

Original Research Article

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Genetic and Anthropometrical Evidences in Infertile Male

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ABSTRACT

Infertility is becoming a major problem among the marital couples and in at least 50% of the cases, the male partners are the only contributor to the problem. Many of these men are diagnosed with no etiology and hence they are idiopathic cases. The current study was planned to generate some markers in infertile males in order to find a preventive measure that could be taken for effective cure of this serious issue. Study conducted in two arms between the age group 18-45. Arm A composed of healthy control male having 150 volunteers and in B arm 50 infertile males. 30 volunteers from arm A having less than two inches AGD therefore finally they were included in arm B. Hence, the total infertile male in arm B was 80 males. A preliminary investigation was done by Physical examination, Anogenital Distance measurement followed by semen analysis, Lymphocyte culture to rule out chromosomal. Among the 60% of infertile + <2 inch AGD) males having Anogenital distance less than 2 inches and having abnormal sperm morphology, less sperm count, motility, viscosity, azoospermia, oligospermia. On the other hand, 70% of the total individuals revealed abnormal chromosomal pattern as compared with the healthy individuals. The study signatures with less anogenital distance and marked chromosomal abnormalities in the infertile male. Moreover, the individuals having less AGD with associated chromosomal defects will certainly identify males at high risk of infertility. This will help the unmarried male individuals get interventional therapy or may undergo another method of therapeutic modality so that the status of a healthy male partner after marriage may be achieved.

Keywords

Anogenital Distance, Chromosomes, Infertility, Semen analysis, Sperm.

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Introduction

The semen quality has been reported declines from the past half century and the births of male child's with genital abnormalities (Carlsen *et al.*, 1992; Davis *et al.*, 1998; Skakkebaek *et al.*, 2007). The aetiology is uncertain that altered testicular development is due to environmental factors which lead to abnormal androgen action and disrupt the normal endocrine signaling (Greenham *et al.*, 1977; Velde *et al.*, 2010). Anogenital distance has been used by the investigators as

a major development of genital and status of androgen in both Investigators have used the anogenital distance AGD) as a measure of genital development and androgen status to determine the reproductive toxicities in both humans and experimental animals (Jaime *et al.*, 2011; Zabarenko *et al.*, 2011; Eisenberg *et al.*, 2012). At the rate of 3% and 8% primary and secondary infertility have been reported respectively (WHO, 1980). In Maharashtra a village level study revealed

that the population shows 6 to 7 % cases of infertile couples Bang *et al.*, 1989). In other states of India, the rate of infertility was found as Haryana 2.25%, Rajasthan 3.57%, Madhya Pradesh 4.23%, Punjab 6.73%, Karnataka 8.72%, Arunachal Pradesh 10.92% (Datta *et al.*, 2010). In some cases, 14% of ovarian problems and 6% of infertile males are having been reported due to chromosomal anomalies.

Couples with primary infertility have never been able to conceive, while on the other hand, secondary infertility does have difficulty in conceiving after being conceived once. Technically secondary infertility will not be presentable if there is a change of partners (Makar *et al.*, 2002).

Materials and Methods

Study population

The present Experimental work was conducted in the Department of Research, JNCH&RC from 2013 to 2016. The research work was approved by the Institutional Ethical Committee, (IEC 546/16.03.16). It is a cross-sectional study and the subjects were enrolled from OPD, referred cases from IVF centers, camps, and volunteers from different section of society, based on the selection criteria study enrolled 200 subjects in two arms between the age group 18-45. Arm A composed of healthy control male having 150 volunteers and in B arm 50 infertile males. 30 volunteers from arm having less than two inches AGD therefore finally they were included in arm B. Hence, the total infertile male in arm B was 80 males. A Preliminary investigation was done by Physical examination, Anogenital Distance measurement followed by semen analysis, Lymphocyte culture to rule out chromosomal Anomalies.

Among the registered subjects, 25% were having the history of primary and secondary infertility and 34.5% having less anogenital distance and the rest of the individuals 60% were healthy with normal sperm count and AGD more than 2 inches. Before taking a sample the subject was asked to fill the informed consent, all other questionnaires and pedigree charts were prepared to know the medical history to calculate the risk factor of infertility if any (Williams *et al.*, 2001).

Physical examination and anogenital distance measurement

Before the physical examination, subjects were asked to remove the pubic hairs and wash the inguinal area to maintain the hygiene for the aseptic conditions. Physical examination was performed to find out any sexual ambiguity, scrotum disorder, undescended testis, varicocele, and micropenis if any. The measurement of Anogenital Distance (AGD) was taken from the center of the anus to the base of the scrotum and the center of the anus to the lower base of the penis with the help of vernier caliper (span diagnostic) on the examination table and then the mean was calculated. At the same day inguinal swab, semen, and blood samples were also taken for bacterial culture, semen analysis for sperm morphology and sperm count and blood sample for cytogenetic to rule out numerical and structural chromosomal abnormalities.

Semen sample analysis

A semen sample was collected in a sterile container by masturbation method in a sample collection room of the hospital by keeping abstinence of 3-5 days. Semen analysis was done for semen volume, sperm concentration and morphology for both the groups as per the WHO guidelines of 2010.

Lymphocyte culture for chromosomal assay

In the heparinized vacutainers two ml of Peripheral venous blood was collected in the aseptic condition and the culture was set up as per the standard modified protocol (Moorhead *et al.*, 1960; Ganesh *et al.*, 2004; Tahir Mohiud-Din Malla *et al.*, 2011; Chinnu Sugavanam Senthilkumar *et al.*, 2015). Then the blood sample was transferred into sterile culture tubes having 5 to 8 ml RPMI 1640 culture medium Himedia AT028) supplemented with 15 % Fetal bovine serum Himedia Labs, India), antibiotics Penicillin - streptomycin solution Invitrogen, California, USA), Gentamicin Ranbaxy) and phytohaemagglutinin Gibco life technologies 10576-015). Once the culture was set it was incubated at 37⁰C for 72 hours in CO₂ incubator Heraeus). Before harvesting usually 2 to 3 hours) 50 µl of colchicines Himedia CMS342-14) was added to arrest the cells at metaphase for 40 to 45 minutes as per standardization of JNCHRC. After incubation of 72 hours, the sample was transferred into the centrifuge tubes and centrifuged for 10 minutes at 2100 rpm. The supernatant was discarded and the pellets were treated with freshly prepared freshly prepared hypotonic solution 0.57% KCl suspended by flushing gently and cyclomixed. Then the centrifuged tubes were incubated for 17 minutes at 37⁰C. Then the processes of centrifugation were repeated followed by the addition of freshly prepared pre-chilled 3:1 methanol and acetic acid Carnoy's fixative) was added to the pellet while mixing on cyclomixer. After the overnight stand, the carnoy's fixative wash was repeated as many times as necessary until clear cells were obtained at the bottom of the tube. Then the chromosomes were prepared by dropping the cell suspension on a clean grease free slide, where the drop spreads out and chromosome get fixed to the slide. Once the slides prepared the slides were stained

with 1% freshly prepared Giemsa stain (Seabright 1971). All the Giemsa-stained slides were observed under Phase contrast, Olympus BX60) microscope of 10X objective lens and 100X oil immersion) lens was used for the minute chromosomal aberration. Thirty to fifty well-spread metaphases were analyzed and the aberrations were recorded.

Results and Discussion

The Anthropometry measurement of healthy and infertile male individuals was measured and it was found that the mean AGD) anogenital distance of infertile individuals was less 1.68 ± 0.117 when compared to healthy control individuals whose mean AGD was 2.86 ± 0.29 as shown in table 1 and graph 1. Out of 200 registered cases, 25% was registered for the primary and secondary infertility and 15% of healthy cases were found to have AGD less than two inches followed by a less sperm count, azoospermia, oligospermia and chromosomal anomalies same as infertile subjects. The result of the above study revealed that Infertile males and subjects with less AGD semen sample experienced abnormal sperm morphology and less sperm count as compared to healthy males as shown in figures 2 and Graph 2. In addition, Infertile males also scored higher frequency of average Chromosomal aberrations than the healthy individuals as shown in Graph 3 and showed both types of aneuploidy, i.e. Hyper and Hypodiploid, in the case of infertile males as shown in Figure 3 and a few infertile male sample experienced chromosomal fragments which may lead them towards infertility.

In conclusion fertility is the boon in the society of mammalian because motherhood has been glorified, welcomed and given special position in the family as well in the society.

Table.1 showing the average AGD of healthy and infertile subjects in which infertile subjects showing less AGD as compared to healthy individuals

Avg. Anogenital Distance AGD) of Healthy and infertile Subjects				
Age group n=120	HC n=120)	Avg. AGD of HC Subjects	INF n=80)	AGD Infertile Subject
18-21	25	2.31±0.24	7	1.61±0.35
22-25	19	2.33±0.25	14	1.595±0.30
26-29	17	2.33±0.26	16	1.593±0.28
30-33	19	2.31±0.25	19	1.58±0.250
34-37	25	2.41±0.29	11	1.63±0.22
38-41	14	2.65±0.38	7	1.58±0.25
42-45	10	2.72±0.44	6	1.47±0.17

Table.2 Showing average sperm count morphology of normal control and infertile subjects. HC- Healthy control, HCNSC- Healthy control Normal control sperm count, AbSC- Abnormal sperm count, INF- Infertile)

Average Sperm count Morphology of Normal Control and Infertile Subjects						
Age group	HC n=120	Avg. HC NSC million/ml	Avg. HC Ab SC million/ml	INF n=80	Avg. INF NSC million/ml	Avg. INF Ab SC million/ml
18-21	25	6379.99	264.75	7	2208.1	270.55
22-25	19	7410.66	415.26	14	1466.02	332.05
26-29	17	7601.68	268.96	16	829.33	121.47
30-33	19	8364.55	248.251	19	1224.64	151.66
34-37	16	21857.97	420.29	11	1465.62	157.33
38-41	14	9038.41	188.905	7	1898.02	318.468
42-45	10	7487.59	220.998	6	980.35	163.61

Fig.1 A landmark of AGD Measurement from center of the anus to the base of the scrotum and from the base of the penis to the center of the anus. Redrawn from (Fowler *et al.*, 2011a, Papadopoulou *et al.*, 2013a, Vafeiadi *et al.*, 2013, Barrett *et al.*, 2014, Sathyanarayana *et al.*, 2015, Thankamony *et al.*, 2009, and Swan *et al.*, 2015

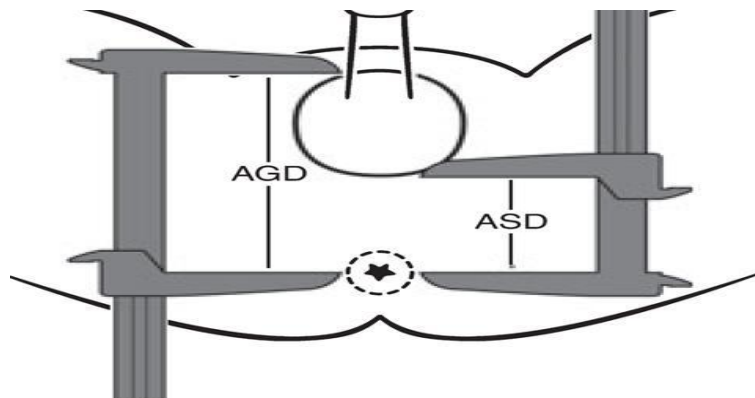
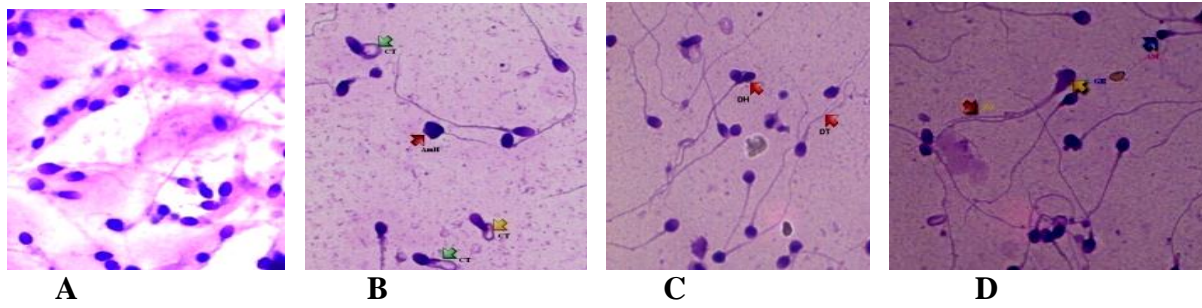


Fig.2 Showing sperm morphology of healthy control and infertile individuals



A) Normal Sperm B) Tail coiling and amorphous head C) Double headed & Double Tail, D) Pin & amorphous head

Fig.3 Numerical chromosomal aberrations in infertile males

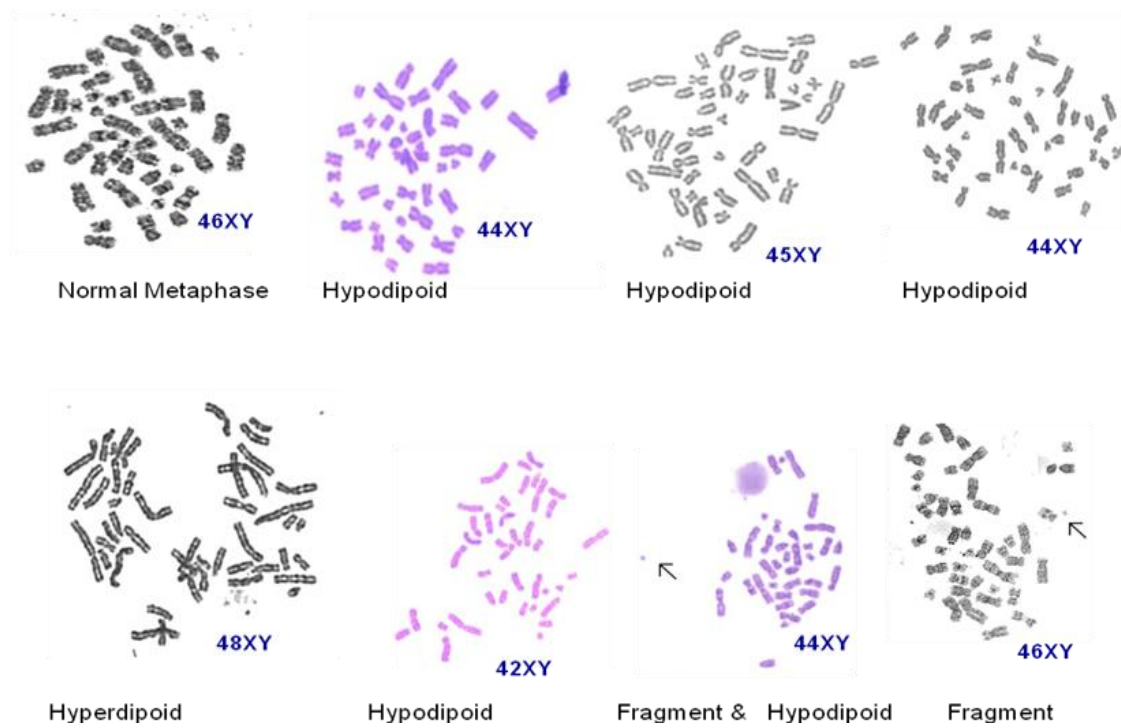
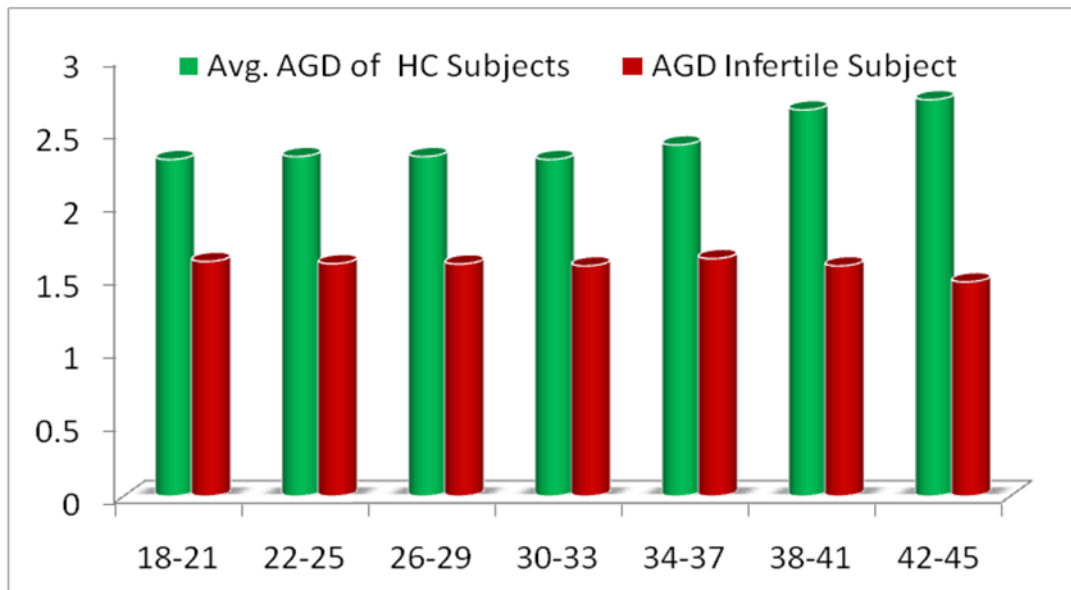


Table.3 Showing Normal and abnormal metaphase count of healthy and infertile subjects

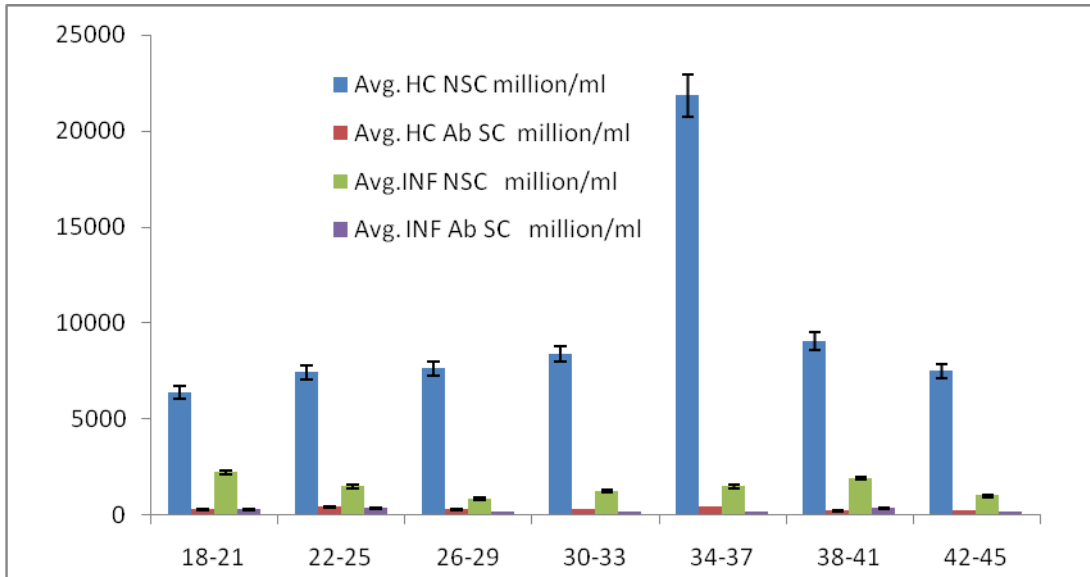
Age Group Healthy and Infertile individuals	Total Avg. metaphase counted	Avg. Normal Metaphase	Average Abnormal Metaphase	Avg. FRG	Avg. Hyper aneuploidy	Avg. Hypo Aneuploidy
18-21 HC n=14)	30	28.93	1.07	0.5	0.57	0
18-21 INF n=12)	30	13.44	16.56	7.22	5.3	4.04
22-25 HC n=19)	30	28.53	1.47	0.6	0	0.87
22-25 INF n=14)	30	12.57	17.6	6.84	5.73	5.03
26-29 HC n=16)	30	29.69	0.31	0	0.11	0.2
26-29 INF n=18)	30	10.27	19.73	9.78	3.73	6.27
30-33 HC n=13)	30	29.1	0.9	0	0.44	0.46
30-33 INF n=22)	30	8.24	21.76	4.55	11.51	5.7
34-37 HC n=17)	30	30	0	0	0	0
34-37 INF n=15)	30	8.75	21.25	4	6.66	10.59
38-41HC n=12)	30	30	0	0	0	0
38-41 INF n=13)	30	10.25	19.75	7.32	8.11	4.32
42-45 HC n=9)	30	26.4	3.6	1.36	0.65	1.59
42-45 INF n=06)	30	6.15	23.85	11.3	7.12	5.43

Graph.1

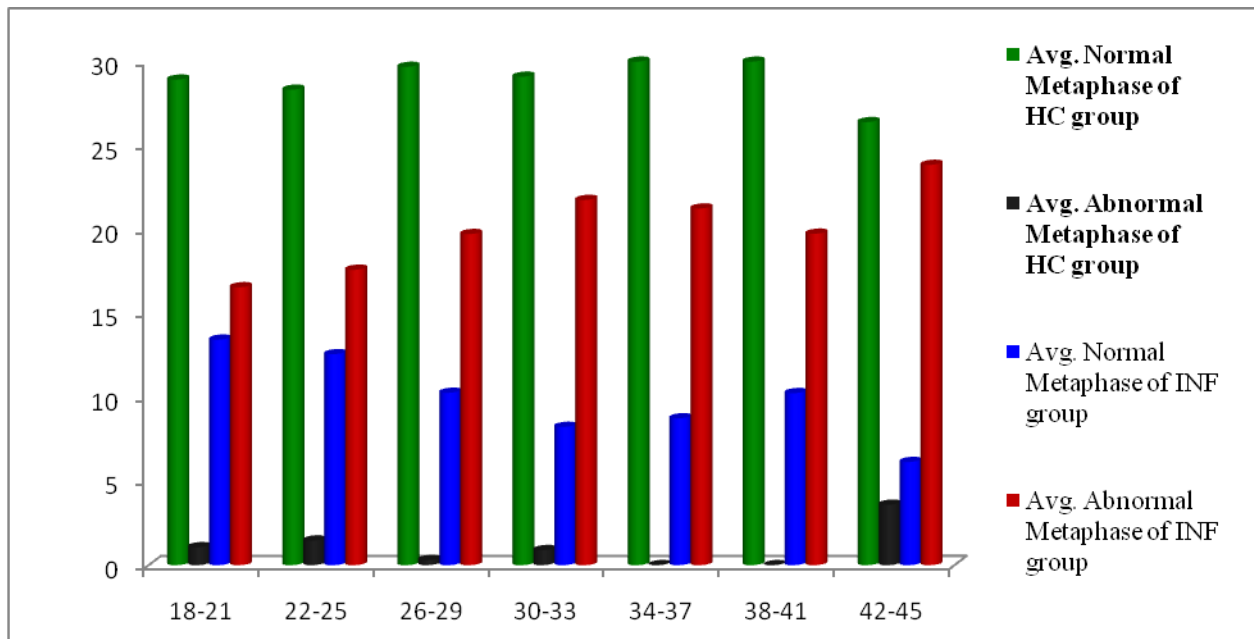


Graph :1 showing anogenital distance(AGD) of Healthy and infertile subjects

Graph.2 Showing average sperm count morphology of normal control and infertile subjects



Graph.3 Showing average normal and abnormal metaphase count of healthy and infertile individuals



Infertility is not a curse rather it is a medico-social event which should be handled in such a way so that, the sentiments and ethics should not be hurt of an individual. In male infertility Anogenital Distance and chromosomal abnormalities plays an

important role. In this study, it has been revealed that infertile males coupled with less AGD as well as abnormal sperm morphology along with chromosomal anomalies might have an effect on spermatogenesis. All these factors, alone or in combination may lead to

infertility in a male as shown by this study. The study showed that males having AGD of less than 2 inches are prone towards infertility. This study is supported by a study done by Deborah Zabarenko *et al.*, in 2011 published in EHP Environmental health perspectives) in which they found that men having AGD less than 2 inches (52mm) have seven times more chances of being sub-fertile as those with longer AGD. In other research work published in the same year by Michael Eisenberg *et al.*, revealed that fatherhood is associated with the longer AGD and may predict the normal male potential and AGD was significantly correlated with total motile sperm count and with sperm density (Michael *et al.*, 2011). Hsieh *et al.*, (2008) revealed that boys having less anogenital distance have genital anomalies (i.e. Cryptorchidism and Hypospadias) establishing a link between normal genital development (Hsieh *et al.*, 2008). Another aspect of this study was a chromosomal assay of the infertile males, which showed that infertile males had a higher frequency of numerical as well as structural aberrations as compared to normal healthy males which are an important indicator towards infertility. This study is supported by Van Assche *et al.*, in their study; they recognized chromosomally consequential sterility has long been recognized (Van Assche *et al.*, 1996). In another study done by Sreenivasa *et al.*, in 2013 revealed that out of 15 infertile cases Four (2%) were 47, XXY and mosaic 47, XXY; Two (1%) were structural autosomal abnormalities; Two (1%) were inversion Y; Seven (3.5%) cases were Y heterochromatin variants and 185 cases (92.5%) showed normal 46, XY karyotype.

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Declaration of interest

The authors declare that there is no conflict of interest regarding this paper submission.

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