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RESEARCH ARTICLE

CHROMOSOMAL ANOMALIES IN INFERTILE COUPLES

Pranav Patel[1], Jigar V Suthar[2], Rajesh K Patel[2] and Jenabhai Chauhan[1]

1. P. G. Department of Genetic, Ashok and Rita Patel Institute of Integrated Studies in Biotechnology and Allied Sciences (ARIBAS), VallabhVidyanagar- 388121, Gujarat, India.
2. Genetic Group of Gujarat Diagnostic Centre (GGGDC), Mehsana-384002, Gujarat, India.

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Abstract

Infertility is a major social and multi-factorial sexual fitness conditionresponsibleforaffecting14%ofIndianpopulation in India. Lack of awareness, literacy, inadequate health care facilities, financial problems, etc, are the principal reasons for infertility especially in rural India. The present study describes the chromosomal aberrations associated with reproductive failure. A total of 68 samples, including 4 controls were collected based on sample selection criteria from the North Gujarat region for the present study. Lymphocyte culture was performed in HiKaryo XL RPMI- 1640 for 72 hrs to obtain metaphase stage for karyotyping. GTG banded chromosomes were analyzed by using an automated microscopy system (Carl Zeiss) using Meta Analysis Software. From 68 samples, 63 samples (92.65%) had no chromosomal anomalies. Four samples exhibited heterochoromatin polymorphic variation; 46,XX,9hq+, 46,XY,14ps+ and 46,XX,21ps+, whereas one sample exhibited paracentric inversion 46,XY,inv (8)(p21:q22). Polymorphic variants in chromosomes like 46,XY,14ps+ and 46,XX,21ps+ observed, which are basically pseudo satellites that contain secondary constrictions. The polymorphic variations and structural chromosomal aberration and their association with reproductive failure are discussed in the present studies.

Introduction:-

Existence of infertility in human society has been known since ages, but during the United Nations International Conference on Population and Development held in 1994, infertility was first officially acknowledged as a core component of reproductive health (UNPF,2014). Infertility is a major social and multi-factorial sexual fitness conditionaffecting14%ofIndianpopulation in India (The World Population Prospects: The2017Revision). Infertility exists in rural as well as urban area. Lack of awareness, literacy, inadequate health care facilities, and financial problems are the principal reasons for infertility in rural areas (Dattaet al., 2016; Sarkar and Gupta, 2016). However, infertility is more observed in urban areas compared to rural areas in India (Sarkar and Gupta, 2016). Present day, infertility is alarmingly high due to changes in lifestyle (Agenor and Bhattacharya, 2015). Infertility is not gender biased; both male and female are equally responsible for reproductive failure. More than 35% male factors, 25% combined male and female factors and 35% of female factors are accountable for reproductive complications (Speroff, 1999; GadaSaxenaet al., 2012).

Corresponding Author:- Rajesh K Patel
Address:- Genetic Group of Gujarat Diagnostic Centre (GGGDC), Mehsana-384002, Gujarat, India.

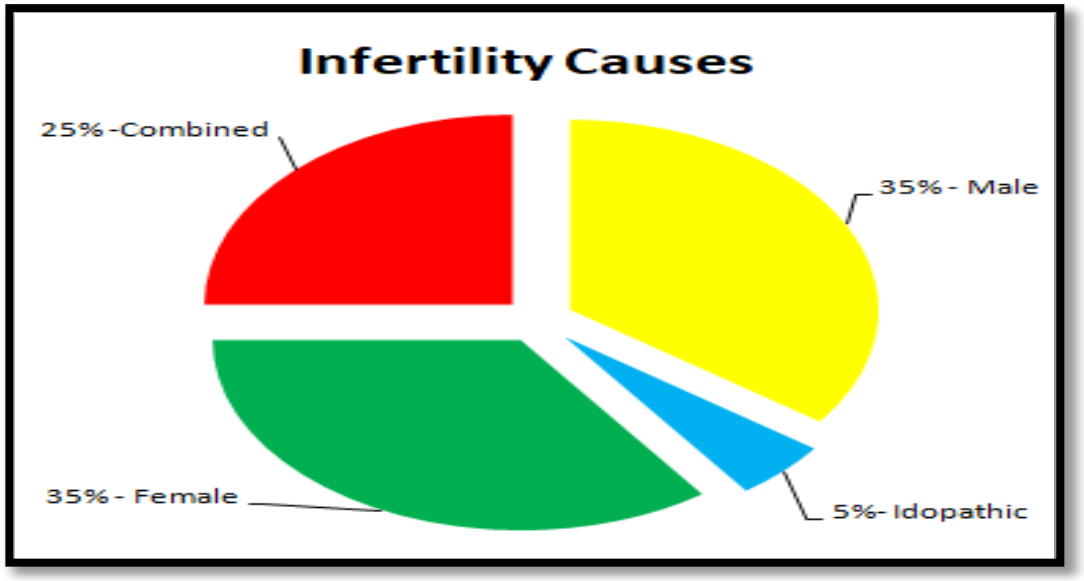

Fig 1:- Distribution of factors responsible for Infertility.

There are 3 major types of infertility: i) Primary Infertility that is inabilitytoconceivewithintwoyears accounts for more than 60% of infertility in women (Masoumi, 2015), ii) Secondary infertility is inability to conceive after first pregnancy accounts for 30% - 40% among infertile patients (Masoumi, 2015) and iii) Idiopathic Infertility is unexplained infertility, where the cause remains unknown even after an infertility analysis (Semen analysis, assessment of ovulation and fallopian tubes, genetic analysis). In idiopathic infertility, couples usually exhibit higher estradiol level and significantly lower levels of FSH and LH (Arafaet al., 2018). Idiopathic infertility has about 1-3% chance of successful conception without treatment. Boivinet al. (2007) estimated global infertility by summarizing prevalence data from seven studies: five from developing countries and two from developing countries. A Demographic and Health Survey (DHS) report also estimated infertility in developing countries using survey data from 47 national DHS surveys (Rutstein and Shah, 2004). The main challenges in generating global estimates of infertility are the scarcity of population-based studies and the inconsistent definitions used in the few high-quality studies available (Gurunathet al., 2011; Dyer, 2009). Infertility in developing and developed countries accounts for 6.9% - 9.3% and 3.5% to 16.7% respectively (Masoumi, 2015). Infertility prevalence is highest in South Asia, Sub-Saharan Africa, North Africa and the Middle East, Central and Eastern Europe, and Central Asia (Inhorn and Patrizio, 2014). Common types of infertility inwomen are; Irregularperiods, painful and heavy periods, no periods, pain during intercourse, hormonal fluctuations associated with skin issues, reduced sexdrive, facial hairgrowth, thinninghair, weightgain, etc. Similarly, Common Signs of Infertility inmen are changes in sexual desire, testicle pain orswelling, erection and ejaculation problem, the small size of testicles etc.

Additionally, common reproductive health problems prevalent among women such as Endometriosis, uterine fibrosis, cancer, interstitial cystitis, polycystic ovary syndrome (POCS), menopause leads to complications in reproduction and/or Infertility (Greilet al., 2016; Valorianiet al., 2016; Henson et al., 2017). Infertility can be caused due to defects in the reproductive system, acquired defects due to damage, environmental factors as well as genetic defects (De novo or passed down from generations). Genetic factor responsible for infertility, mostly involves aberrations or alterations in chromosomes. Several factors responsible for male infertility include anti-sperm antibodies, varicocele, cryptorchidism, semen abnormalities, infections, testicular malignancy, hormonal imbalance, erectile dysfunction, accidental causes, environmental influences, or genetic factors. The diagnosis of infertility is challenging as various factors are responsible for infertility as well as environmental and genetic factors play a crucial role. There are various kinds of tests available to monitor infertility in childhood as well as in adults; however, the present study describes the chromosomal aberrations associated with infertility.

Materials and Methods:-

Total 68 samples were collected (34 couples including 2 couples as a control) from the North Gujarat region of India were selected for the present study. The experimental design of the study was evaluated by the Institutional Committee of ARIBAS. Couples above 18 years and below 45 years of age who are suffering from primary infertility

for a period of more than 1 year or suffered multiple miscarriages were selected after explaining study purpose and signing the consent. Blood samples (3 – 5 ml) were collected in sterile heparinized Vacutainer tubes by the medical practitioners. After collection of samples, they are transported in cool condition and stored at 4^0C till lymphocyte culture. Peripheral blood leukocyte culture of the samples was performed within 24 hours. A few drops of blood (Approximately. 0.5ml) was added to the 15ml falcon tube (Tarson) containing 10 ml of HiKaryo XL RPMI- 1640 complete media (Himedia) in sterile conditions under Laminar Air Flow Unit. Cultures were mixed well after the addition of blood. Cultures were incubated at 37°C for 72 hours. Approximately 60 µl of Colchicine (2 mg/10 ml) was added to the cultures, one hour prior to the harvesting. The cells were separated by centrifugation at 1500 RPM for 5 minutes, followed by hypotonic treatment with 0.075 M KCl for 30 minutes at 37°C and fixed in 3:1 ratio of methanol and acetic acid glacial. The cell suspension was dropped on slides and air dried. Conventional staining by Giemsa and routine GTG banding with little modification (Sugaliet al., 2016) were performed on chromosome slides. Finally, around 100 Giemsa stained and G-banded metaphase plates were screened for chromosomal analysis by using automated microscopy system Meta analysis software (Carl Zeiss).

Results and Discussion:-

A total of 68 samples, including 4 controls were collected based on sample selection criteria. Of 68 samples, 63 samples (92.65%) had no chromosomal anomalies. (Fig. 2 and Fig. 3) whereas, 5 samples (7.35%) had chromosomal abnormalities. Among the 5 abnormal samples, 4 samples (5.88%) showed heterochromatin polymorphism (Fig. 4 & Fig. 5) while only 1 sample (1.41%) had a structural chromosomal abnormality (Fig. 6).

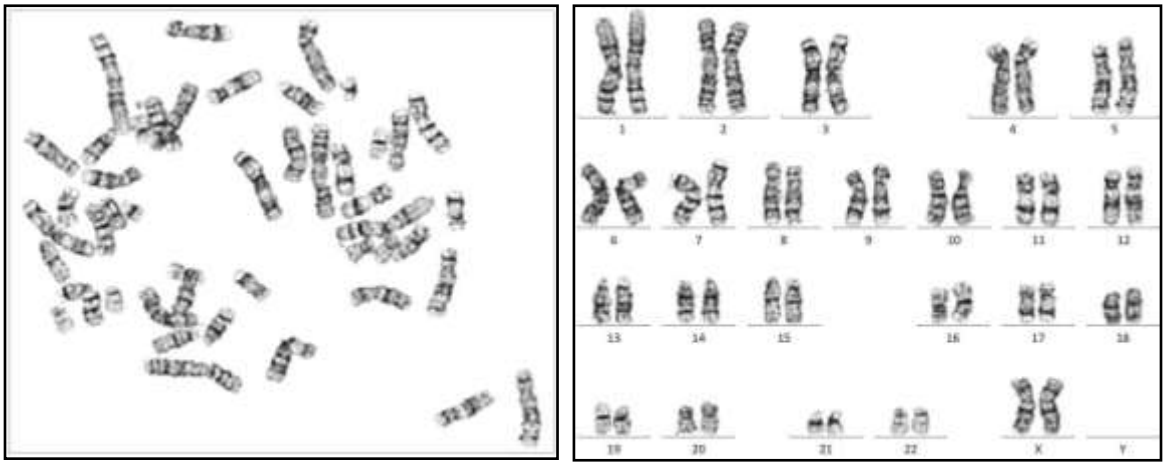

Fig 2:- Normal female metaphase chromosomes (2n = 46, XX) and karyotype.

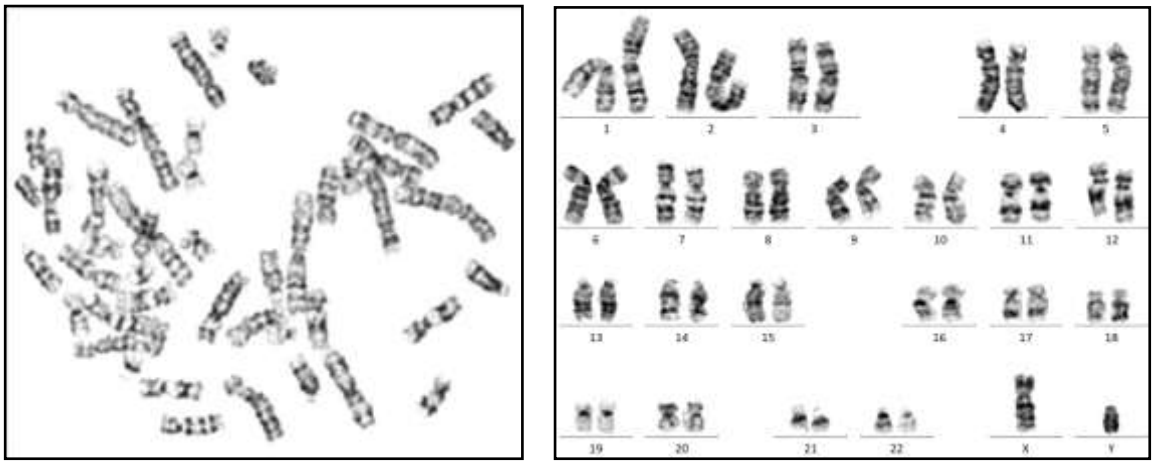

Fig 3:- Normal male metaphase chromosomes (2n = 46, XY) and karyotype.

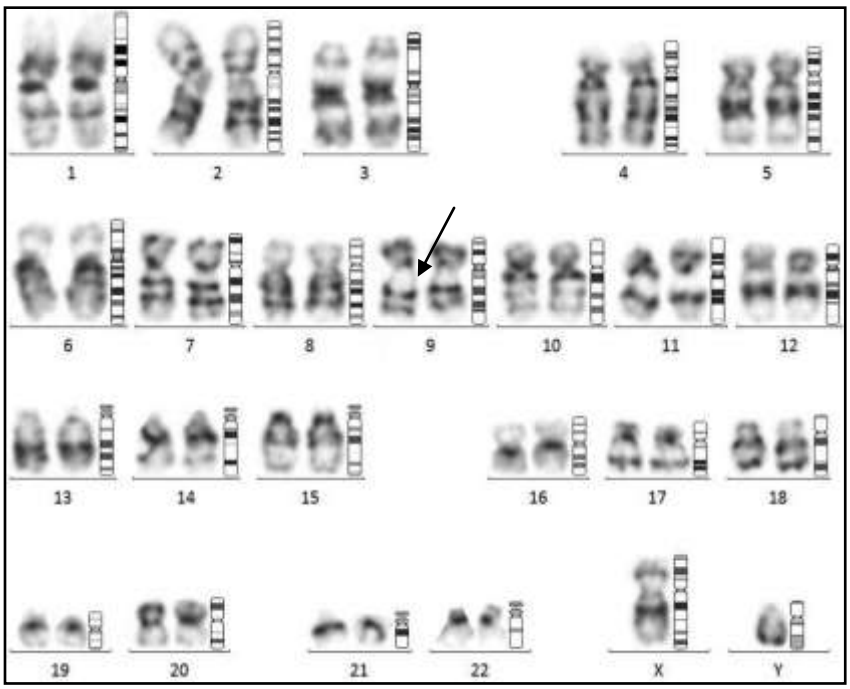

Fig 4:- G-Banded karyotype showing 46,XX,9hq+. Arrow indicates 9hq+ .

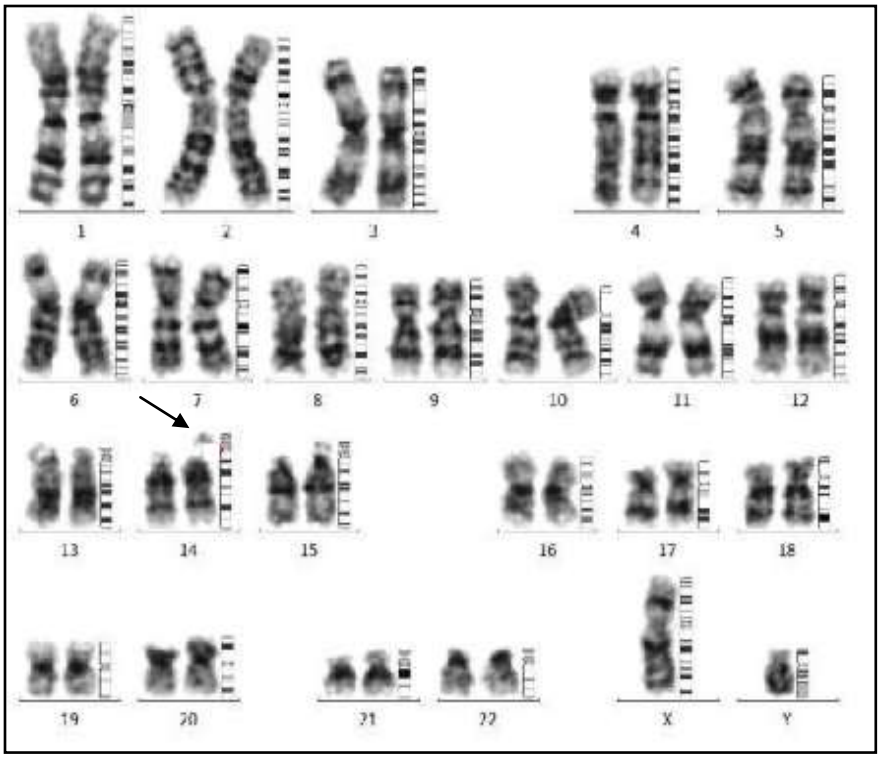

Fig 5: G-Banded karyotype showing 46,XX,14ps+. Arrow indicates 14ps+.

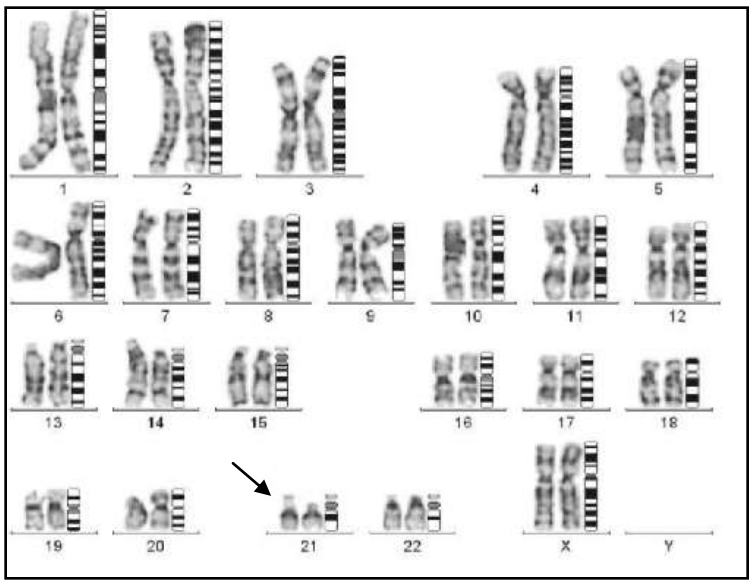

Fig 6:- G-Banded karyotype showing 46, XX,21ps+. Arrow indicates 21ps+.

As per the results noted in the present study, out of 64 samples, 3 samples exhibited structural polymorphic variations; 46,XX,9hq+, 46,XY,14ps+, 46,XY,inv (8)(p21:q22) respectively and two samples exhibited 46,XX,21ps+. The overall prevalence of structural polymorphic variations was found to be 7.8 % in North Gujarat. Polymorphic variants in heterochromatic regions of chromosomes 1, 9, 16 and Y are known to occur in the general population (Borgaonkar, 1997; Shaffer and Tommerup, 2005) however, higher frequencies in these variations have been recently reported with reproductive failure as compare to normal population. Minocherhomjiet al., (2009) reported the frequency of 9qh+ was statistically significantly increased in women with primary infertility and in men with severe male infertility suggests a possible correlation between 9qh+ variant and infertility.

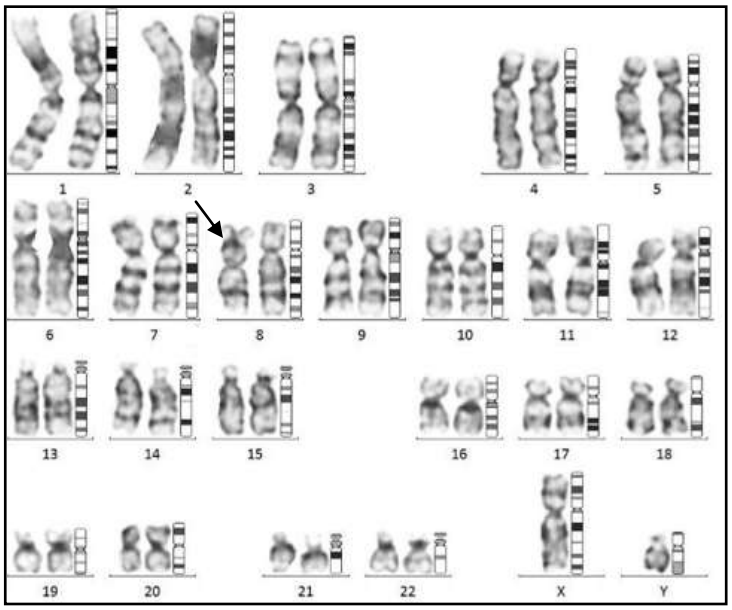

Fig 7:- Karyotype showing 46,XY,inv (8)(p21:q22). Arrow indicates paracentric inversion.

Polymorphic variations in chromosomes like 46,XY,14ps+ and 46,XX,21ps+ observed in the present study, was also observed by many researchers in their studies (Mierla and Stoian, 2012; Minocherhomjiet al., 2009). These variations are basically pseudo satellites that contain secondary constrictions serve as identifying markers. Besides

the centromere one or more secondary constriction can also be observed in some of acrocentric chromosomes at metaphase that contains a segment that is separated from the main body of the chromosome by such a secondary constriction (Sullivan et al., 2001). According to Anuradha et al., (2002) these satellite associations might predispose chromosome to non-disjunction, and hence, lead to translocations as a significant increase of satellite associations in the recurrent miscarriage couples has been reported in their study. One case of paracentric inversion, 45,XY,inv (8)(p21:q22) was also observed in an infertile patient. Various kinds of inversions were also reported in infertile and reduced fertile in males and females (Mierla and Stoian 2012; Minocherhomjiet al., 2009; Clementiniet al., 2005). Precisely, constitutional aberrant karyotypes can account for infertility or recurrent pregnancy loss. When present in the germ lineage, chromosomal abnormalities can be segregated in gametes and transmitted to the offspring, while in other cases, they can hamper meiosis up to the arrest of gametogenesis, or may give rise to unbalanced gametes (Makino et al., 1990; McFadden and Friedman, 1997; Wilkins-Hauget al., 1997; Lawler and Gearhart, 1998; Pao-Lin Kuo, 2002; Gekas et al., 2003). In mammals, gametes carrying chromosomal aberrations have a poor chance of successfully undergoing fertilization.

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