that the mammalian X chromosome is enriched in genes expressed in early spermatogenesis.

Male infertility is most commonly caused by spermatogenic defects to which X chromosome dosage is closely linked; for example, any supernumerary X chromosome as in Klinefelter syndrome will lead to male infertility.

Furthermore, because males normally only have a single X chromosome and because X-linked genetic anomalies are generally only present in a single copy in males, any loss-of-function mutations in single-copy X-chromosomal genes cannot be compensated by a normal allele. These features make X-linked genes particularly attractive for studying male spermatogenic failure. However, to date, only very few genetic causes have been identified as being definitively responsible for male infertility in humans.

Although genetic studies of germ cell-enriched X-chromosomal genes in mice suggest a role of certain human orthologs in infertile men, these genes in mice and humans have striking evolutionary differences. Furthermore, the complexity and highly repetitive structure of the X chromosome hinder the mutational analysis of X-linked genes in humans. Therefore, we conclude that additional methodological approaches are urgently warranted to advance our understanding of the genetics of X-linked male infertility[21].

Cytogenetic in infertility

Infertility can be caused by defects in the development of the urogenital system or its function by genetic defects of the endocrine system, including the hypothalamic-pituitarygonadal axis, or by defects in gametogenesis, sexual function, fertilization or early embryonic development.

Secondary or acquired infertility, such as after-tubal diseases, vasectomy or exposure to gonadotoxins, may occur. Cytogenetic abnormalities (both somatic and meiotic) are a major cause of male infertility. Cytogenetic studies have been reported to determine the contribution of chromosomal abnormalities in parents with reproductive failure from various countries[28.]

A standardized nomenclature is critical for the accurate and consistent description of genomic changes as identified by karyotyping, fluorescence in situ hybridization and microarray.

The International System for Human Cytogenomic Nomenclature (ISCN) is the central reference for the description of karyotyping, FISH, and microarray results, and provides rules for describing cytogenetic and molecular cytogenetic findings in laboratory reports. These laboratory reports are documents to the referring clinician, and should be clear, accurate and contain all information relevant for good interpretation of the cytogenetic findings[24].

Figure 6 Female Metaphase. Laboratorio de Citogenética del Centro Especializado en Genética Médica

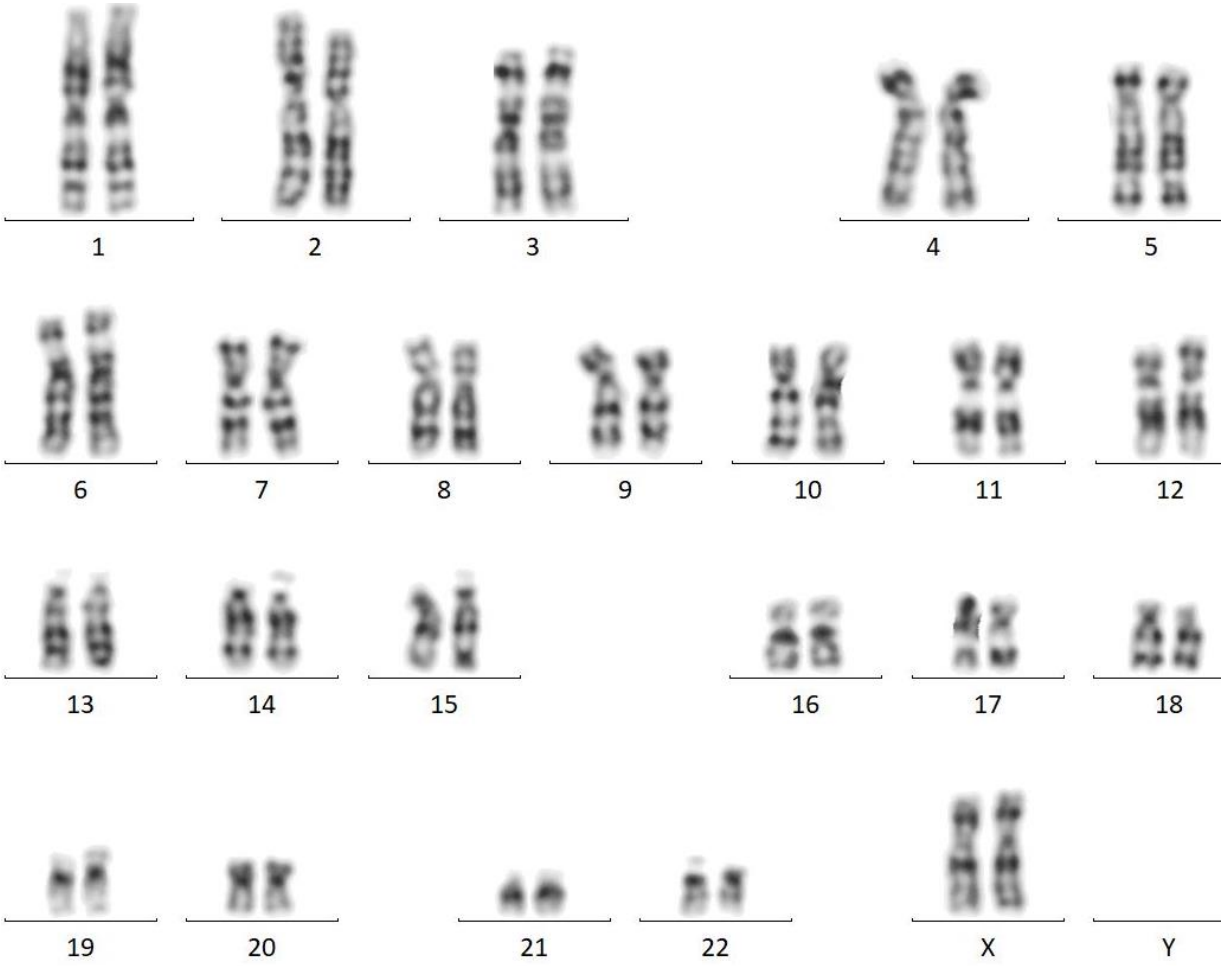


Figure 7 Normal Female Karyotype. Laboratorio de Citogenética del Centro Especializado en Genética Médica

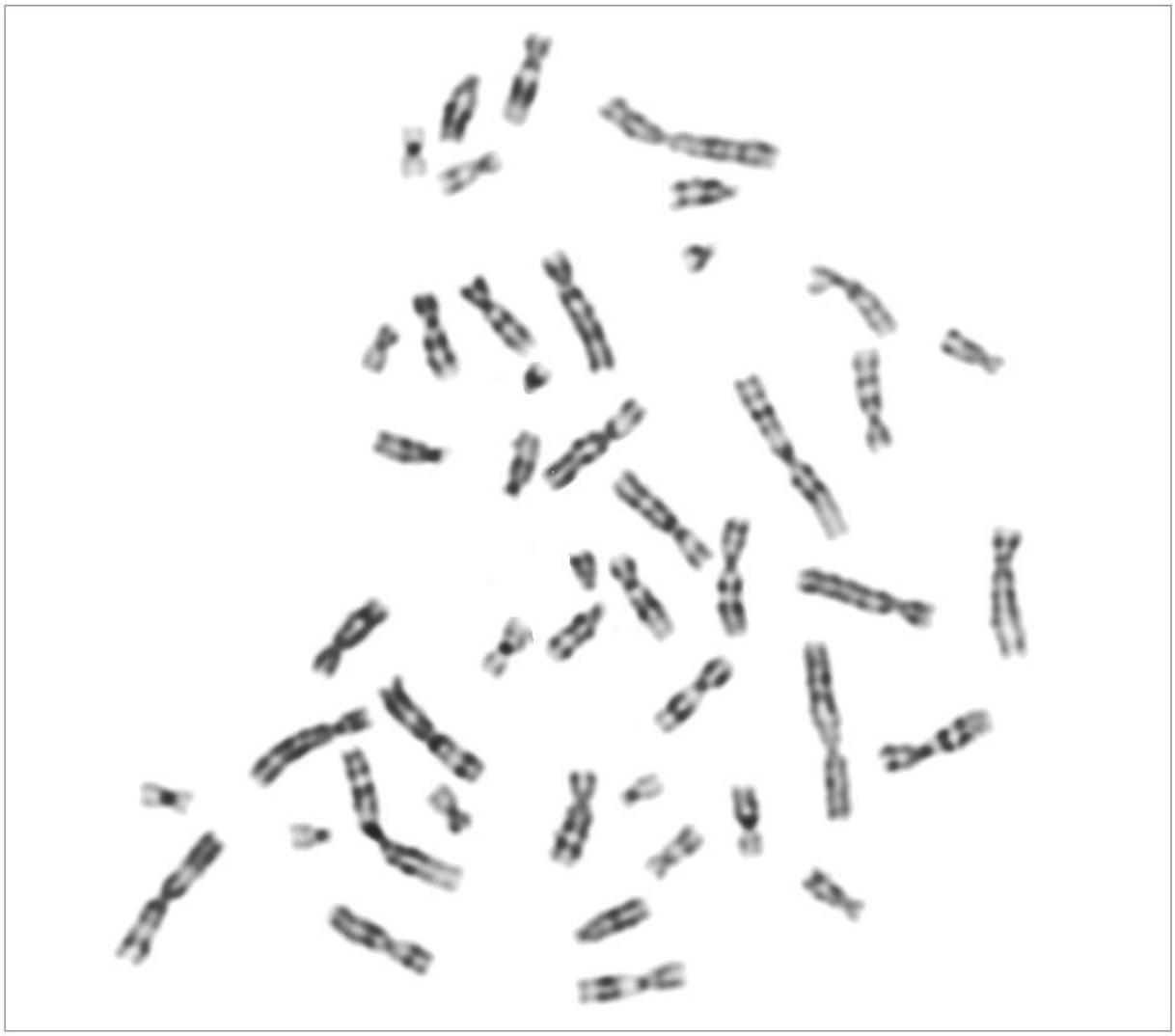


Figure 8 Male Metaphase. Laboratorio de Citogenética del Centro Especializado en Genética Médica

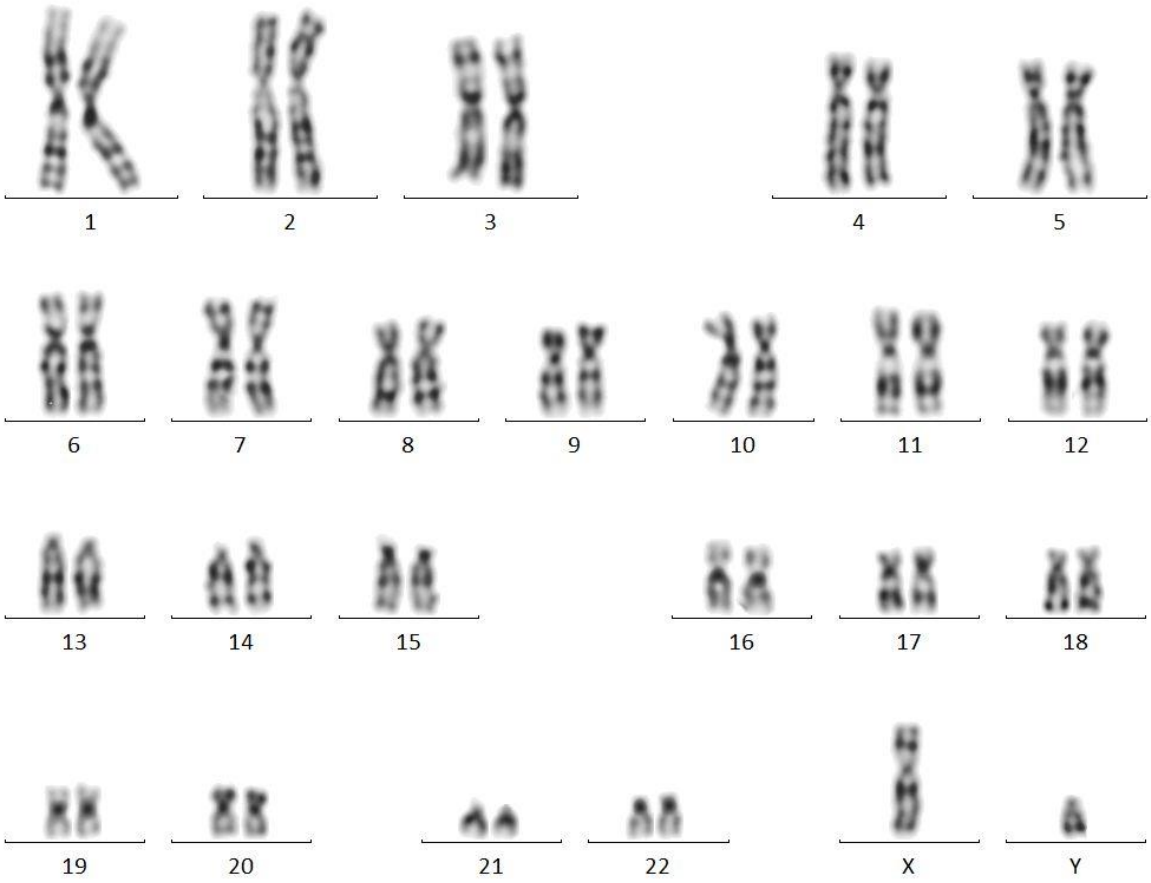


Figure 9 Normal Male Karyotype. Laboratorio de Citogenética del Centro Especializado en Genética Médica

Treatment of infertility

As it has been stated above, the reproductive process in humans is very complicated, therefore a hypothesis on the causes of infertility is better than a diagnosis of infertility.

In the clinical practice, the most common medical issues are: normal or disturbed ovarian function (anovulation, oligoovulation, luteal phase defects), normal or abnormal anatomy of the oviducts and uterus, normal or disturbed spermatogenesis, endometriosis, but also a situation when all parameters are normal (un-explained infertility).

When the disturbed function of the ovaries is hypothesised then pharmacotherapy should be advised. There is a great number of various proposals of the ovarian stimulation.

It should be remembered that ovarian stimulation for achieving pregnancy always requires 'healthy oviducts' and normal parameters of the semen. Therefore, conducting such treatment without described requirements can be considered as 'stealing' a woman's reproductive time.

The pathology of uterus and oviducts is diagnosed by ultrasonography, HSG and most precisely by laparoscopy which should be not only diagnostic but also operative. If the anatomical correction is possible, it should be performed. However, in cases of oviducts' pathology the method of choice should be in vitro fertilization (IVF).

In cases of disturbed spermatogenesis there is no proven pharmacological and surgical treatment. Varicocele as the cause of male infertility is one of the most controversial issues in the field of andrology.

When oligoasthenozoospermia of the first degree is diagnosed the intrauterine insemination (IUI) can be applied (only in young women).

The most rationale procedure is IVF together with intracytoplasmic sperm injection (ICSI). As the ART increases the risk of genetic disorders, some genetic counselling should be desired and advised.

Endometriosis remains an enigmatic disease. The diagnosis is most frequently obtained during laparoscopy. The minimal and mild endometriosis could be treated with IUI, but the most sensible approach would be waiting for two years and later on applying the in vitro fertilization-embryo transfer (IVF/ET).

Unexplained infertility indicates the inability to recognize the true cause of infertility. From the practical point of view the best strategy would be waiting for two years (providing the woman is under 35 years old) and later on IVF/ET is recommended[22].

Infertility counseling, whether provided by a psychiatrist or another health care professional, involves the treatment and care of patients, not simply when they are

undergoing fertility treatment but also with their long-term emotional well-being, and that of their children and the reproductive helpers who may assist them in achieving biologic or reproductive parenthood.

They can educate patients about the side effects of infertility treatment medications and the impact of hormone shifts on psychologic well-being. They are also helpful with differential diagnoses among grief, depressions, and stress; in assessing psychologic preparedness; and in determining the acceptability and suitability of gamete donation, a gestational carrier, or surrogacy as a family-building alternative for individuals, couples, and reproductive collaborators[23].

CHAPTER 2

Materials and methods

MATERIALS AND METHODS

This investigation is in base to the results development around one year in the Cytogenetic Laboratory of Center Specialized in Medical Genetics in Ecuador.

All the results obtained are karyotypes of people who come for infertility or recurrent abortions.

Currently, cytogeneticists are developing molecular approaches for deciphering the structure, function and evolution of chromosomes. Conventional cytogenetics using regular banded chromosomal analysis remains a simple and popular technique to get an overview of the human genome.

Cytogenetics involves the examination of chromosomes to identify structural abnormalities. Chromosomes of a dividing human cell can be analyzed clearly in white blood cells, specifically T lymphocytes, which are easily collected from blood.

Cells from other tissues such as bone marrow, amniotic fluid, and other tissues can also be cultured for cytogenetic analysis. Following several days of cell culture, chromosomes are fixed, spread on microscope slides, and stained.

The staining methods for routine analysis allow each of the chromosomes to be individually identified. The distinct bands of each chromosome revealed by staining allow for analysis of the chromosomal structure.

Routine banded karyotype analysis can now be combined with M-FISH and various other molecular techniques, leading to more precise detection of various syndromes in children. The combination of CGH with multicolour FISH was seen from the beginning to be a powerful combination for 7 characterising complex karyotypes.

More recently, microarray-based formats using large insert genomic clones, cDNAs or oligonucleotides have replaced metaphase chromosomes as DNA targets, providing higher resolution and the ability to directly map the copy number changes to the genome

sequence. In other words, chromosomal abnormalities exist as nature's guide to the molecular basis of many unexplained human disorders.

Thus, techniques of cytogenetics are bound to continue to be indispensable tools for diagnosing genetic disorders and indicating possible treatment and management[25].

Process of Karyotyping

Every human pregnancy that goes to full term in North America and other modern industrialized nations has a small risk (about 2-3%) of serious birth defects. This is the case even when the mother is young and healthy. However, some pregnancies are at a higher risk due to a variety of factors.

Fortunately, it is now possible to connect some of the inherited genetic defects to specific chromosome irregularities. This can be done by examining small tissue samples from adults, children, or even unborn babies.

The samples are cultured to induce mitosis so that the chromosomes become visible. In this state, the chromosomes can be photographed. The images are then converted into a karyotype of the individual from whom the sample was taken. This involves the precise measurements of chromatid length ratios and other morphological features so that they can be placed into homologous pairs.

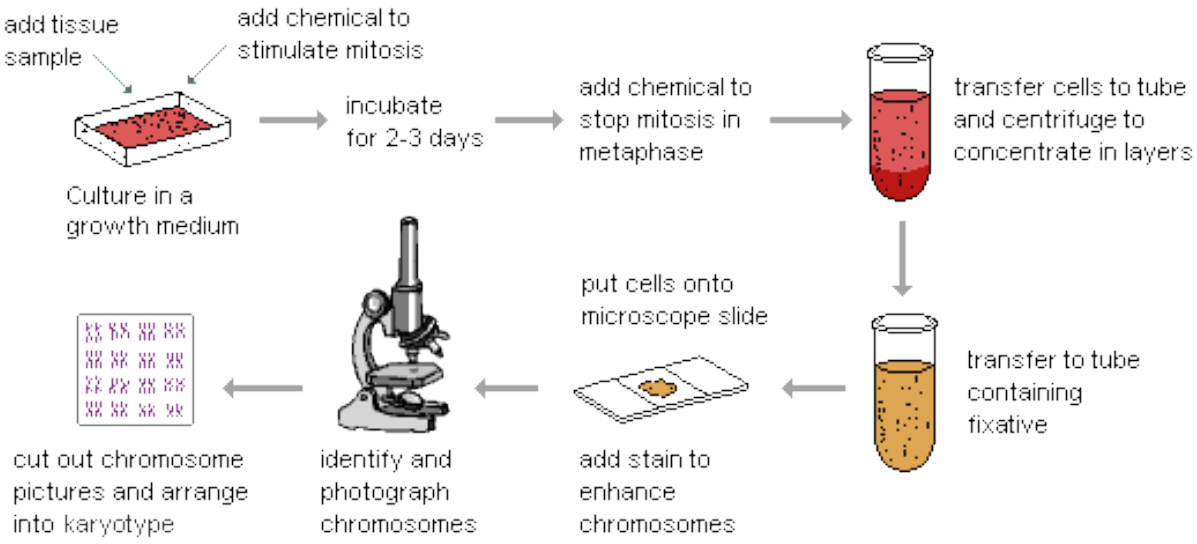


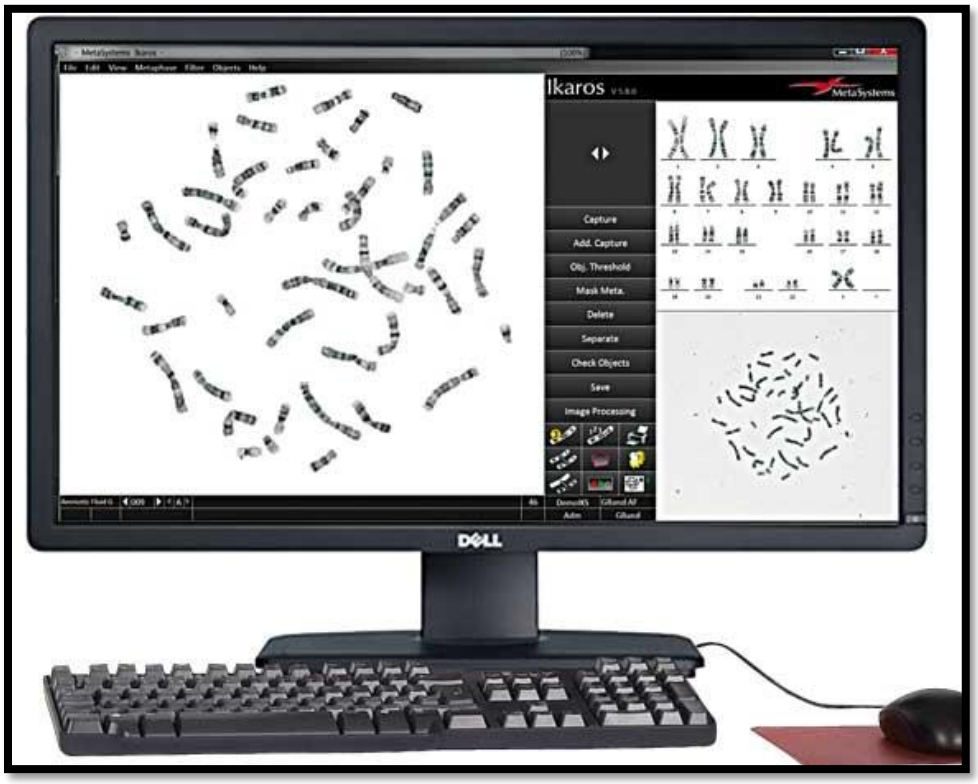
Figure 10 Process of Karyotyping. https://www2.palomar.edu/anthro/abnormal/abnormal_1.htm

Software used for karyotype analysis

In the laboratory, the MetaSystems software "IKAROS" was used, software that allows the analysis of karyotypes and the study of cases sent to the laboratory from geneticists nationwide.

The software supports many different banding techniques and resolutions. Numerous pre-defined karyogram and idiogram forms for human are available. There is the possibility to create individual templates with the integrated karyogram form editor makes Ikaros applicable to many different fields of research.

The possibility to run chromosome comparisons by chromosome classes or by karyograms facilitates the on-screen analysis of chromosome. Aberrant chromosomes can be illustrated.



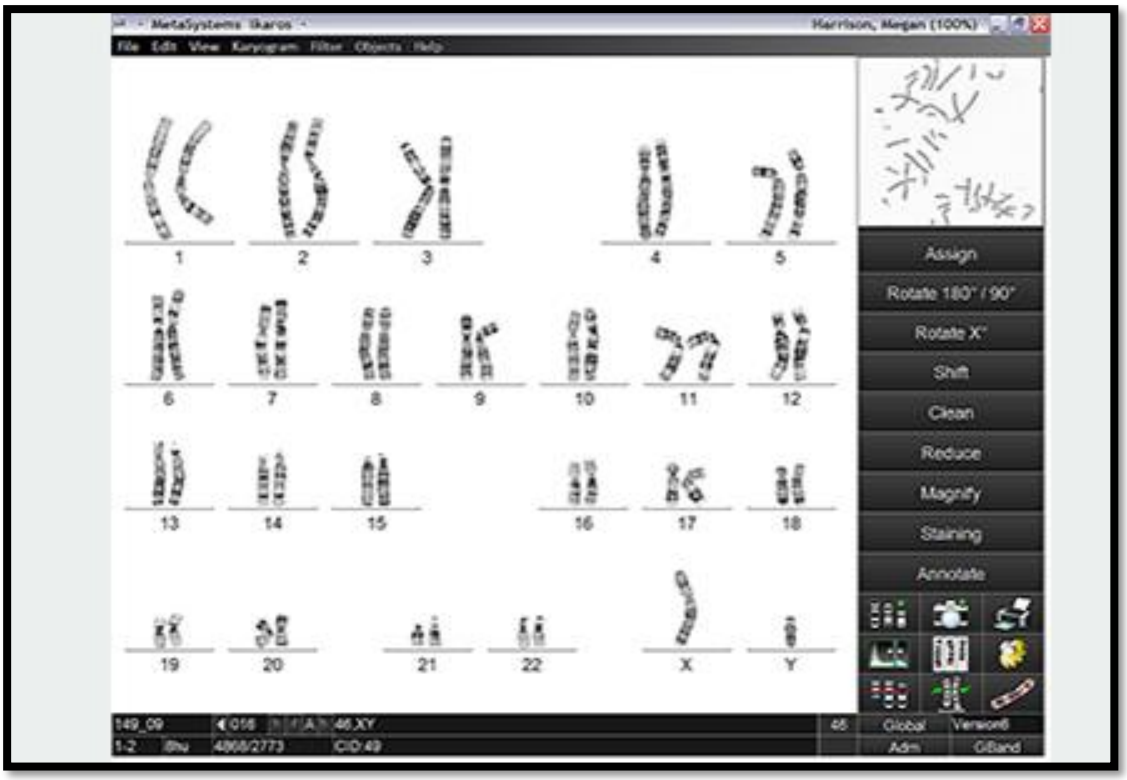


Figure 11 Software used. IKAROS of Metasystem.

Data base

For the present study, data were collected from patients who attended the Cytogenetics Laboratory for Genetic Counseling, or reason for consultation due to infertility. All data were collected in the Laboratory and subsequently analyzed for interpretation.

The karyotype and nomenclature, the reason for consultation, age and sex of the patients, as well as complementary information such as city, where they live and occupation are registry.

The cytogenetics laboratory analyzes 25 metaphases from each patient, and studies 5 karyotypes of each of them. Subsequently, the result report issued is recorded in the laboratory results matrix with the other patient data.

Ministerio de Salud Pública
Especializado en Genética Médica CEGEMED

CENTRO ESPECIALIZADO EN GENÉTICA MÉDICA

AGENDAMIENTO DE PACIENTES																	RESULTADO	
DATOS GENERALES DEL PACIENTE																		
Fecha dd/mm/aa	Documento Identificación	Apellidos y Nombres	Sexo	Fecha de nacimient	Nacionalida	Autoidentificaci ón Étnica	Estado civil	Nivel de educació	Cobertura	Contactar					Procedencia	Nro. de Historia	Fecha Entrega Paciente	Diagnóstic
										Teléfono 1	Teléfono 2	Dirección	Correo	Agendado				
29/09/2020	1759865676	SIMBAÑA YASQUEZ JULIETH AITANA	Femenina	8/10/2019	Ecuatoriana		N/A	N/A	MSP	0995466971			robert.davila@hqdc.qnb.ec	FP	1759865676	12/10/2020	46,XX,der(14;21)(q10	
29/09/2020	1752113520	USULLA QUILLIGANA DANIELA ESTEFANIA	Femenina	1/01/2003			N/A	N/A	MSP	0230774446			robert.davila@hqdc.qnb.ec	GG	1752113520	12/10/2020	46,XX	
2/10/2020	1760206011	ALBAN BAÑO ADRIANA VALENTINA	Intersexo	17/07/2020			N/A	N/A	IESS	-	-		juan.pazo@hbe.qnb.ec	FP	1760206011	19/10/2020	46,XY	
6/10/2020		MEDINA ORTIZ RN	Femenina	1/10/2020			N/A	N/A	MSP	-	-		robert.davila@hqdc.qnb.ec	FP	0	16/10/2020	46,XX	
6/10/2020	1760025863	RAMIREZ GUALOTO GENESIS SARAHI	Femenina	4/01/2020	Ecuatoriana	Mestiza	N/A	N/A	MSP	0992283049	-	Liliana Chica	robert.davila@hqdc.qnb.ec	GG	1760025863	17/10/2020	47,XX,+21	
16/10/2020	1760307696	MALES SANTELLAN LAYLA YAMILE	Femenina	2/10/2020			N/A	N/A	IESS	-	-		robert.davila@hqdc.qnb.ec	GG	1760307696	17/10/2020	Cultivo no obti	
23/10/2020	1352305626	RUIZ MACIAS ELIAN JHOSUE	Masculina	16/03/2019	Ecuatoriana		N/A	N/A	MSP	0967765333	-	San Alejo Calle 20 Diciembre	ingrid.mtr@hotmail.com	GG	1352305626	20/10/2020	47,XY,+21	
23/10/2020	-	BAME CHILA RN	Femenina	20/10/2020	Ecuatoriana		N/A	N/A	MSP	0994670523	-	Cantón Sucre	ingrid.mtr@hotmail.com	GG	-	20/10/2020	46,XX	
27/10/2020	1720464633	PINO SOLIS-ROSAS CATALINA PAZ	Femenina	19/03/1988	Chilena		Casada/o	Tercer Nivel	MSP	0995630296	-	La primavera	hectorp.aulym11@gmail.com	GG	1720464633	12/11/2020	46,XX	
27/10/2020	1723400750	PAZMIÑO PIEDRA MAURICIO MIGUEL	Masculina	22/05/1989	Ecuatoriana		Casada/o	Tercer Nivel	MSP	0984737846	-	La primavera	hectorp.aulym11@gmail.com	GG	1723400750	12/11/2020	46,XY,9qh+	
6/11/2020	1717232232	VERA PINUEZA ROSA ELVIRA	Femenina	10/02/1982	Ecuatoriana		Casada/o	Secundaria comp	MSP	0984865963	-	Panciana Baja	robert.davila@hqdc.qnb.ec	KT	1717232232	12/11/2020	46,XX	
6/11/2020	1713551075	CARVAJAL MANTA LUIS ENRIQUE	Masculina	8/09/1972	Ecuatoriana	Mestiza	Casada/o	Secundaria comp	IESS	0984647700	0984868963	Panciana	robert.davila@hqdc.qnb.ec	FP	1713551075	12/11/2020	46,XY	
6/11/2020	1003314630	ARELLANO VILLARREAL PAOLA ALEXANDRA	Femenina	3/06/1990	Ecuatoriana	Mestiza	Soltera/o	Tercer Nivel	IESS	0980420762	-	Ibarra	robert.davila@hqdc.qnb.ec	FP	1003314630	19/11/2020	45,X	
6/11/2020	1757646602	CRUZ GROYA WILLIAM JESUS	Masculina	8/09/2009	Cubana	Blanca	Soltera/o	Primaria	IESS	0982103728	6015809	Labradar	robert.davila@hqdc.qnb.ec	FP	1757646602	12/11/2020	46,XY	
6/11/2020	1760221951	HARO FERNANDEZ ZOE SCARLETT	Femenina	22/05/2020	Ecuatoriana	Mestiza	Soltera/o	N/A	MSP	0997665676	0959630265	Sangolquí	juan.pazo@hbe.qnb.ec	FP	1760221951	12/11/2020	47,XX,+21	
6/11/2020	1760399228	ARIAS PARRA ITZAYANA MIKAELA	Femenina	1/10/2020	Ecuatoriana	Mestiza	Soltera/o	N/A	MSP	0982337159	-	Cayambe	robert.davila@hqdc.qnb.ec	KT	1760399228	12/11/2020	47,XX,+21	
9/11/2020	1757471535	LIMA ORTIZ RAFAELA MONSERRAT	Femenina	27/05/2016	Ecuatoriana	Mestiza	N/A	Educación Básic	IESS	022499759	-	Machala y Manizal Sucre Cata	robert.davila@hqdc.qnb.ec	GG	1757471535	17/11/2020	46,XX	
10/11/2020	1316977972	AGUAYO MENDOZA IRINA VICTORIA	Femenina	29/06/2012	Ecuatoriana	Mestiza	N/A	Educación Básic	IESS	0990767035	-	Detrás Del Tia	hectorp.aulym11@gmail.com	FB	1316977972	20/11/2020	46,XX	
10/11/2020	1353106709	TOYAR AVILA LIAM DARIEL	Masculina	4/04/2019	Ecuatoriana		N/A	N/A	MSP	0969963545	0980792517	Cerca De Tienda Libertad	hectorp.aulym11@gmail.com	FB	1353106709	23/11/2020	47,XY,+21	
10/11/2020	1352469750	ALCIVAR DUEÑAS RUBI MARISOL	Femenina	15/09/2020	Ecuatoriana		N/A	N/A	MSP	0999169267	0991123460	Cementerio Jardines de Manab	hectorp.aulym11@gmail.com	FB	1352469750	20/11/2020	47,XX,+21	
10/11/2020	1313884021	CEDEÑO MACIAS GRACE YURITH	Femenina	24/04/2000	Ecuatoriana	Mestiza	N/A	N/A	MSP	0997919139	-	San Pablo Mz 4	hectorp.aulym11@gmail.com	99	1313884021	20/11/2020	46,XX	
10/11/2020	1352460921	DE LA CRUZ CEVALLOS AMY MAITE	Femenina	6/07/2017	Ecuatoriana		N/A	N/A	MSP	0969351552	0962092092	Centro	hectorp.aulym11@gmail.com	FB	1352460921	20/11/2020	46,XX	
10/11/2020	1352102246	LUCAS QUIROZ JENNIFER LESLY	Femenina	24/04/2015	Ecuatoriana	Mestiza	N/A	N/A	MSP	0980541095	0993109000	Calle Tamar Larrea	hectorp.aulym11@gmail.com	99	1352102246	20/11/2020	47,XX,+21	
10/11/2020	1352460461	MERA CEDEÑO BOSCO ZADKIEL	Masculina	20/05/2014	Ecuatoriana		N/A	Educación Básic	MSP	0980286131	-	rav de Iglesia Cristo del Consue	hectorp.aulym11@gmail.com	FB	1352460461	20/11/2020	46,XY	
10/11/2020	1352046087	QUIJANO ZAMORA SAILENY VALERIA	Femenina	11/01/2019	Ecuatoriana	Mestiza	N/A	N/A	MSP	0962280348	0996950239	Puebla Nueva, Av Juma	hectorp.aulym11@gmail.com	GG	1352046087	19/11/2020	47,XX,+21	
10/11/2020	104648537	REYES ODALIS FRANCISCA	Femenina	22/10/2000	Venezolana	Mestiza	N/A	N/A	MSP	0962923718	-	Recafuerte y 10 de Agosto	hectorp.aulym11@gmail.com	99	104648537	19/11/2020	47,XX,+21	

Figure 12. Data base Centro Especializado en Genética Médica - Ecuador. CEGEMED

CONTENTS AND RESULTS

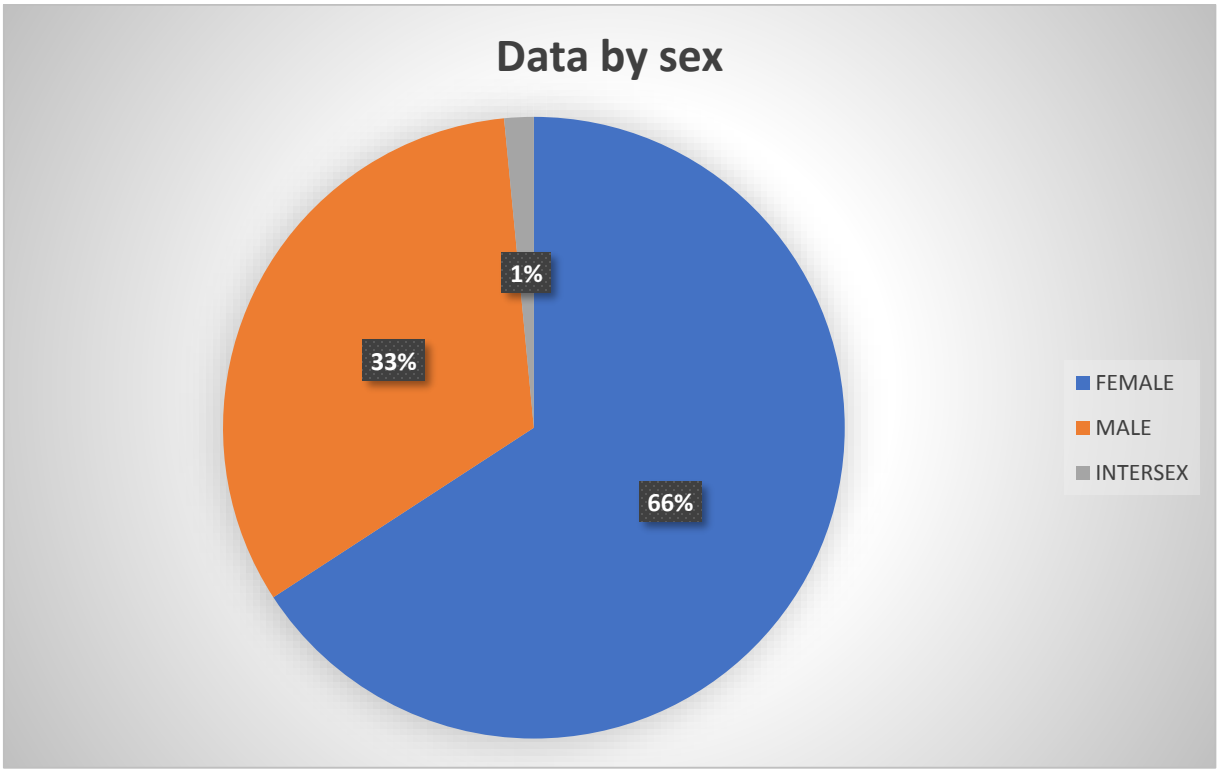
CONTENTS AND RESULTS

Once the database obtained in the Cytogenetics Laboratory had been analyzed, the results were classified according to sex, age and indication for the test.

Table 3 Data by sex

SEX	NUMBER OF PATIENTS FOR TESTING
FEMALE	129
MALE	64
INTERSEX	3

Figure 13 Data by sex

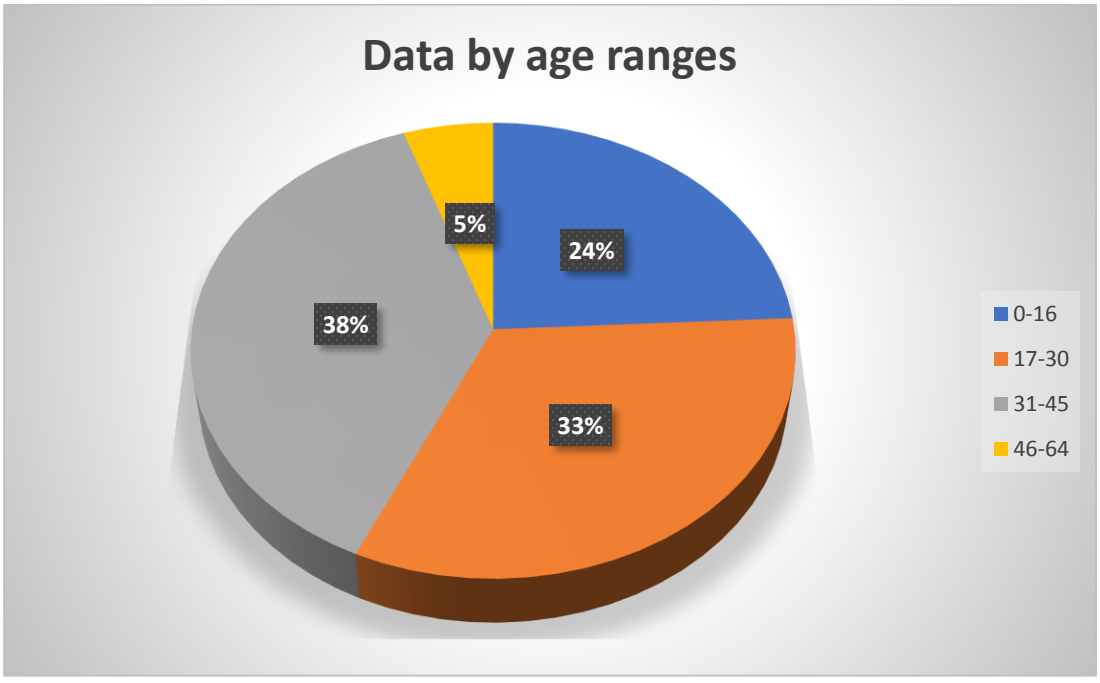


As we can see, the data indicate a higher incidence in the number of female patients compared to male patients.

Table 4 Data by age ranges

AGE	NUMBER OF PATIENTS FOR TESTING
0-16	46
17-30	62
31-45	72
46-64	10

Figure 14 Data by age ranges

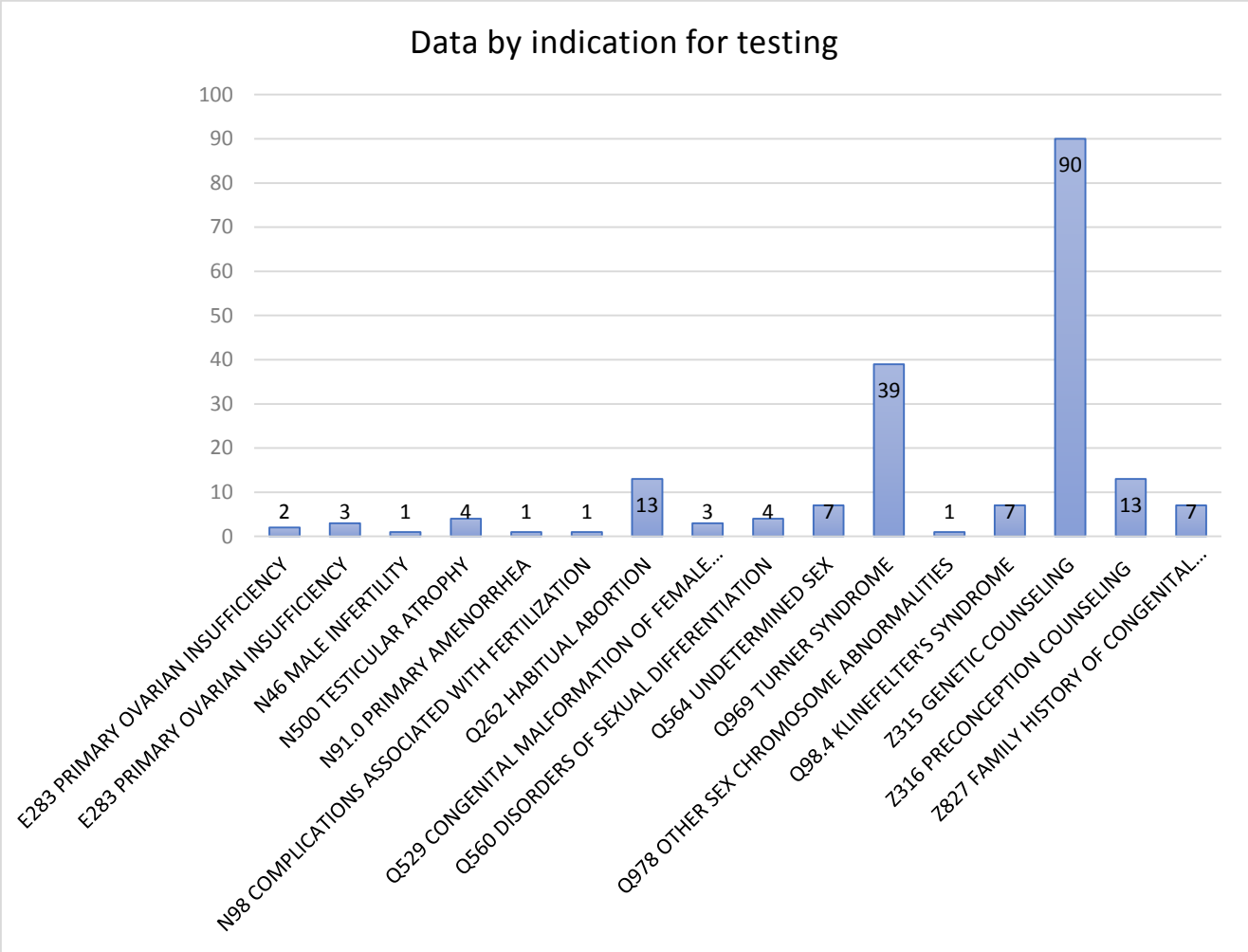


The largest number of patients is between 17 and 45 years old, that is, fertile ages.

Table 5 Data by indication for testing

INDICATION FOR TESTING	NUMBER OF PATIENTS FOR TESTING
E283 PRIMARY OVARIAN INSUFFICIENCY	2
E283 PRIMARY OVARIAN INSUFFICIENCY	3
N46 MALE INFERTILITY	1
N500 TESTICULAR ATROPHY	4
N91.0 PRIMARY AMENORRHEA	1
N98 COMPLICATIONS ASSOCIATED WITH FERTILIZATION	1
Q262 HABITUAL ABORTION	13
Q529 CONGENITAL MALFORMATION OF FEMALE GENITALIA / UNSPECIFIED	3
Q560 DISORDERS OF SEXUAL DIFFERENTIATION	4
Q564 UNDETERMINED SEX	7
Q969 TURNER SYNDROME	39
Q978 OTHER SEX CHROMOSOME ABNORMALITIES	1
Q98.4 KLINEFELTER'S SYNDROME	7
Z315 GENETIC COUNSELING	90
Z316 PRECONCEPTION COUNSELING	13
Z827 FAMILY HISTORY OF CONGENITAL MALFORMATIONS/DEFORMITIES AND OTHER CHROMOSOMAL ABNORMALITIES	7

Figure 15 Data by indication for testing



According to the data analyzed, most patients go to the laboratory with indication of Genetic Counseling.

Results

Table 6 Results of patients

RESULTS	NUMBER OF PATIENTS FOR TESTING
45,X	17
45,XX,der(13;14)(q10;q10)	2
45,XY,der(14;21)(q10;q10)	1
46,X,del(X)(q25)	1
46,X,del(X)(q25),9qh+	2
46,X,i(X)(q10)	2
46,XX	90
46,XX,16qh+	1
46,XX,1qh+	1
46,XX,9qh-	1
46,XX,9qh+	2
46,XY	61
46,XY,16qh+	1
46,XY,1qh+	1
46,XY,22ps+	2
46,XY,9qh-	1
47,XXY	1
Negative culture	4
mos 46,X,del(X)(q25)/46,XX	1
mos45,X/46,XX	2
mos45,X/46,XY	1
mos47,XX,+mar/46,XX	1
TOTAL	196

Figure 16 Normal and Altered Karyotype

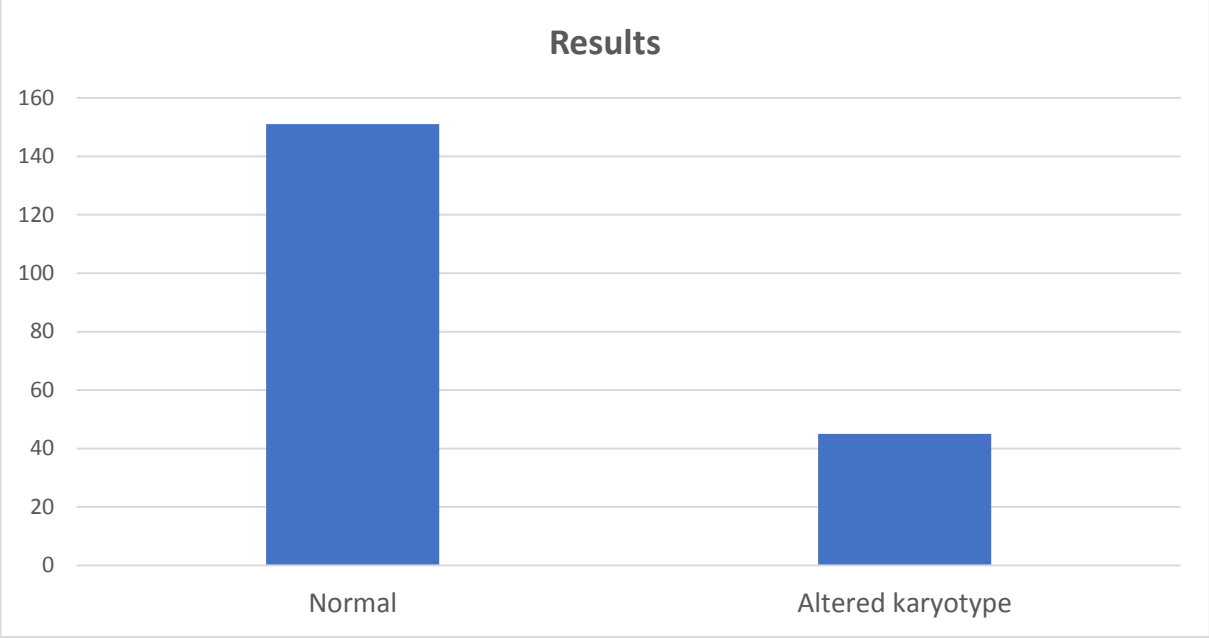


Figure 17 Altered Results

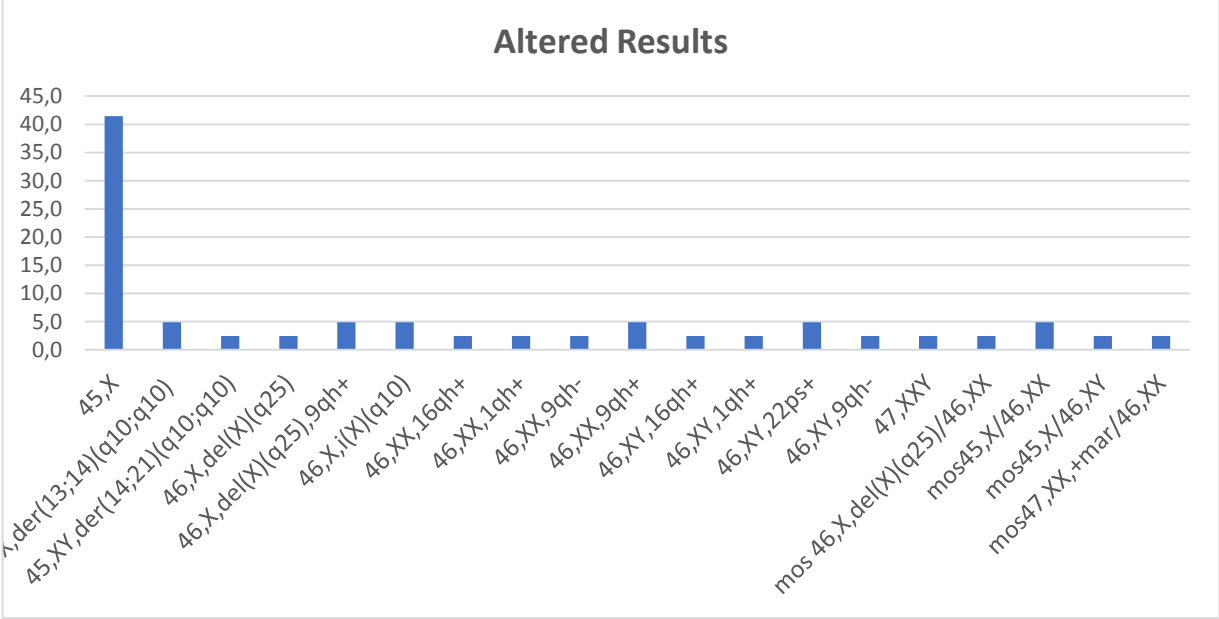


Figure 18 Polymorphisms founded

RESULTS	NUMBER OF PATIENTS FOR TESTING
Polymorphisms	12
Normal	184

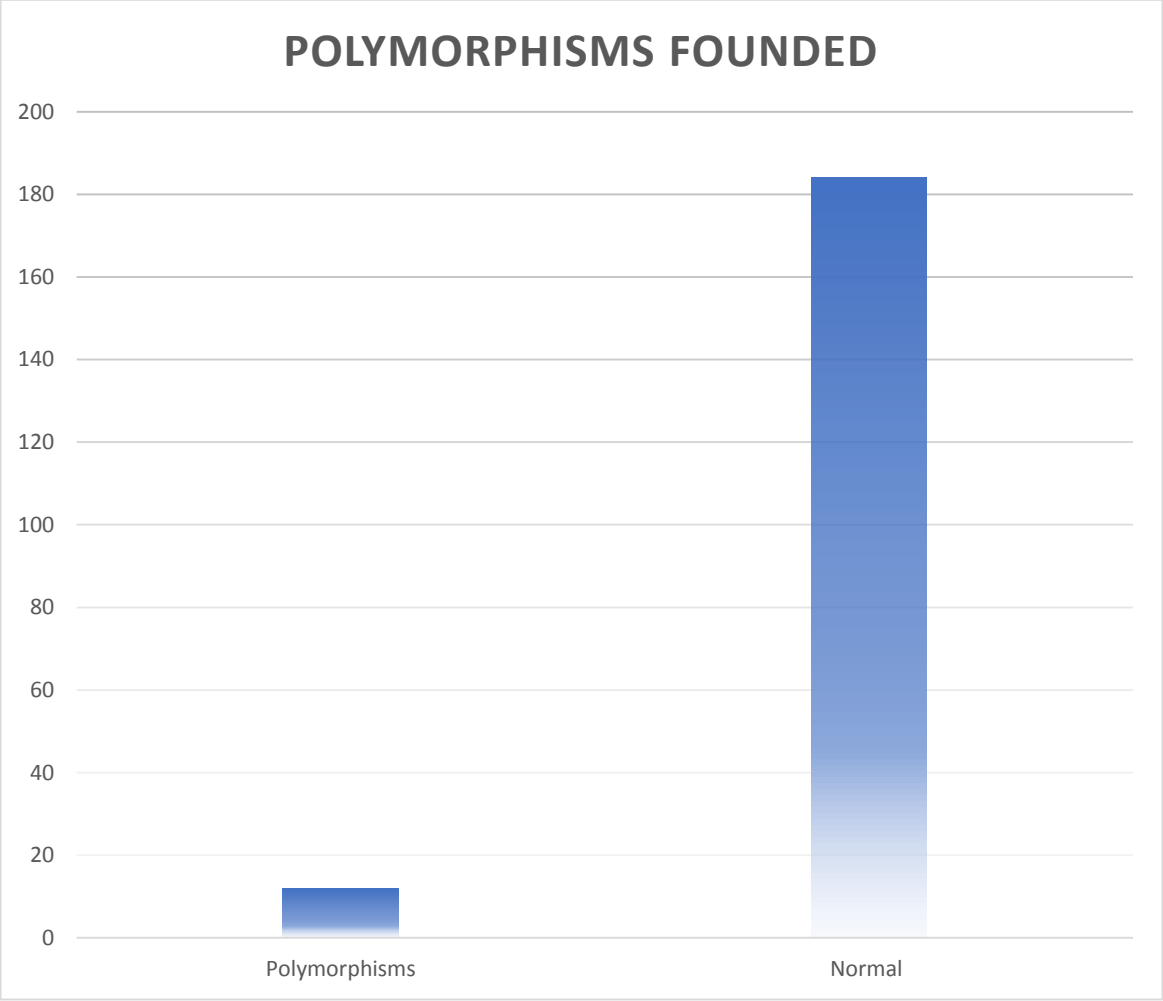
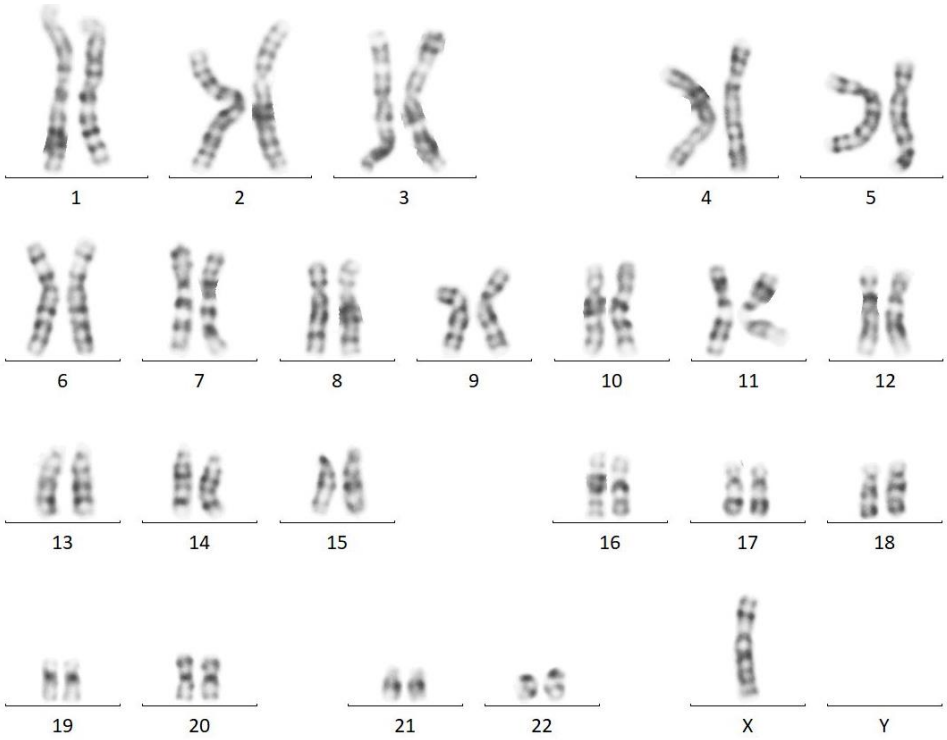
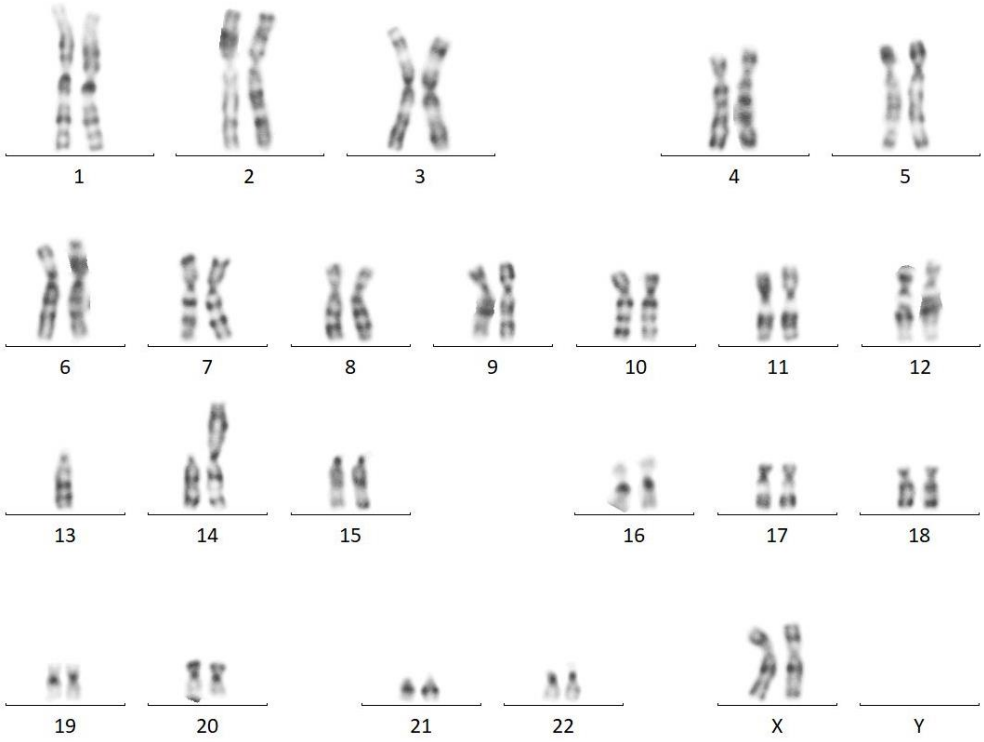


Table 7 karyotypes with alterations, found in the present study.

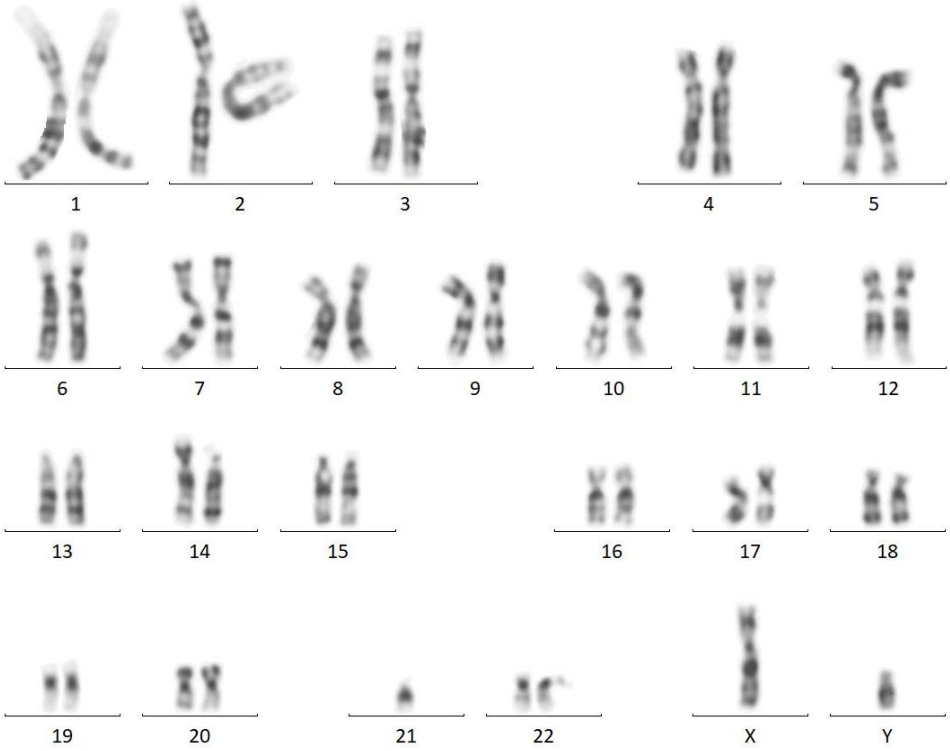
45,X



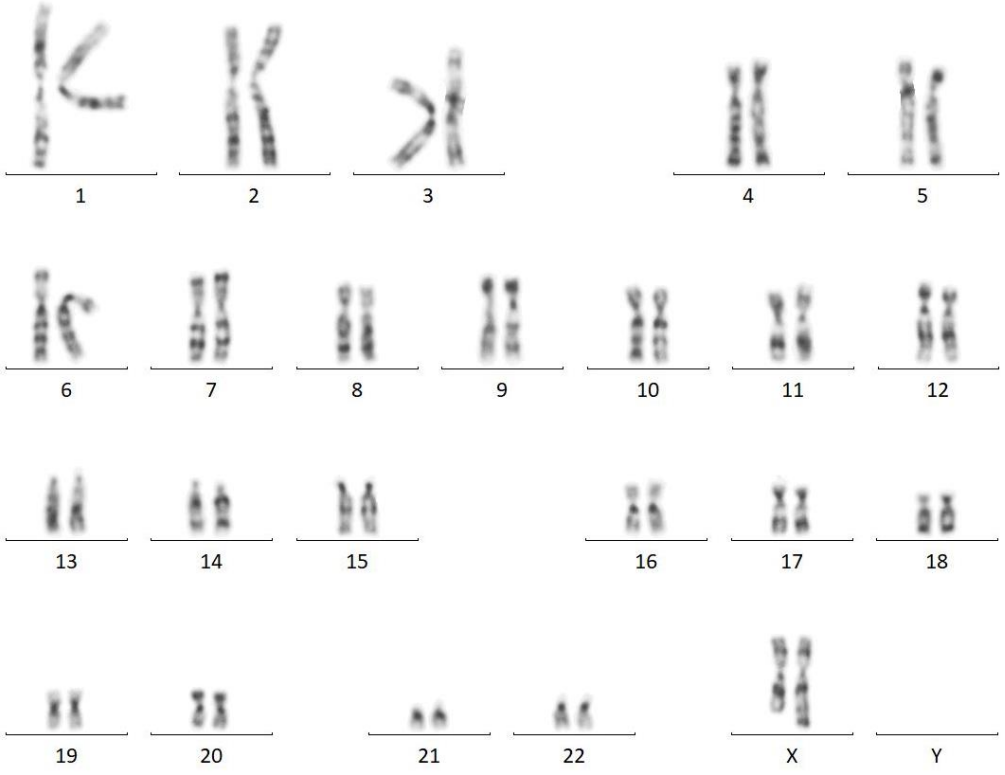
45,XX,der(13;14)(q10;q10)



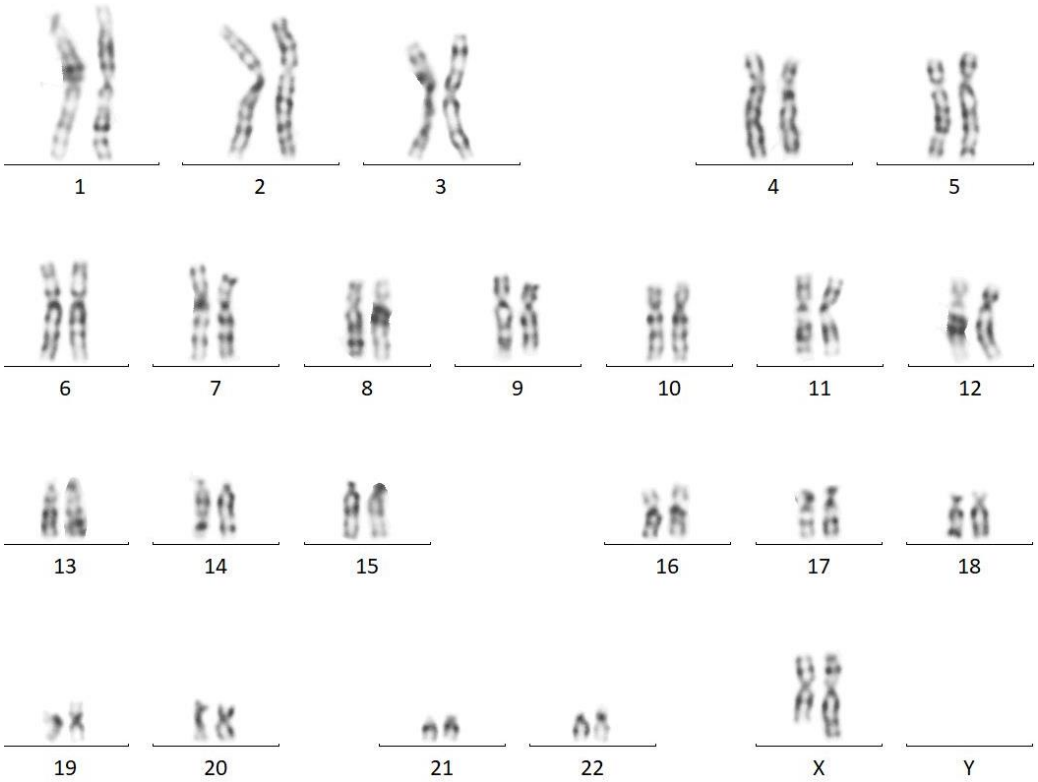
45,XY,der(14;21)(q10;q10)



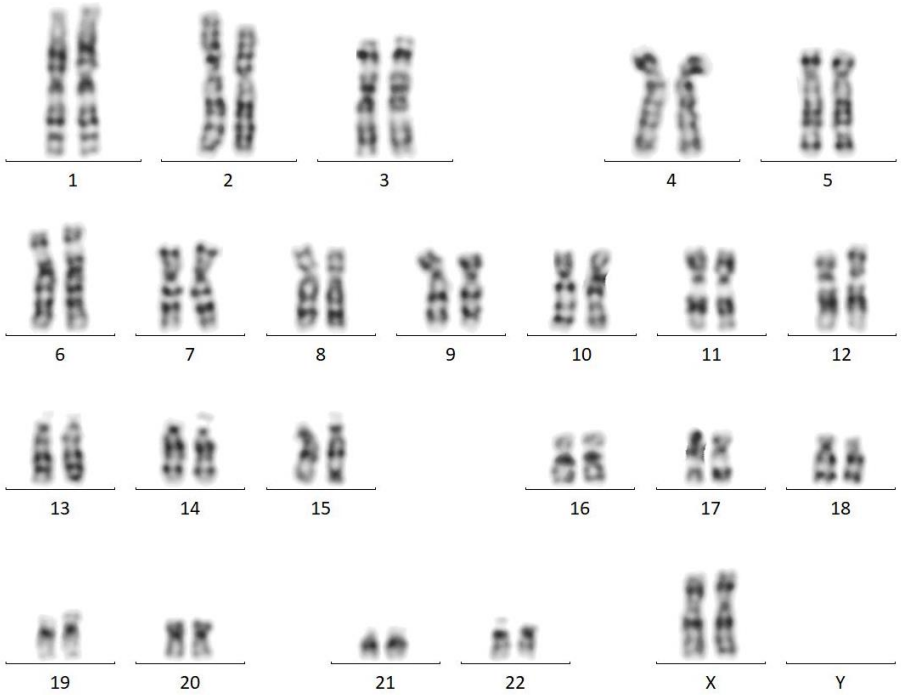
46,X,del(X)(q25)



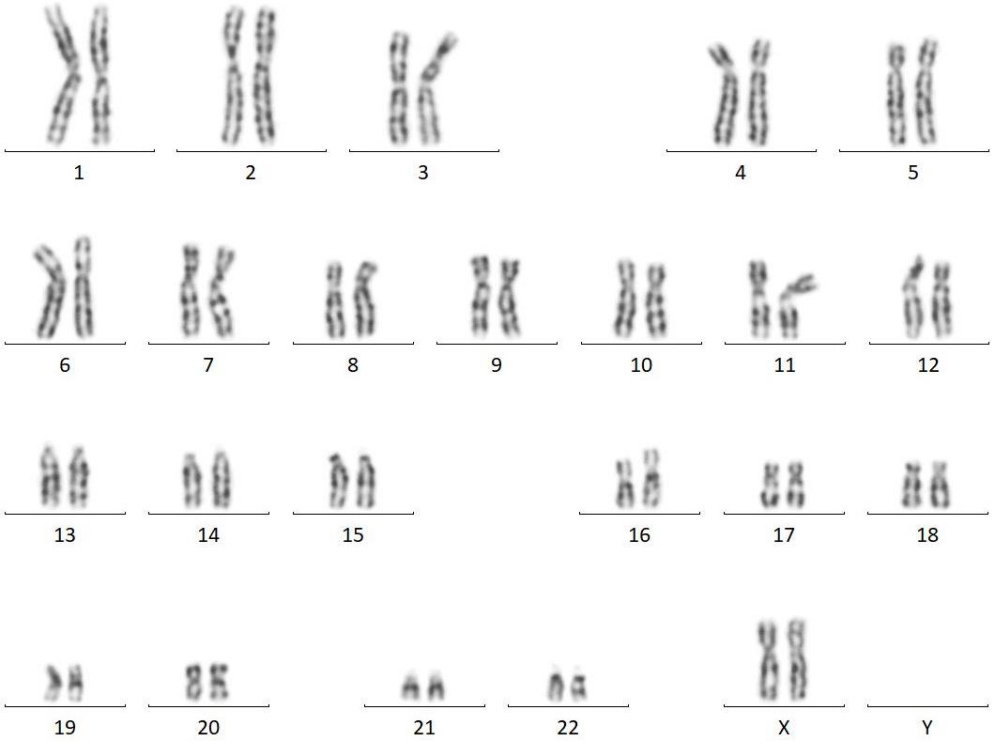
46,X,del(X)(q25),9qh+



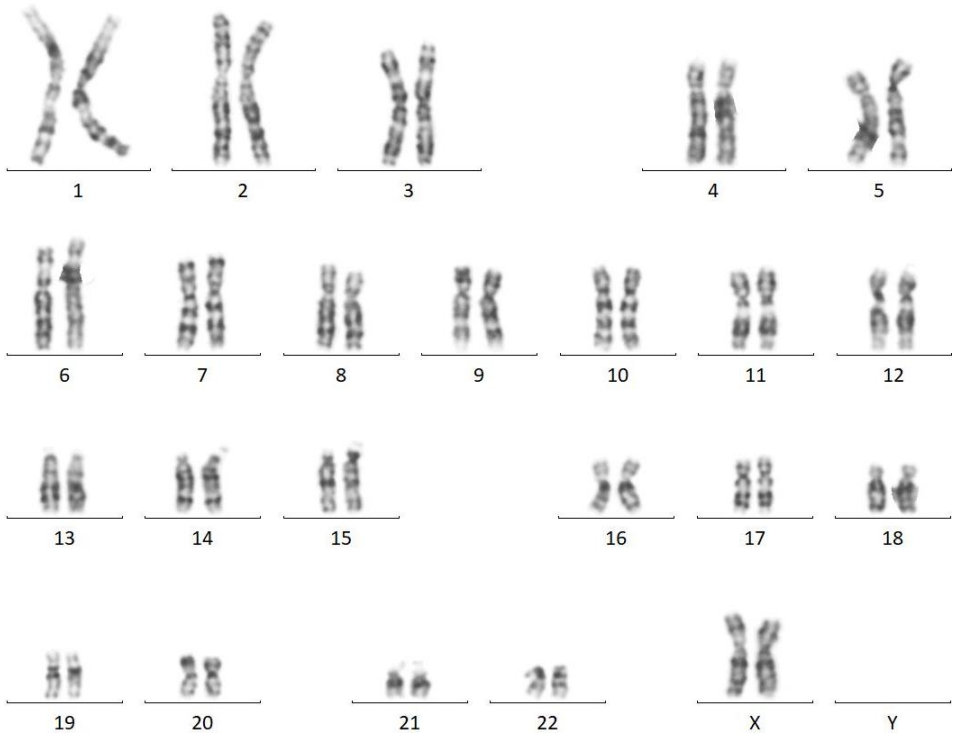
46,XX



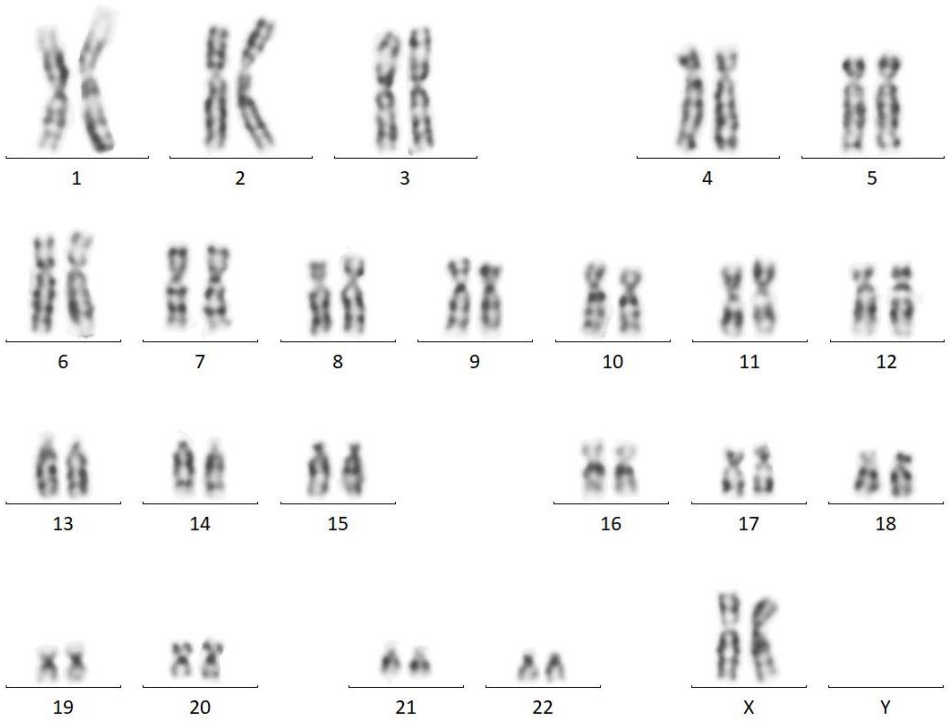
46,XX,16qh+



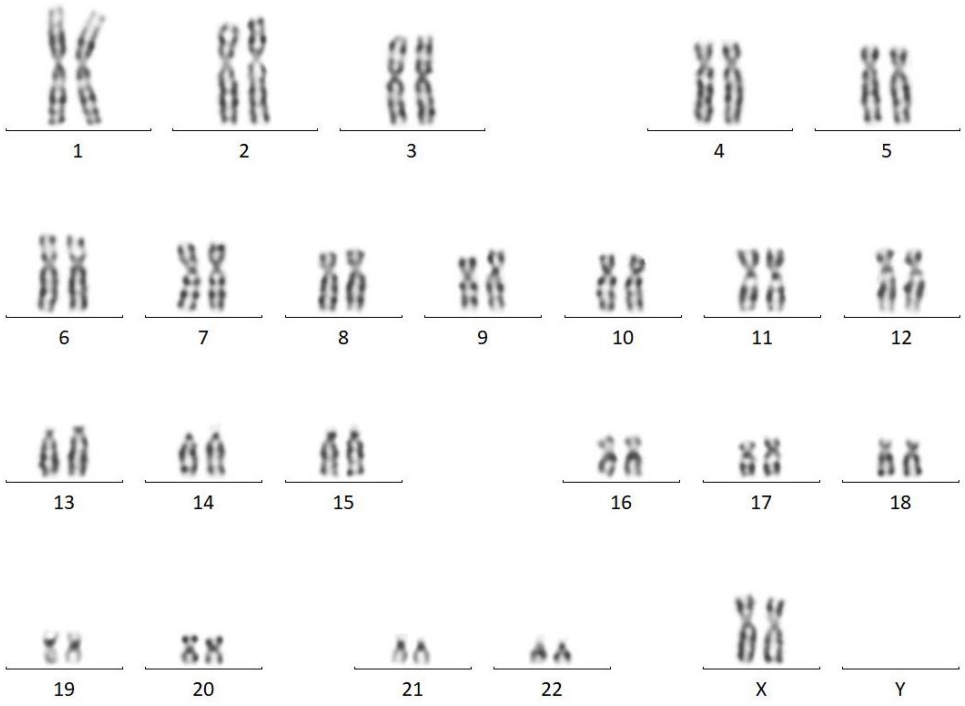
46,XX,1qh+



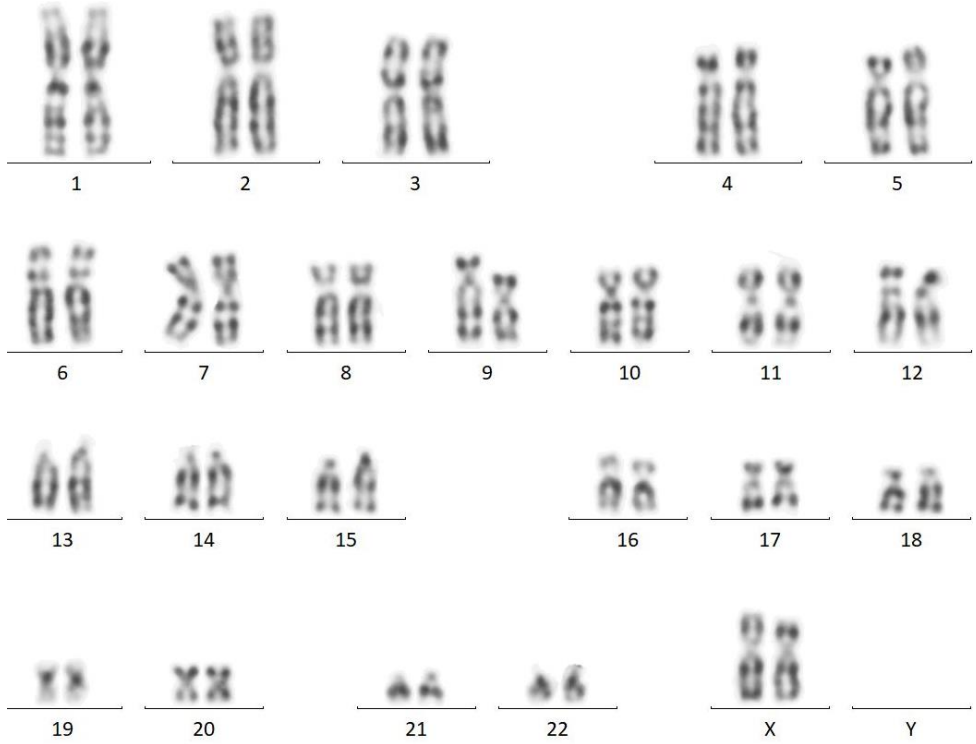
46,XX,1qh-



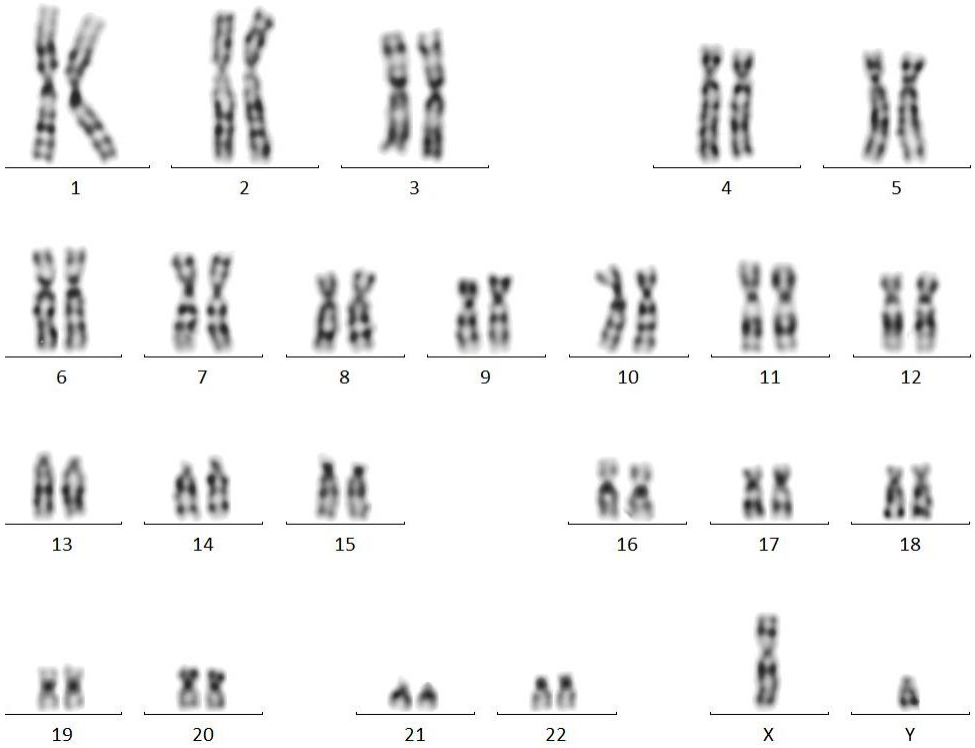
46,XX,9qh-



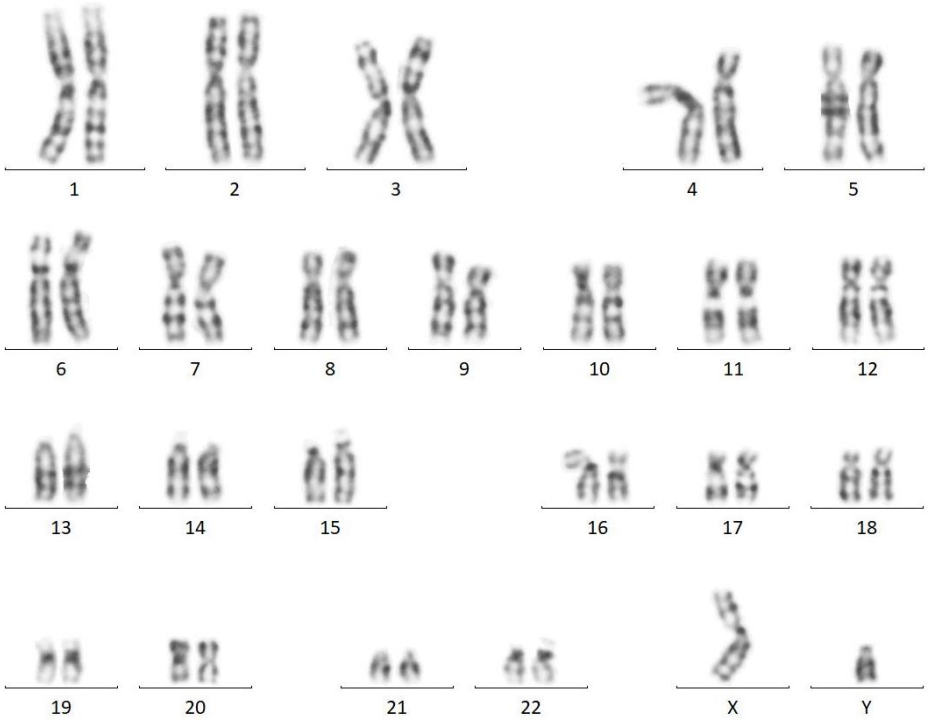
46,XX,9qh+



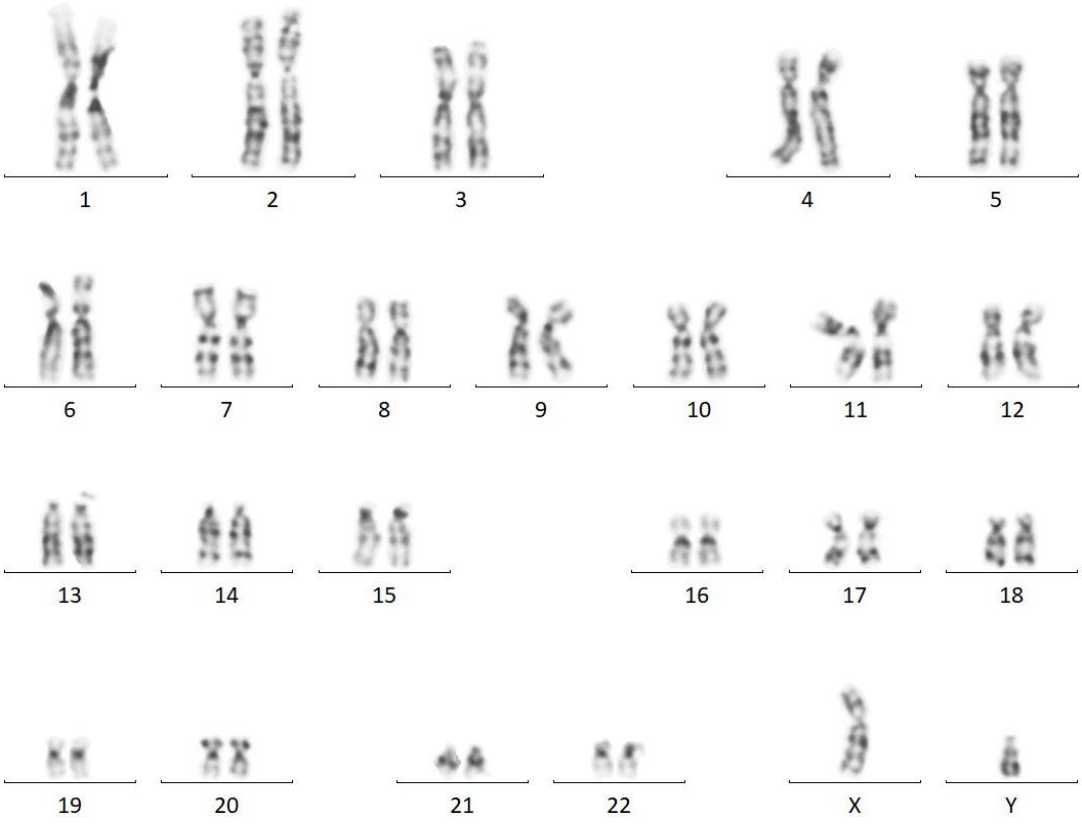
46,XY



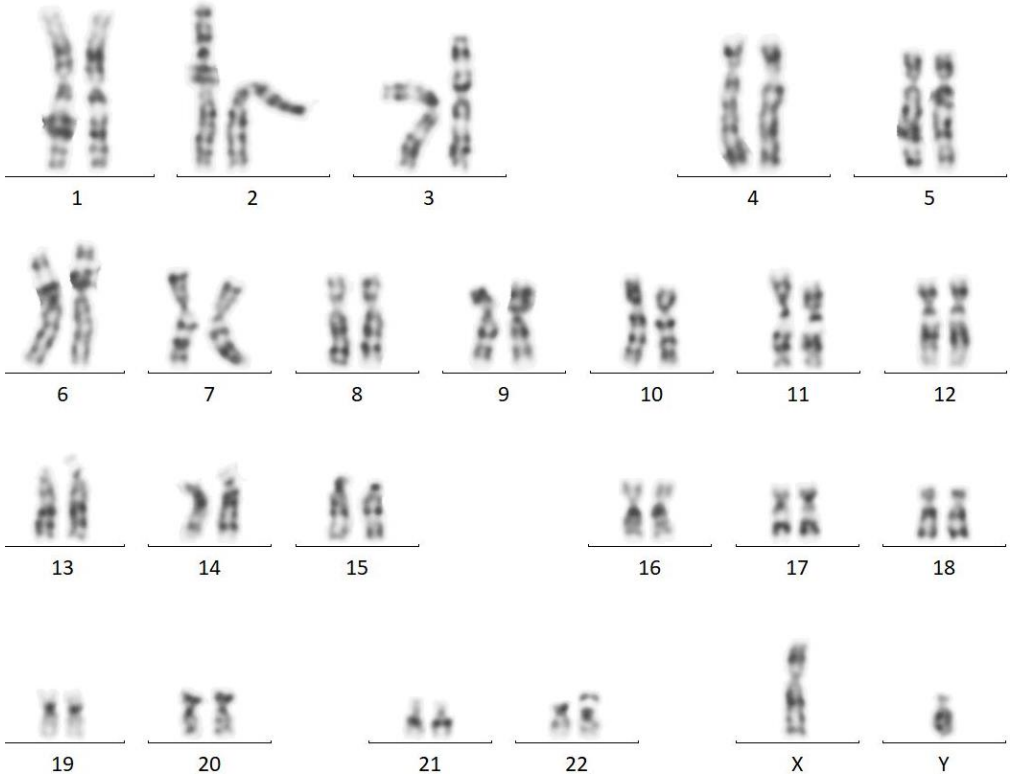
46,XY,16qh+



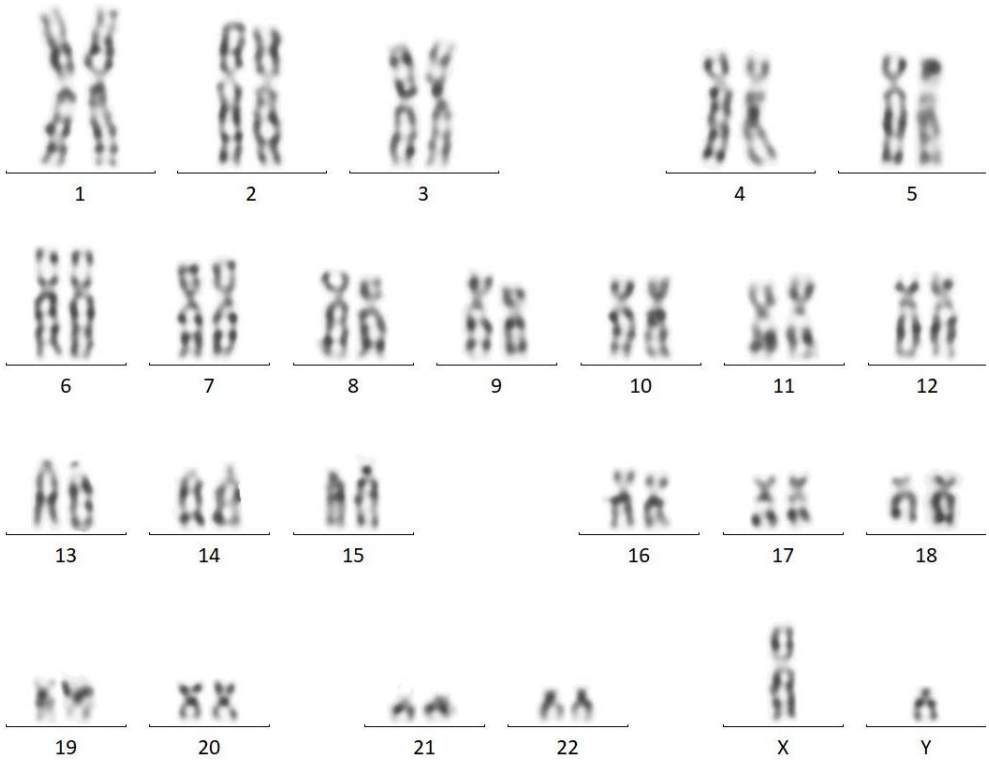
46,XY,1qh+



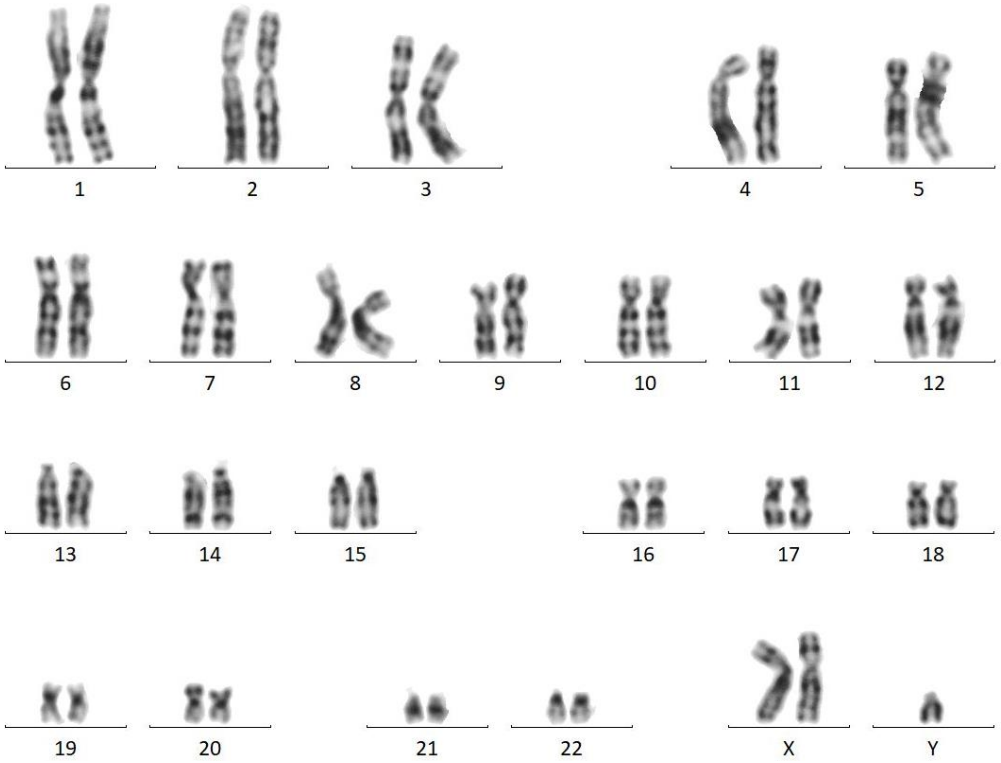
46,XY,22ps+



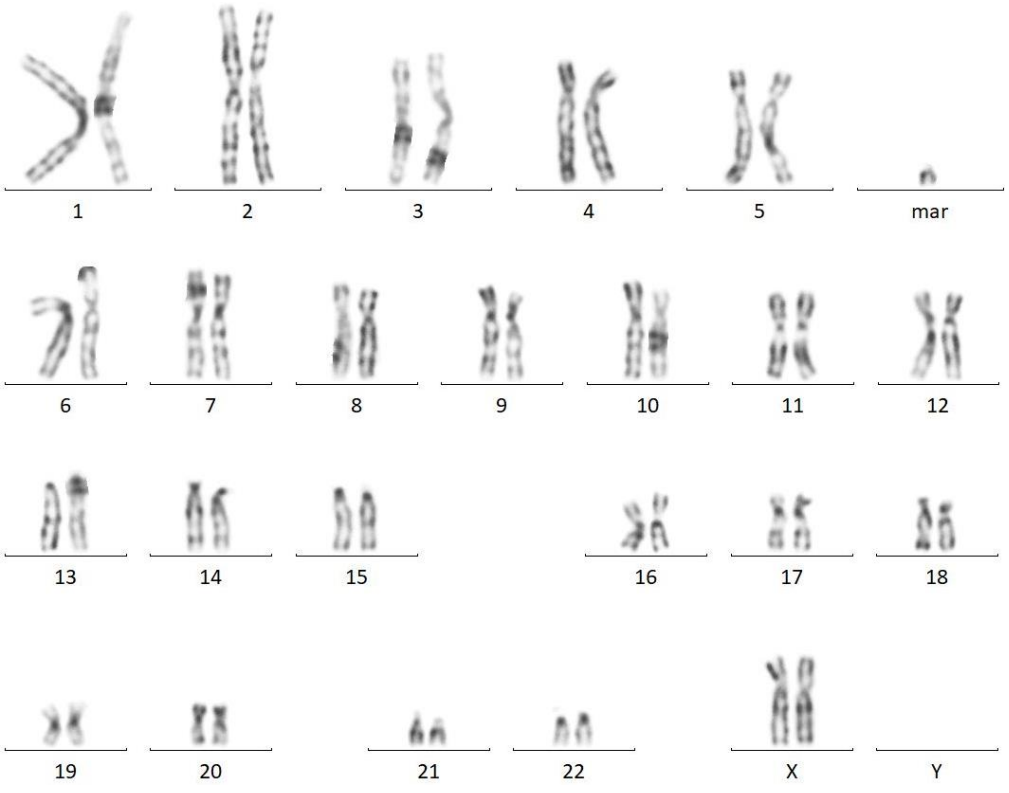
46,XY,9qh-



47,XXY



mos47,XX,+mar/46,XX



TABLES AND FIGURES

TABLES

FIGURES

CHAPTER 3

DISCUSSION

DISCUSSION

1. In this work, karyotype data have been collected from people who come to genetic care due to infertility or genetic counseling due to a family history of a disease or condition of genetic origin.

   The results of 196 people with these characteristics were analyzed, of which only 51 people had a karyotype with some structural or numerical abnormality. which means that of 100% of cases only 23% have a chromosomal alteration. A low percentage, considering the large number of couples who do not find an apparent cause for their infertility problems.

2. The analysis of the results in this study showed that of the 100% of patients who attended the genetic consultation, 66% were female patients, which indicates that there is still a belief that whoever is responsible for infertility problems is the woman. If the studies were carried out on both the woman and the man in the couple, it is certain that more accurate data would be found on infertility problems related to chromosomal alterations.

3. 38% of people who attended genetic consultation for infertility in this study, are in the age range of 31 to 45 years, this is because in this age range people begin to see infertility problems by not being able to conceive, In the case of women over 35 years of age, the reason for attending a genetic consultation is due to the increased risk of chromosomal alterations in pregnancy.

4. The infertility is a complex multifactorial condition that presents with highly heterogeneous phenotypes. The chromosome alterations plays a central role in regulation of gametogenesis as it harbors sexual chromosomes-linked genes that are involved in various processes during gametogenesis. The importance of these genes is evident from the observations that the removal of these genes causes distinct pathological testis phenotypes.

5. One of the chromosomal abnormalities found most frequently in this study was monosomy of the X chromosome, corresponding to Turner syndrome. 12% percent of patients showed a karyotype related to Turner Syndrome and its variants, among these variants are X chromosome deletions and 46,X/45,X mosaicism. Turner syndrome is the most common sex chromosome abnormality in women, occurring in approximately 1 in 2500 live births.

   Women with Turner syndrome are at extremely high risk for primary ovarian insufficiency and infertility. Although approximately 70%-80% have no spontaneous pubertal development and 90% experience primary amenorrhea, the remainder might possess a small residual of ovarian follicles at birth or early childhood.

6. In this study, chromosomal translocations were also found, including derived chromosomes 13;14 and 14;21. The important thing about finding these alterations is that adequate genetic counseling can be done to the parents, since, being carriers, they can transmit it to their children, increased risk for aneuploidy, and be the cause of abortions or possible malformations in their children.

   In men with oligozoospermia, Robertsonian translocations (RobTs) are the most common type of autosomal aberrations. The most commonly occurring types are rob(13;14) and rob(14;21), and other types of RobTs are described as 'rare' cases. Based on molecular research, all RobTs can be broadly classified into Class 1 and Class 2. Class 1 translocations produce the same breakpoints within their RobT type, but Class 2 translocations are predicted to form during meiosis or mitosis through a variety of mechanisms, resulting in variation in the breakpoint locations.

7. The second chromosomal alteration in sex chromosomes found was 47,XXY, corresponding to Klinefelter Syndrome. Klinefelter's syndrome is characterized by progressive testicular failure causing aspermatogenesis and androgen deficiency.

Klinefelter patients classically have complete male sex differentiation, and genital anomalies are generally not recognized as associated features of the syndrome.

8. Alterations were also found in the lengthening or shortening of heterochromatin, especially in chromosomes 1, 9, 16, although these alterations are constantly found in apparently normal people, it is possible that they have some relationship with infertility and it would be important that they be performed complementary studies in order to find or not a relationship between these abnormalities and alterations in fertility. Certain regions in the genome are subject to heteromorphisms due to their repetitive DNA content. Chromosome localizations of these regions may be identified by several methods. Each of the methods reveals typical staining patterns implying constitutional differences in heterochromatin.

   The term heteromorphism is used synonymously with polymorphism or normal variant. Common cytogenetic polymorphisms detected by G-banding are considered as heteromorphisms and include heterochromatin regions of chromosomes 1, 9, 16 and Y and also prominent acrocentric short arms, satellites and stalks. The role of chromosome heteromorphisms in infertility has been studied previously. There seems to be an increased incidence especially in infertile men but the mechanism underlying this association needs to be elucidated.

9. In this study, a patient with a part of a chromosome of unknown origin was also found. In these cases, it is important that complementary studies be carried out to determine its origin and possible involvement in infertility.

10. These are the first Cytogenetics data collected in Ecuador during a year, to be related to infertility, it is important that more studies are carried out on the subject, in addition to an adequate follow-up of possible new cases during the diagnoses that are issued later.

CONCLUSIONS

CONCLUSIONS

1. Although the results show that patients with infertility related to chromosomal abnormalities are few, this work is of great importance to demonstrate the role of cytogenetics in the diagnosis and follow-up of patients with infertility and other related chromosomal abnormalities, which otherwise would remain in complementary studies that would not allow its opportune diagnosis.

   The importance of cytogenetic diagnosis also implies adequate counseling for couples who wish to have children and possibly are carriers of a structural alteration that can be inherited in their progeny. Genetics has made great progress in the past decades, and prenatal diagnosis, predictive genetic testing, and genetic counseling have drawn the limelight of public attention. The subject of genetic counseling is of crucial consequence for both the short and long term, its ethical aspects are paramount. The question is whether mankind is mature enough to use this extraordinary knowledge in the right way for the benefit of the society. In the center of ethical questions is the comprehensiveness of information provided to the couples or patients and counseling them about results and making informed educated decisions. In addition, it is crucial how sensitive personal information is treated and whether and how it should be made public[26].

2. In this study, several numerical and structural alterations were found, among these the most frequent were: Monosomy of the X chromosome (45,X), deletions of the X Chromosome, Klinefelter Syndrome (47,XXY), translocations (13;14) , (14;21), heteromorphisms on chromosomes 1, 9, and 16, in addition to mosaics of normal male and female cell lines and monosomies of the X chromosome (mos 45,X/46XX), (mos 45,X/46XY).

3. The present work has the first data on the relationship between infertility and cytogenetics collected in Ecuador. It is important that these data be updated every year, to access more information on this relationship.

   The data was collected during a year in the Cytogenetics Laboratory of the Genetics Center in Ecuador, if possible, more institutions could join the data necessary for more information about the topic raised.

CHAPTER 4

APPENDICES

APPENDICES

Blood sampling procedure

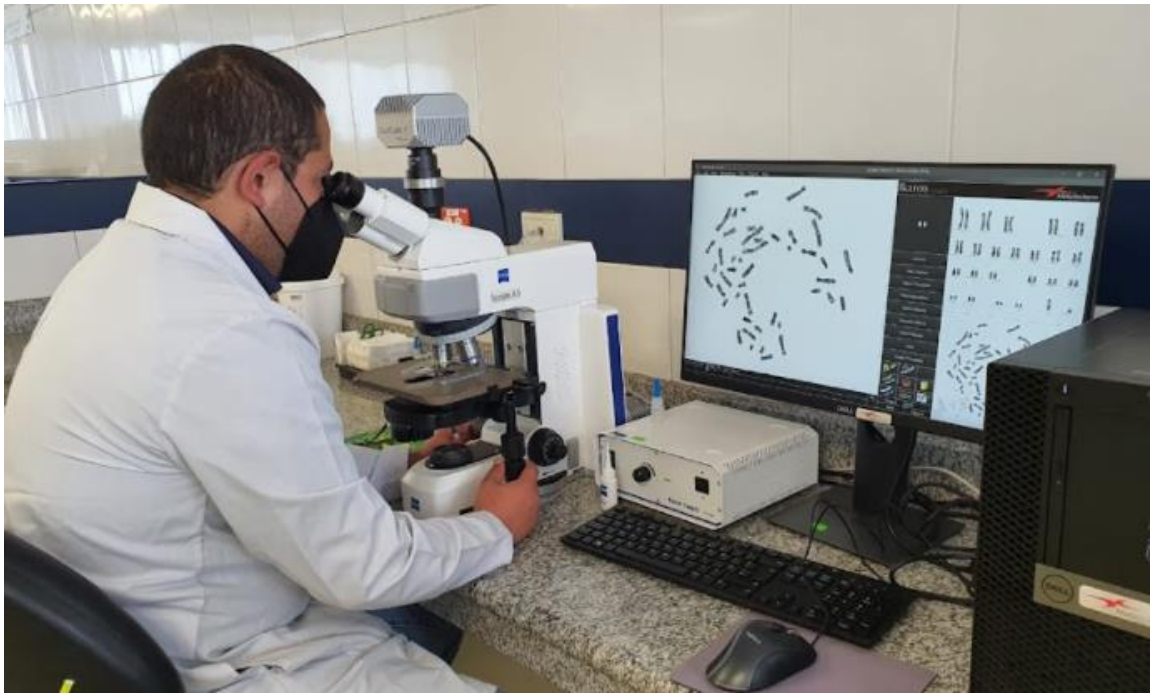
The process for obtaining metaphases is:

1.-Lymphocyte culture

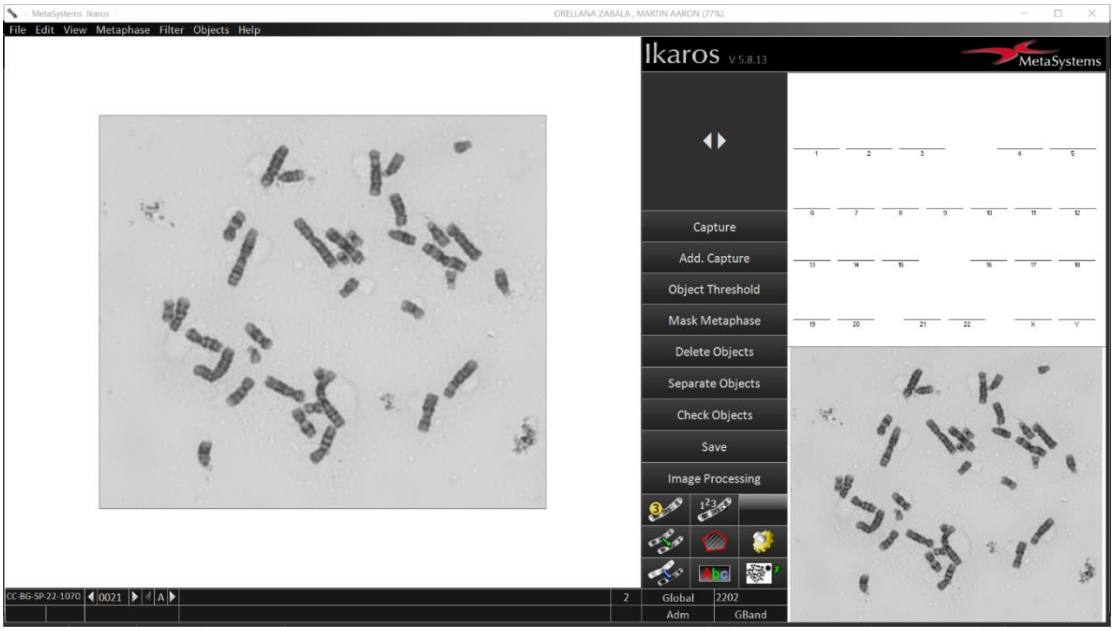
2.-Process to culture (72:00 Hours)

3.-Slides of metaphases

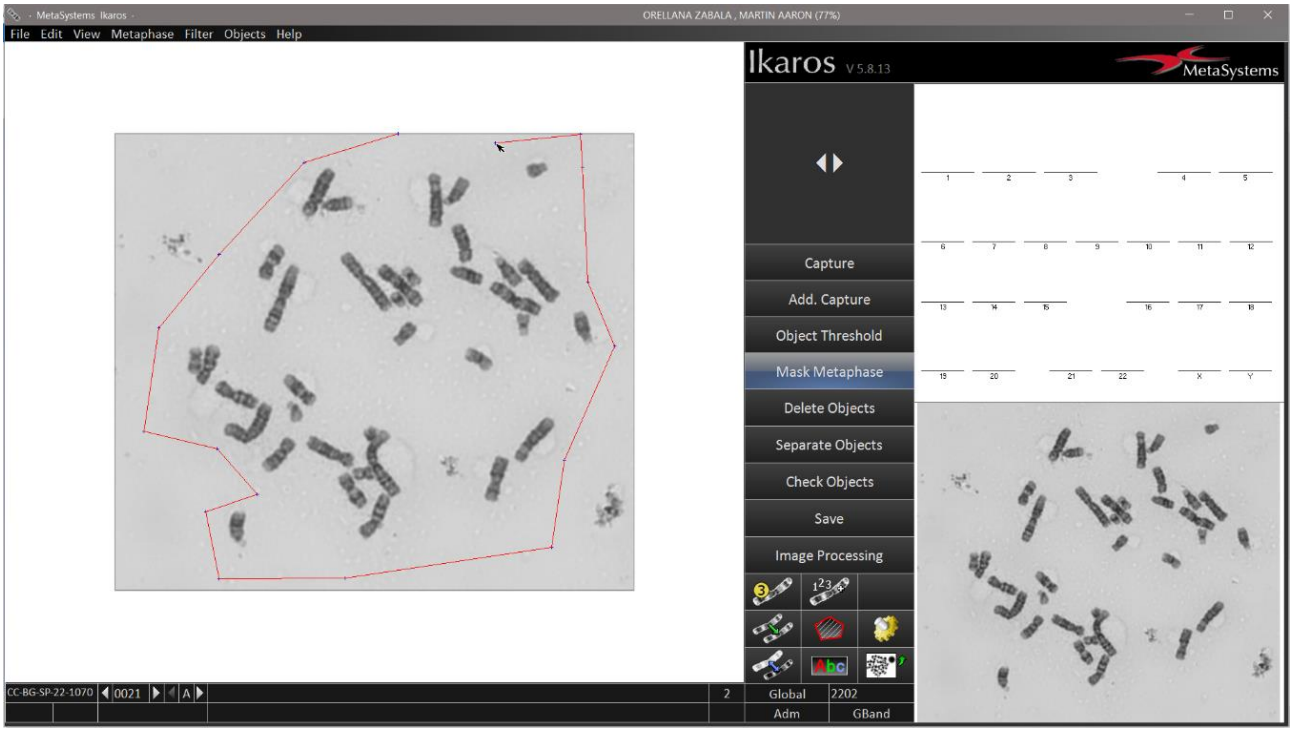
The process by which the metaphases obtained are analyzed using the IKAROS software, available at the CEGEMED Cytogenetics laboratory, is detailed below.



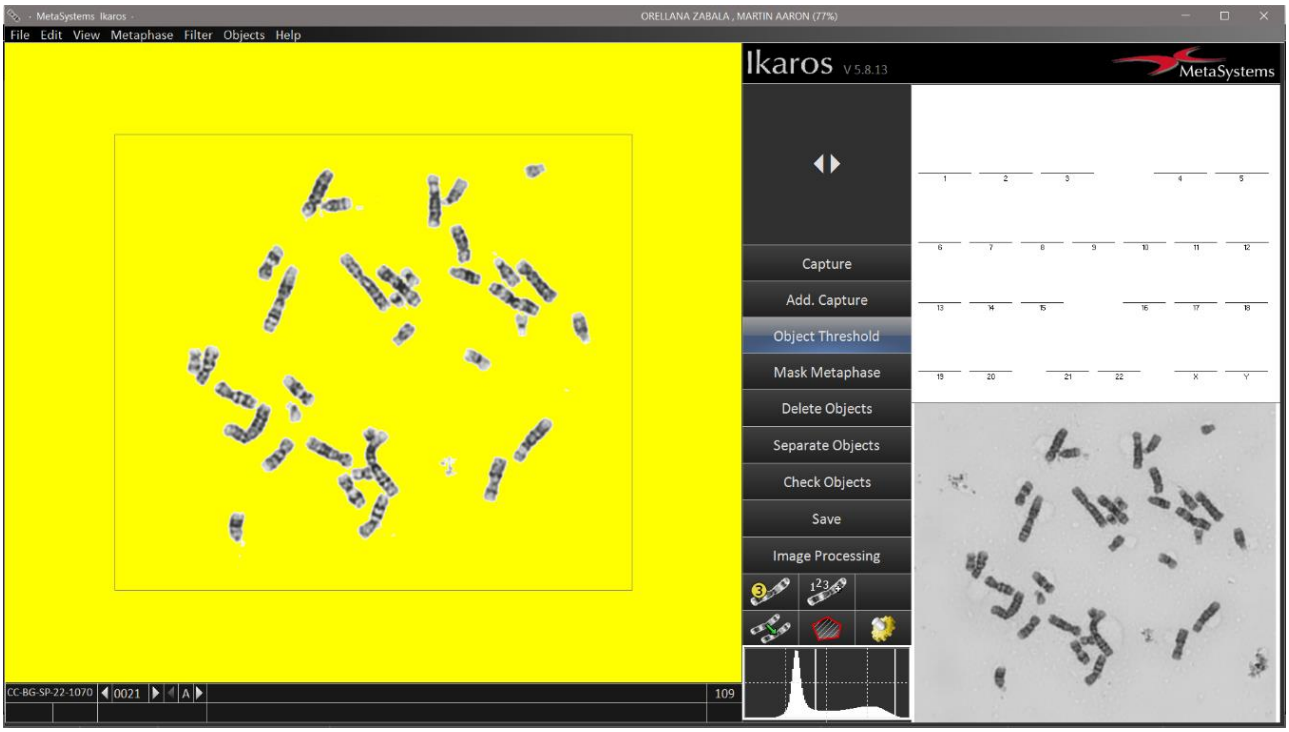
1.- The slides are observed under a microscope with a magnification of 100X and later captured by a monochrome camera to the system for analysis



2.- The section of interest where the metaphase is located is selected



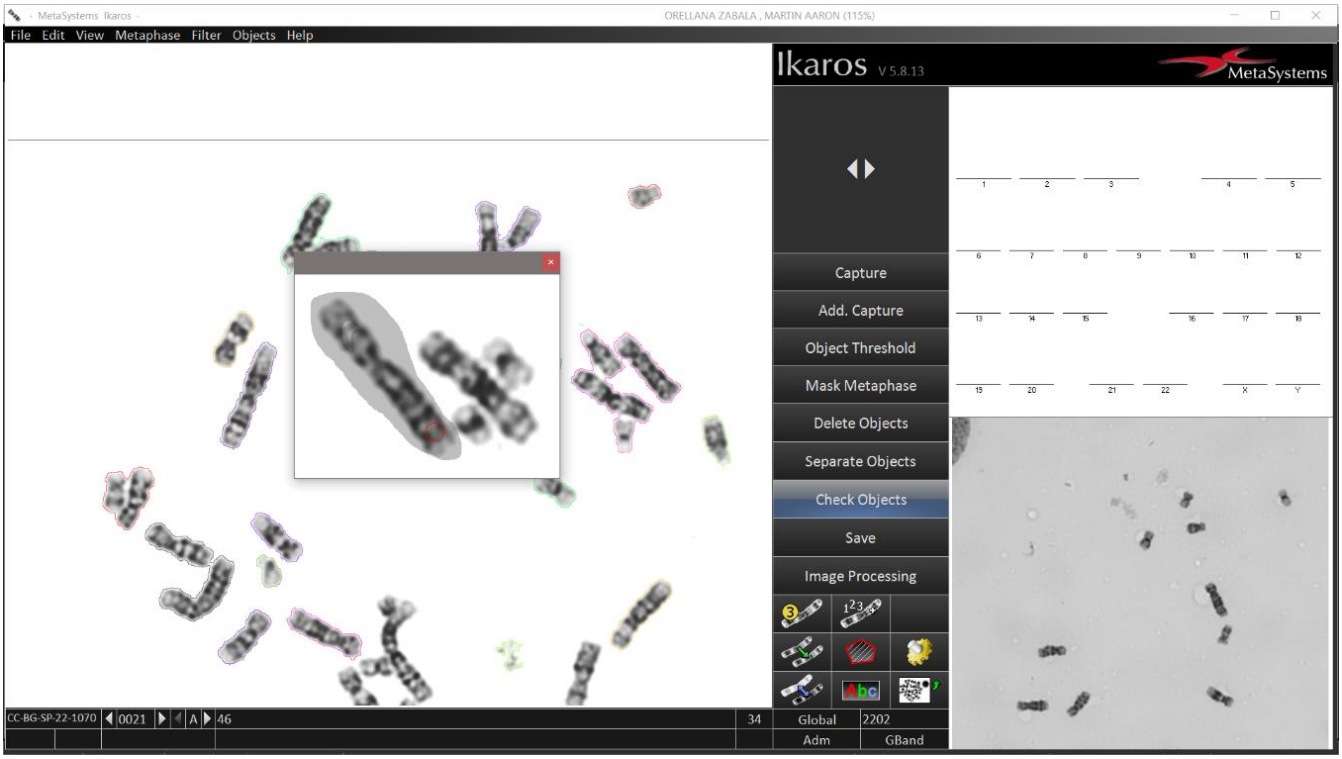
3.- We remove the background, so that the chromosomes are visible and easy to observe



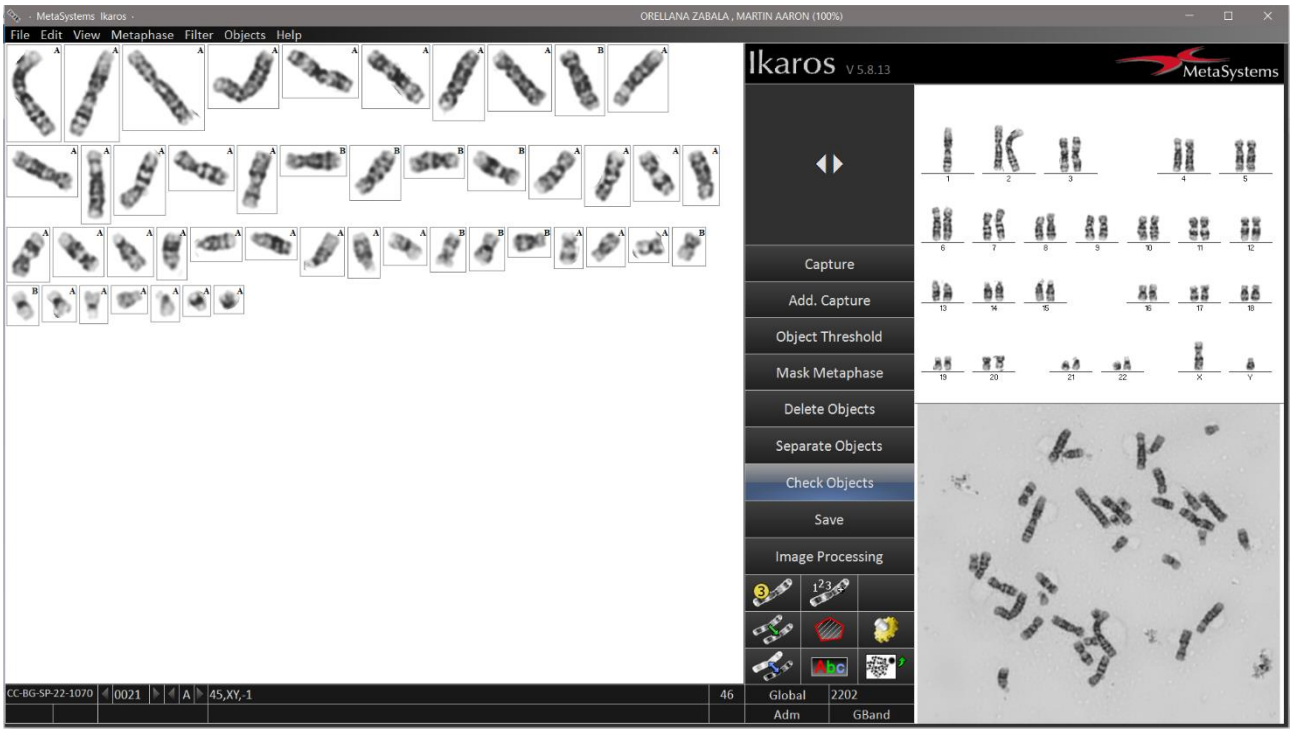
4.- The first step is to know if the chromosomes are in the correct number, so we count the photographed chromosomes. For the normal Karyotypes the correct number is 46 chromosomes, this is the preliminary analysis and it is important to do it in at least 25 metaphases, in order to rule out mosaics.

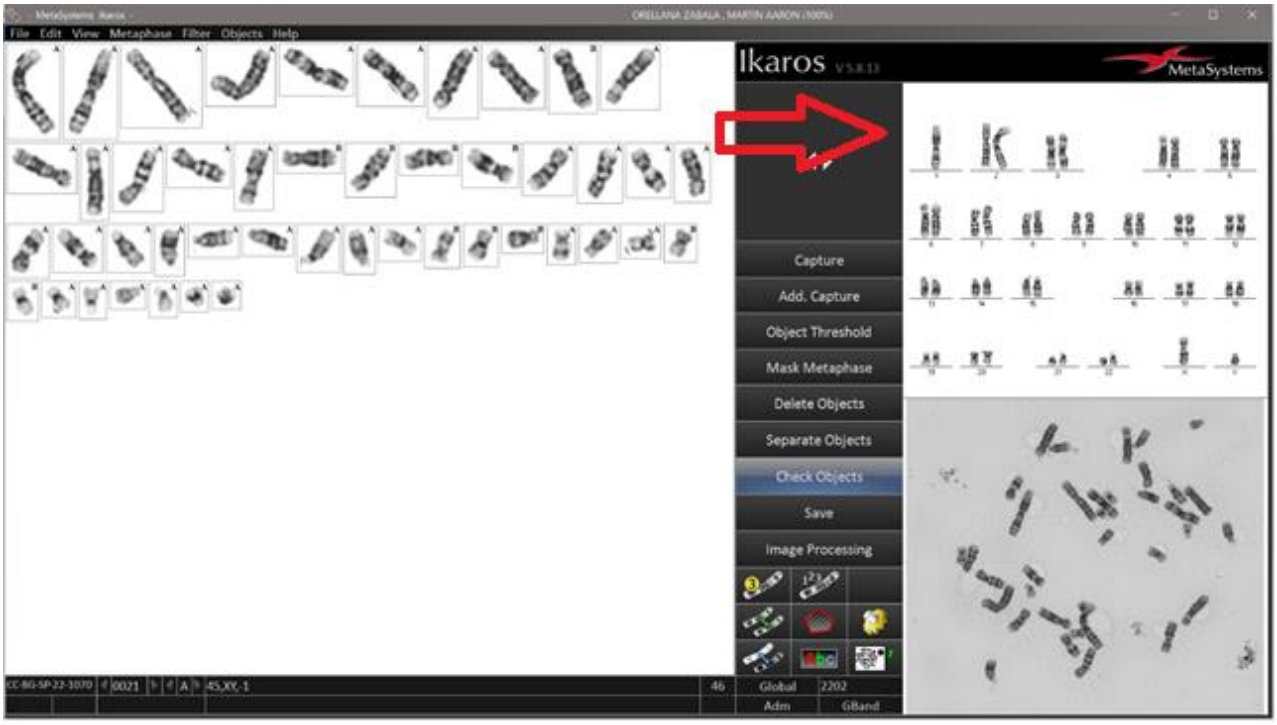
5.- We separate the chromosomes so that later they can be organized.

For this process it is important to obtain metaphases in which the chromosomes are not overlapped, in this way their separation and analysis will be easier.



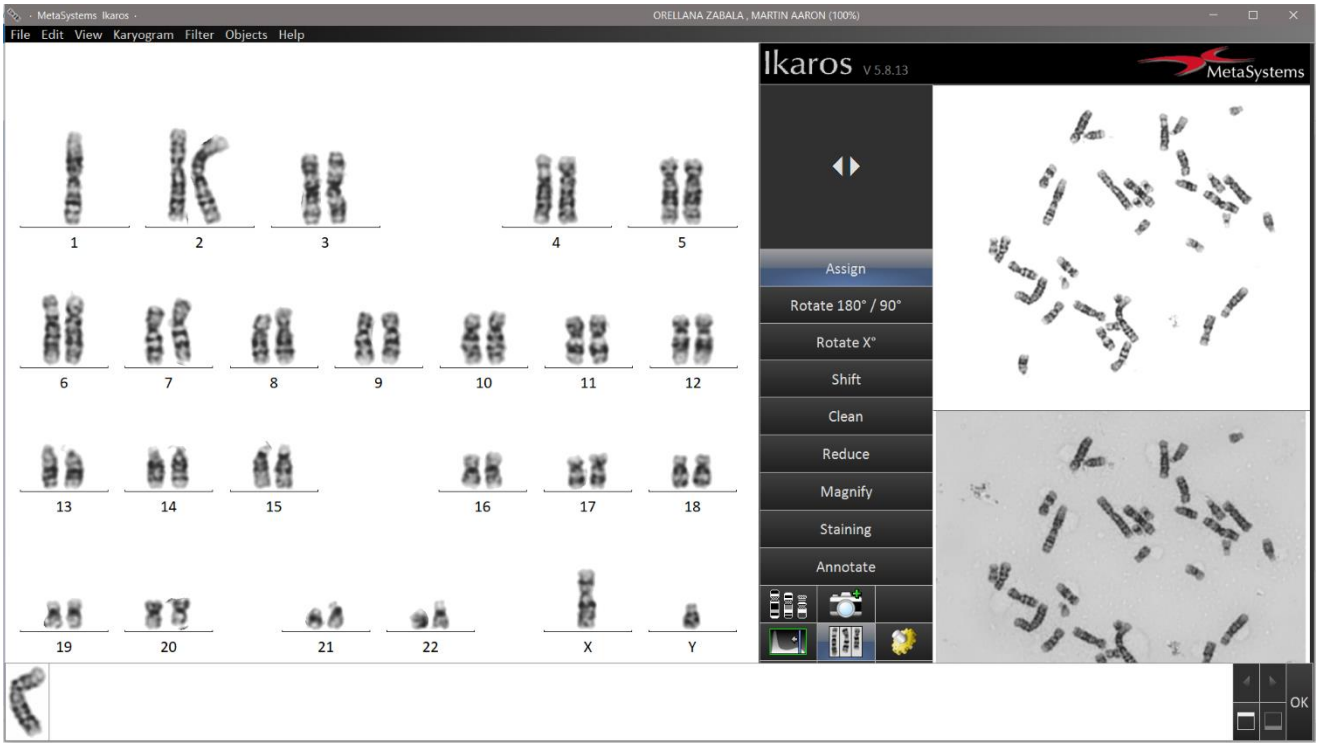
6.- once the chromosomes have been separated, we take them to the template available in the software used for their analysis.





7.- We organize each of the chromosomes in pairs from chromosome one to 22 and end with the sex chromosomes.

For its analysis we observe the number, size and pattern of bands, in addition to any visible structural alteration.



Results report

CENTRO ESPECIALIZADO EN GENÉTICA MÉDICA

LABORATORIO DE CITOGENÉTICA CONVENCIONAL

Informe de Resultado

Fecha de Entrega: 17/2/2022 **Código CEGEMED:** CC-BG-SP-21-0819

Establecimiento de Salud Solicitante: Hospital de Especialidades, Portoviejo - Portoviejo

DATOS DEL PACIENTE

Apellidos del Paciente: BRIONES ALCIVAR **Nombres del Paciente:** IVANA MARIA

Cédula de Ciudadanía / Pasaporte: 1315796357 **Edad:** 13

Sexo: Femenino

Motivo de solicitud: ASESORAMIENTO GENÉTICO

DATOS DE LA MUESTRA

Fecha de la Toma de Muestra: 15/10/2021 **Tipo de Muestra:** Sangre Periférica

Calidad de la Muestra: Buena **Volumen de la Muestra:** 3mL

DATOS DE LA TÉCNICA

Tipo de Bandeo: Bandeo G **Metafases Analizadas:** 3

Resolución de Bandeo: 400 **Cariotipos Analizados:** 2

RESULTADO

46,XX **Analista Responsable:** Génesis García D.

Interpretación:

El análisis citogenético mostró un cariotipo femenino 46,XX en todas las células examinadas. No se encontraron aberraciones numéricas o estructurales al nivel de resolución de bandas indicado.
No se excluyen mosaicos pequeños ni enfermedades genéticas causadas por mutaciones puntuales u otros cambios no detectables con el método usado.

Se sugiere asesoramiento genético.

GENESIS DANIELA GARCIA DIAZ
Firmado digitalmente por GENESIS DANIELA GARCIA DIAZ

MSc. Génesis García D.

FERNANDO PAUL CRUZ QUISHPE

Dr. Fernando Cruz Q.

CENTRO ESPECIALIZADO EN GENÉTICA MÉDICA

DATOS GENERALES

Apellidos del Paciente: BRIONES ALCIVAR

Nombres del Paciente: IVANA MARIA

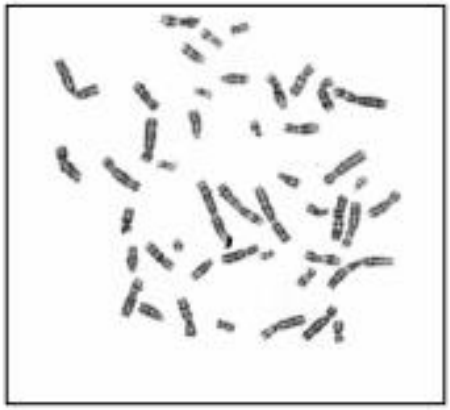
Código CEGEMED: CC-BG-SP-21-0819

Tipo de Bandeo: Bandeo G

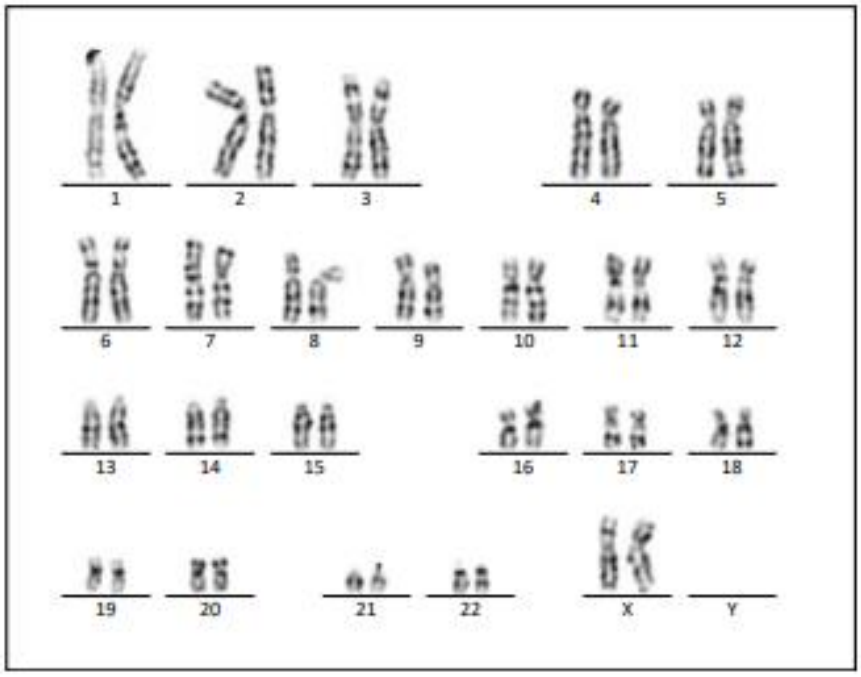
Resolución de Bandeo: 400

Resultado: **46,XX**

REPRESENTACIÓN - METAFASE



REPRESENTACIÓN - CARIOTIPO DIAGNÓSTICO



GENESIS DANIELA GARCIA DIAZ
Firmado digitalmente por GENESIS DANIELA GARCIA DIAZ

MSc. Génesis Garcia D.
LÍDER DEL LABORATORIO DE CITOGENÉTICA

FERNANDO PAUL CRUZ QUISHPE

Dr. Fernando Cruz Q.
ESPECIALISTA EN GENÉTICA CLÍNICA

THANKS

Thanks

I am infinitely grateful to my family, friends, and co-workers for their support in this work. I hope it will be useful for future projects and research in this field in Ecuador.

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