American Journal of Science, Engineering and Technology
2026, Vol. 11, No. 3, pp. 239–245
https://doi.org/10.11648/j.ajset.20261103.23

SciencePG
Science Publishing Group

Research Article

Chromosomal Heteromorphisms and Primary Male Subfertility: A Case–Control Cytogenetic Study from South India

Santhosh Kumar Jamithireddy[1, *], Rabi Prasad[1], Pavani Upendram[2]

[1]Department of Biotechnology, Gandhi Institute of Engineering and Technology University (GIET University), Gunupur, India

[2]Department of Genetics, Krishna Institute of Medical Sciences, Hospital (KIMS), Secunderabad, India

Abstract

Chromosomal heteromorphic variants are usually regarded as benign; nevertheless, some studies have found an association with subfertility, and their clinical importance is still uncertain. The present case–control study was carried out in order to assess the link between chromosomal heteromorphisms and male subfertility in a South Indian population. A total of 1,200 South Indian men were included in the study, comprising 600 subfertile men who had been referred for chromosomal analysis and 600 fertile men who served as controls. For the chromosomal assessment, standard cytogenetic methods were used, such as culturing peripheral blood lymphocytes, G-banding, and karyotyping. The results indicated that the frequency of chromosomal heteromorphisms was significantly greater in the group of subfertile men than in the control group (18.3% versus 7.5%), with a statistically significant association (OR = 2.77, 95% CI: 1.92–3.99; $p < 0.001$). Of the variants, Yqh− and 9qh+ were found to have statistically significant associations with male subfertility, while inv (9) showed increased odds although this was not statistically significant. The findings thus suggest a possible association between chromosomal heteromorphic variants, especially those involving chromosomes Y and 9, and male subfertility.

Keywords

Subfertility, Heteromorphisms, G-Banding, Chromosomal Analysis, Karyotyping, Cytogenetics, Spermatogenesis

1. Introduction

According to the World Health Organisation (WHO), subfertility is the failure to achieve pregnancy after a minimum of 12 months of consistent, unprotected sexual activity [1]. Subfertility is considered one of the major clinical issues in all societies, affecting approximately 8–12% of couples globally and around 4–6% of couples in India, according to the 1982 census [2]. Subfertility is considered a public health concern by the World Health Organisation (WHO) [3]. Male factors are one of the major causes and are responsible for approximately 50% of cases in the subfertile population. Among these, idiopathic (unknown) factors account for about 30–50% of cases [4]. Other factors include various sperm abnormalities (obstructive or non-obstructive spermatogenesis), hormonal imbalances, anatomical issues, infections, injuries, exposure

*Correspondence: Santhosh Kumar Jamithireddy (jsanthoshkumar@giet.edu)

Received: 3 September 2026; **Accepted:** 15 September 2026; **Published:** 28 September 2026

to gonadotoxins, abnormal sperm DNA fragmentation, or genetic abnormalities [5]. Idiopathic male subfertility can be affected by several genetic abnormalities, according to previous studies. Among these numerical (Aneuploidy), structural (Balanced/Unbalanced translocations), and hetero/polymorphic (satellites, stalks) variants of chromosomes are seen in both Autosomes (Somatic chromosomes) and Allosomes (sex chromosomes) [6, 7].

Among all genetic disorders, the prevalence of chromosomal abnormalities in the normal population is around 0.6%. However, various studies have reported an increased prevalence ranging approximately between 2% and 14% in subfertile men. This difference significantly highlights the important role of chromosomal abnormalities and their contribution to male subfertility. Microscopically visible variations in these sections are commonly observed during standard karyotyping. These are defined as chromosomal heteromorphisms, these variations include size, morphology, banding patterns at the constitutive heterochromatin regions of chromosomes 1, 9, 13, 14, 15, 16, 21, 22, Y. These variants present in 2–5% of the general population are considered normal variants, in clinical practice of cytogenetics [8, 9]. The role of these chromosomal heteromorphic variants in the clinical practice during the interpretation of the karyotyping reports remains unclear, and their clinical significance is not fully understood [10]. According to the latest edition of the International System for Human Cytogenetic Nomenclature (ISCN-2024), these observations are classified as polymorphisms. Earlier studies considered heterochromatin regions as repetitive, non-coding gene sequences with minimal clinical significance and undetermined disease-causing effects [11]. Current research shows that the association and prevalence of these variants in male subfertility are three to five times higher than in the fertile population and women [12]. Cytogenetic studies enable us to understand the association between these heteromorphisms and male subfertility, and contemporary studies propose that the significance of these heteromorphisms in male subfertility should not be denied [13, 14].

In this study, we identified the association of chromosomal heteromorphisms present in the subfertile male population and to compare these frequencies with those of a control group consisting of fertile males by conducting chromosomal analysis study.

2. Materials and Methods

2.1. Study Population

This case control study included 1200 cases in that 600 men diagnosed with primary subfertility and 600 fertile men with proven paternity of south Indian region. The average age of the patients was 32.2 (±5.5). Individuals with a history of obstructive Sub-Fertility, systemic illness, or exposure to gonadotoxic agents were excluded. Additionally, cases showing numerical or structural chromosomal abnormalities were excluded from the analysis. The Ethics Committee of KIMS (Krishna Institute of Medical Sciences) in Hyderabad granted approval (KIMS/ECBMHR/2020/07-6) for the study, and informed consent forms were obtained from all participating patients.

2.2. Cytogenetic Analysis

Peripheral blood samples were collected in sterile heparinized vacutainers under aseptic conditions. Lymphocyte cultures were established using standard cytogenetic protocols. 0.5 mL of whole blood was cultured in 5 mL of RPMI-1640 medium supplemented with 15–20% fetal bovine serum, antibiotics (penicillin and streptomycin), and phytohemagglutinin (PHA) to stimulate lymphocyte proliferation. Cultures were incubated at 37°C in a humidified atmosphere with 5% CO_2 for 72 hours.

Two hours prior to harvesting, colcemid was added to arrest cells at metaphase. The cultures were then subjected to hypotonic treatment using 0.075 M potassium chloride (Kcl) solution and incubated at 37 ℃ for 15–20 minutes. This was followed by fixation using a freshly prepared fixative (methanol: acetic acid in a 3:1 ratio), with multiple changes to ensure proper fixation.

Metaphase spreads were prepared by dropping the fixed cell suspension onto clean glass slides and air-drying. Chromosomes were stained using Giemsa after trypsin digestion to obtain G-banding (GTG-banding). Well-spread metaphases were captured and analysed using an automated karyotyping system. 20 metaphases per individual were analysed and Karyotypes were interpreted and reported according to the guidelines of the International System for Human Cytogenomic Nomenclature (ISCN, 2024). Cytogenetics flow chart has been illustrated in Figure 1.

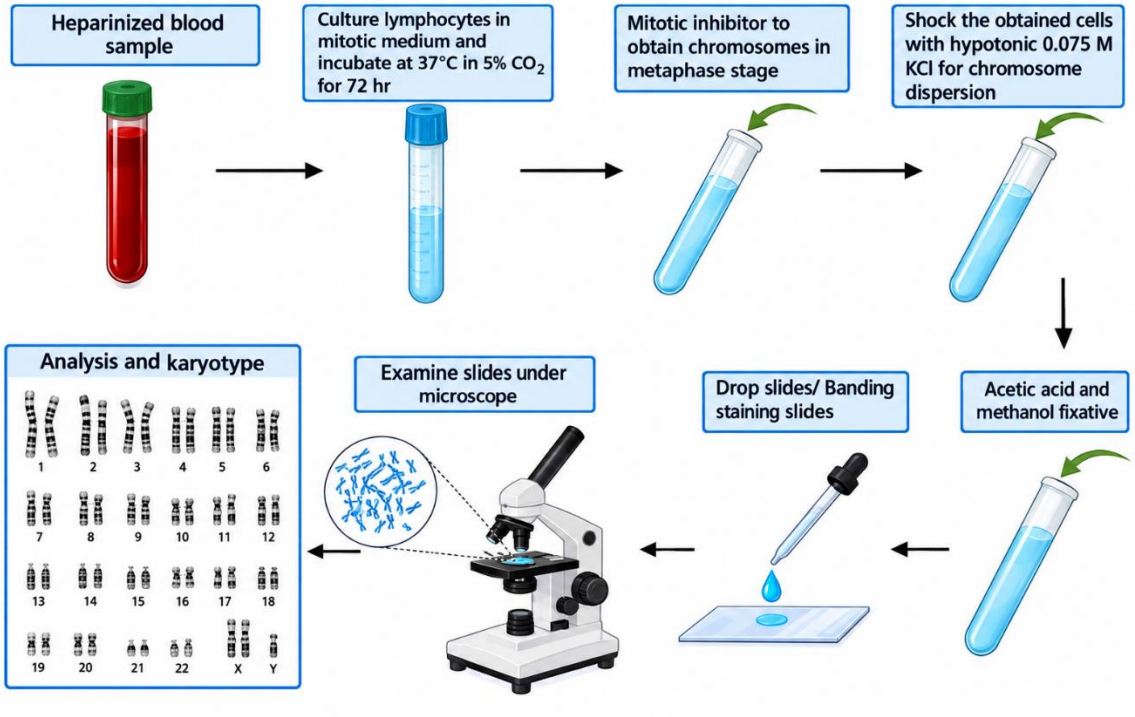

Figure 1. *The flow chart of Karyotyping procedure. Reproduced from ScienceDirect Topics (Karyotyping), courtesy of BioRender.*

2.3. Statistical Analysis

Statistical analysis was performed to evaluate the association between chromosomal heteromorphisms and primary male subfertility. The frequency of different chromosomal heteromorphisms was compared between the study group and the control group using the Chi-square (χ^2) test. This test was applied to determine whether there was a statistically significant difference in the distribution of heteromorphisms between the two groups.

To assess the strength and direction of the association, Odds Ratios (ORs) were calculated along with their corresponding 95% Confidence Intervals (CIs). The OR provided an estimate of the likelihood of chromosomal heteromorphisms in subfertile males compared to fertile controls. A 95% CI not including the value 1 was considered indicative of a statistically meaningful association.

A p-value of less than 0.05 ($p < 0.05$) was considered statistically significant. All statistical analyses were performed using appropriate statistical software.

3. Results and Discussions

Table 1 summarises the frequency of chromosomal heteromorphisms in the study and control groups. The frequency was markedly higher in the study group compared to the control group. Altogether, chromosomal heteromorphisms were shown high significance in subfertile men (18.3%) than in fertile controls (7.5%). Statistical analysis was done by using the chi-square (χ^2) test, and odds ratio analysis (OR) with 95% confidence interval (CI) was calculated to assess the strength of association. The difference was statistically highly significant ($\chi^2 = 31.3$, $p < 0.001$), indicating a strong association between chromosomal heteromorphisms and male subfertility.

Table 1. *Frequency of chromosomal heteromorphisms between subfertile and Fertile groups.*

Karyotype	Subfertile Men (n = 600)	Fertil Men (n = 600)	χ^2 value	p-value
Heteromorphisms	110 (18.3%)	45 (7.5%)		
Normal karyotype	490 (81.7%)	555 (92.5%)		
Total	600	600	31.3	<0.0001

Table 1 Represents the comparative study between study and control group with chromosomal heteromorphisms.

Table 2 represents the odds ratio of chromosomal heteromorphisms in the study and control groups. The Odds ratio analysis revealed that men with primary subfertility had 2.77 times higher odds of possessing chromosomal heteromorphisms compared to fertile controls. The confidence interval (1.92–3.99) does not cross 1, confirming statistical significance. This strongly supports the hypothesis that chromosomal heteromorphisms may be associated with impaired fertility. Similar observations have been reported in earlier studies, where an increased prevalence of heterochromatic variants was noted among infertile men. These variants, although cytogenetically classified as polymorphisms, may influence chromosomal behaviour during meiosis. Alterations in heterochromatin structure can interfere with homologous pairing, recombination, and segregation, thereby affecting spermatogenesis.

Table 2. *Odds Ratio Analysis of Chromosomal Heteromorphisms.*

Karyotype	Study Group	Control Group	OR (95% CI)	p-value
Heteromorphism present	110	45		
Normal karyotype	490	555	2.77 (1.92–3.99)	< 0.001

Table 2 represents the odds ratio of chromosomal heteromorphisms in the study and control groups.

Table 3 summarizes the distribution of various chromosomal heteromorphism types in the study and control groups, which is further depicted in Figure 1. Among all variants, Yqh− was the most frequently observed Heteromorphism in the study group and showed a statistically significant association with primary male subfertility (OR = 2.78, p = 0.002). Similarly, 9qh+ showed a significant association (OR = 5.09, p = 0.03), suggesting that chromosome 9 pericentric heterochromatic variation may contribute to defective meiosis or chromosomal instability. Although variants such as inv (Y) and inv (9) demonstrated higher odds ratios, the associations were not statistically significant. In the present study, Y chromosome heteromorphisms showed a notable association with Sub-Fertility.

The Yqh− variant showed a significant statistical association (OR = 2.78; p = 0.002), suggesting a clear association with male subfertility. Certain regions of the Y chromosome comprise genes involved in male fertility, and variations in centromeric regions may alter chromatin organization and gene regulation. These findings are consistent with previous reports indicating the involvement of Y chromosome heteromorphisms in male subfertility [15, 16].

Another significant observation was the association of the 9qh+ variant with subfertility (OR = 5.09; p = 0.03). Variants of chromosome 9 have been reported in a few cytogenetic studies to be associated with reproductive disorders [17, 18]. The high odds ratio observed in the present study supports the possible pathogenic role of this variant.

The pericentric inversion of chromosome 9 showed an increased odds ratio (OR = 2.04) but did not reach statistical significance (p = 0.06). These findings reflect the ongoing controversy regarding its clinical significance [19].

Other heteromorphisms, including Yqh+, 9qh−, and acrocentric short arm variants (13ps+, 14ps+, 15ps+, 21ps+, and 22ps+), did not show significant associations in the present study.

Although some of these variants showed high odds ratios, the lack of statistical significance in terms of number of cases. Similar limitations have been reported in previous studies, suggesting the need for larger sample sizes to establish definitive conclusions. [Figure 2] illustrates various types of chromosomal heteromorphisms identified in the present study.

Table 3. *Distribution of Chromosomal Heteromorphism Types.*

Type of Heteromorphism	Study (n=600)	Control (n=600)	Odds Ratio (OR)	p-value
Yqh+	10	5	2.02	0.18
Yqh−	35	13	2.78	0.002*
Inv (Y)	5	1	5.04	0.10
9qh+	10	2	5.09	0.03*

Type of Heteromorphism	Study (n=600)	Control (n=600)	Odds Ratio (OR)	p-value
9qh−	5	3	1.67	0.47
Inv (9)	20	10	2.04	0.06
1qh−	2	1	2.00	0.56
13ps+	5	2	2.51	0.25
14ps+	3	1	3.01	0.31
15ps+	6	3	2.01	0.31
21ps+	5	2	2.51	0.25
22ps+	4	2	2.01	0.41

Table 3 summarizes the distribution of various chromosomal heteromorphism types in the study and control groups.

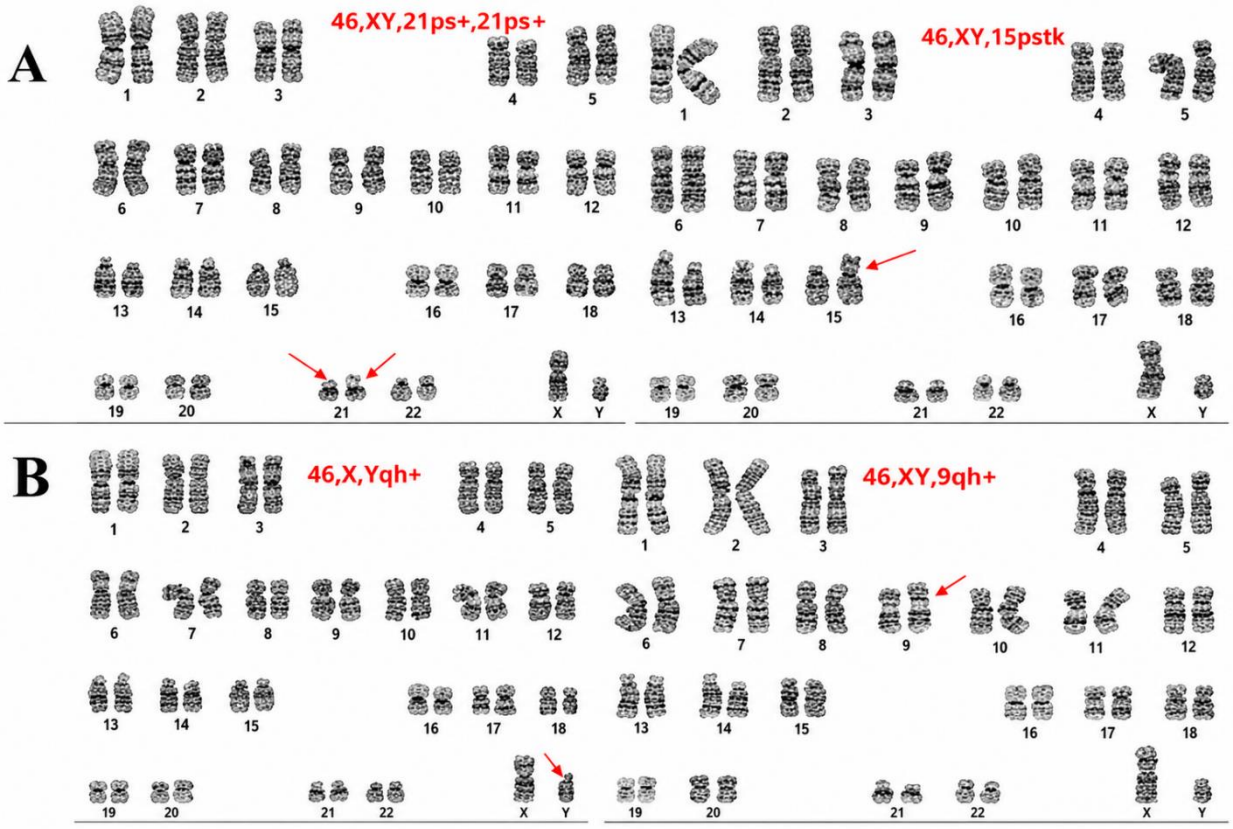

Figure 2. *Shows various types of chromosomal heteromorphisms identified in the present study. A represents (21ps+, 15pstk+), B represents (Yqh+, 9qh+).*

4. Conclusions

Chromosomal heteromorphisms are considered cytogenetic variants; however, their clinical relevance in male subfertility remains unclear. The present case–control study evaluated their association with primary male subfertility using conventional karyotyping in 600 subfertile males and 600 fertile controls. The overall frequency of heteromorphisms was significantly higher in subfertile males compared to controls (18.3% vs. 7.5%), showing a strong association with subfertility (OR = 2.77, 95% CI: 1.92–3.99; $p < 0.001$). Among the individual variants, Yqh− and 9qh+ demonstrated significant associations with subfertility, whereas inv (9) showed an increased trend without statistical significance.

These findings suggest that certain chromosomal heteromorphisms, particularly those involving the Y chromosome

and chromosome 9, contribute to impaired spermatogenesis and male reproductive dysfunction. In conclusion, chromosomal heteromorphisms may act as significant predisposing genetic factors and accurate identification and reporting of heterochromatic variants could support genetic counselling and, in the future, potentially inform personalized therapeutic approaches [20].

Abbreviations

OR	Odds Ratio
CV	Coefficient of Variation
inv	Inversions

Acknowledgments

The authors also wish to acknowledge KIMS Hospital, Secunderabad for utilizing the lab services.

Author Contributions

Santhosh Kumar Jamithireddy: Data curation, Investigation, Methodology

Rabi Prasad: Formal Analysis, Project administration, Writing – review & editing

Pavani Upendram: Conceptualization, Investigation, Methodology, Writing – original draft

Data Availability Statement

The data supporting the outcome of this research work has been reported in this manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

References

[1] Purkayastha N, Sharma H. Prevalence and potential determinants of primary infertility in India: Evidence from Indian demographic health survey. Clin Epidemiol Glob Health. 2021; 9: 162-170. https://doi.org/10.1016/j.cegh.2020.08.008

[2] Agiwal V, Madhuri RS, Chaudhuri S. Infertility Burden Across Indian States: Insights from a Nationally Representative Survey Conducted During 2019-21. J Reprod Infertil. 2023 Oct-Dec; 24(4): 287-292. https://doi.org/10.18502/jri.v24i4.14156

[3] Agarwal A, Baskaran S, Parekh N, Cho CL, Henkel R, Vij S, Arafa M, Panner Selvam MK, Shah R. Male infertility. Lancet. 2021 Jan 23; 397(10271): 319-333. Epub 2020 Dec10. https://doi.org/10.1016/S0140-6736(20)32667-2

[4] Kundu S, Ali B, Dhillon P (2023) Surging trends of infertility and its behavioural determinants in India. PLoS ONE 18(7): e0289096. https://doi.org/10.1371/journal.pone.0289096

[5] Hamada AJ, Montgomery B, Agarwal A. Male infertility: a critical review of pharmacologic management. Expert Opin Pharmacother. 2012 Dec; 13(17): 2511-31. Epub 2012 Nov 3. https://doi.org/10.1517/14656566.2012.740011

[6] Arumugam M, Shetty DP, Kadandale JS, Kumari SN. Y chromosome microdeletion and cytogenetic findings in male infertility: A cross-sectional descriptive study. Int J Reprod Biomed. 2021 Feb 21; 19(2): 147-156. https://doi.org/10.18502/ijrm.v19i2.8473

[7] Sreenivasa G, Malini SS, Kumari P, Dutta UR. Cytogenetic abnormalities in 200 male infertile cases in the southern region of India. Open J Genet. 2013; 3(2): 33-37. https://doi.org/10.4236/ojgen.2013.32A3005

[8] Šípek A Jr, Mihalová R, Panczak A, Hrčková L, Janashia M, Kaspříková N, Kohoutová M. Heterochromatin variants in human karyotypes: a possible association with reproductive failure. Reprod Biomed Online. 2014 Aug; 29(2): 245-50. Epub 2014 May 16. https://doi.org/10.1016/j.rbmo.2014.04.021

[9] Pires S, Jorge P, Liehr T, Oliva-Teles N. Challenges in classifying human chromosomal heteromorphisms using banding cytogenetics: From controversial guidelines to the need for a universal scoring system. Hum Genome Var. 2024 Oct 24; 11(1): 38. https://doi.org/10.1038/s41439-024-00295-8

[10] Arafa MM, Majzoub A, AlSaid SS, El Ansari W, Al Ansari A, Elbardisi Y, Elbardisi HT. Chromosomal abnormalities in infertile men with azoospermia and severe oligozoospermia in Qatar and their association with sperm retrieval intracytoplasmic sperm injection outcomes. Arab J Urol. 2017 Dec 23; 16(1): 132-139. https://doi.org/10.1016/j.aju.2017.11.009

[11] Pang, H., Zhang, T., Yi, X. et al. Exploring the link between chromosomal polymorphisms and reproductive abnormalities. Reprod Health 21, 130 (2024). https://doi.org/10.1186/s12978-024-01854-6

[12] Rodriguez, F., Cruz, M. & Requena, A. Impact of parental chromosomal polymorphisms on the incidence of congenital anomalies and perinatal complications in a cohort of newborns conceived after ICSI + PGT-A. Reprod Biol Endocrinol 20, 145 (2022). https://doi.org/10.1186/s12958-022-01012-2

[13] Ralapanawe, M. S. B., Gajaweera, S. L., Karunaratne, N. et al. A comprehensive analysis of chromosomal polymorphic variants on reproductive outcomes after intracytoplasmic sperm injection treatment. Sci Rep 13, 1319 (2023). https://doi.org/10.1038/s41598-023-28552-w

[14] Shen L, Sun Q, Shen L, Zhu Y, Sun Y. Impact of chromosomal polymorphisms on pregnancy outcomes after in vitro fertilization or intracytoplasmic sperm injection: A systematic review and meta-analysis. Pak J Med Sci. 2025 Dec; 41(12): 3542-3557. https://doi.org/10.12669/pjms.41.12.13037

[15] Mottola F, Roversi V, Ferrari R, et al. Polymorphic rearrangements of human chromosome 9 and male infertility: new evidence and impact on spermatogenesis. Biomolecules. 2023; 13(5): 729.

[16] Pal AK, Ambulkar PS, Sontakke BR, Talhar SS, Bokariya P, Gujar VK. A Study on Chromosomal Analysis of Patients with Primary Amenorrhea. J Hum Reprod Sci. 2019 Jan-Mar; 12(1): 29-34. https://doi.org/10.4103/jhrs.JHRS_125_17

[17] Naasse Y, Charoute H, El Houate B, Elbekkay C, Razoki L, Malki A, Barakat A, Rouba H. Chromosomal abnormalities and Y chromosome microdeletions in infertile men from Morocco. BMC Urol. 2015 Sep 18; 15: 95. https://doi.org/10.1186/s12894-015-0089-3

[18] Frouzandeh M, Saeideh S, Sanaz M. Chromosomal abnormalities in infertile men referred to iran blood transfusion organization research center. J Reprod Infertil. 2010 Oct; 11(3): 175-8.

[19] Tuerlings JH, de France HF, Hamers A, Hordijk R, Van Hemel JO, Hansson K, Hoovers JM, Madan K, Van der Blij-Philipsen M, Gerssen-Schoorl KB, Kremer JA, Smeets DF. Chromosome studies in 1792 males prior to intra-cytoplasmic sperm injection: the Dutch experience. Eur J Hum Genet. 1998 May-Jun; 6(3): 194-200. https://doi.org/10.1038/sj.ejhg.5200193

[20] Pires, S., Fran ça, I., Oliveira, P. et al. The role of satellite DNA-enriched heterochromatic variants in reproductive disorders: Insights from standardized cytogenetic analysis. Chromosome Res 34, 9 (2026). https://doi.org/10.1007/s10577-026-09800-x

Biography

Santhosh Kumar Jamithireddy is an Assistant Professor in the Department of Biotechnology, GIET University, Gunupur, Odisha, India. He holds M. Sc. in Biotechnology and has research experience in human cytogenetics, and research area in the study of chromosomal heteromorphisms and polymorphisms using conventional karyotyping. His research focuses on understanding the possible clinical significance of chromosomal variants and their potential association with male subfertility and reproductive health.

Rabi Prasad is the Head of the Department of Biotechnology, GIET University, Gunupur, Odisha, India. He is an academician and researcher actively involved in teaching, research, academic leadership, and student mentoring. His scholarly contributions include peer-reviewed research publications, and he promotes research, innovation, interdisciplinary collaboration, and academic excellence in biotechnology and allied disciplines.

Pavani Upendram is a Senior Cytogeneticist in the Department of Genetics and Molecular Pathology at Krishna Institute of Medical Sciences (KIMS). She has expertise in clinical cytogenetics, chromosomal analysis, and genetic diagnostics, with applications in genetic, hematological, and oncological disorders. She is also a certified Genetic Counselor, combining cytogenetic expertise with genetic counseling to support accurate interpretation of genetic findings and their clinical implications.