



Impact of Histidine rich glycoprotein gene polymorphism in infertile male on the outcome of intracytoplasmic sperm injection (ICSI)

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Abstract

Introduction: Infertility is a major concern among married couples when they fail to achieve conception. The presence of genetic anomalies both at chromosomal and gen level is a major concern in the couples opting for assisted reproductive techniques (ARTs). Male infertility may be related to various genetic polymorphisms and their combinations and not solely on a single one. Perhaps in the future, a panel of polymorphisms may guide the clinicians and patients to the best treatment alternatives of infertility.

Study design: This is a cohort prospective study including 100 infertile male (oligozoospermia) to determine the impact of Histidine rich glycoprotein gene polymorphism HRG (C633T) in infertile male on the outcome of intracytoplasmic sperm injection (ICSI).

Results: In this study 56, 52 % of infertile males with succeeded ICSI have C/T genotype, 34.78% of them have C/C genotype and 8.69% of them have T/T genotype. While 33.33% of infertile male with failed ICSI have C/T genotype, 37.03 % of them have C/C genotype and 29.62 % of them have T/T genotype. There was significant increase of C/T mutation (56.52%) in infertile males with successful ICSI compared to failed ICSI (33.33%) p 0.05. There was significant decrease of T/T mutation (8.69%) in infertile males with successful ICSI compared to failed ICSI (29.26%) p 0.05. There were significant differences between HRG C/T genotype compared to C/C and T/T genotypes regarding sperm count, sperm concentration and total motility p 0.05.

Conclusion: In this study the distribution of HRG (C633T) genotypes was similar to that reported in European population , the heterozygous C/T infertile males have high success rate after ICSI treatment while homozygous T/T have the lowest success rate.

Keywords: Infertility, Histidine rich glycoprotein gene, Single nucleotide polymorphism, Oligozoospermia

Introduction

Infertility is a major concern among married couples when they fail to achieve conception even after one year of regular unprotected intercourse (WHO 2000) (1). About 25% of couples don't achieve pregnancy within one year, 15% seek medical treatment for infertility and less than 5% remain unwillingly childless. Approximately in 50% of the cases underlying etiology lies in men alone. In addition, no causal factors are found in 60-70% of these men, therefore the cause is idiopathic (2). Idiopathic male infertility may be caused by several factors, such as chronic stress, immunological, endocrine disruption due to environmental pollution, reactive oxygen species and genetic abnormalities (WHO 2000) (1). The cause of an abnormal sperm sample is unknown in about 26% of infertile men. It is estimated that more than 10% of spermatogenic impairment is explained by genetic defects (3).

The presence of genetic anomalies both at chromosomal and gen level is a major concern in the couples opting for assisted reproductive techniques (ARTs), which provide the last hope for these couples to have their own offspring. However, if infertile men harbor any genetic anomaly, it may be transmitted to the offspring through in vitro fertilization (IVF) or intra cytoplasmic sperm injection (ICSI) (4).

Genetic anomalies not only impair the fertility (natural conception) of men but also prevent artificial conception. These abnormalities may be transmitted to the offspring in case of an artificial conception. With an increasing number of infertile couples opting for ART, it is now mandatory to analyze cases with severe oligozoospermia and azoospermia for genetic defects and counsel them who have these disorders accordingly (5).

Several studies have reported novel mutation and high frequency of single nucleotide polymorphisms (SNPs) in sperm nuclear genes to be one of the important causes of male infertility (6). In addition, several reports have linked SNPs in different genes to idiopathic recurrent miscarriage which is perhaps more likely to

depend on the right combination of SNPs, and not just a single one (7).

An estimated 2000 or more genes are involved in spermatogenesis and spermiogenesis and they are not completely known. Furthermore, association studies propose gene polymorphism to affect spermatogenesis which in turn may lead to oligozoospermia or azoospermia induced male infertility (8).

Histidine – rich Glycoprotein (HRG) is a multi – domain glycoprotein that is synthesized mainly by the liver and is present at high concentrations in the plasma. The protein is transported as a free protein but it is also stored in the () granules of platelets and released upon thrombin activation (9). HRG is composed of 525 amino acids that form three domains: N terminal domain with two cystatin like region (N1 and N2), a central Histidine – rich region (HRR) which has a proline – rich region (PRR1 and PRR2) on each side of it, and a C terminal domain which together forms the multi domain structure of the protein (10).

Human HRG gene is localized at chromosome in position 3q28-29 and contains seven exons and six introns. HRG C633 SNP is a cytosine (C) nucleotide while HRG 633T is a thymine (T) nucleotide, a cytosine (C) at this position will form a proline at amino acid position 204, a thymine (T) however will code for serine instead. Individuals can be homozygous for HRG SNP (C/C) and produce protein with proline amino acid at position 204; homozygous for HRG SNP (T/T) and produce protein with serine amino acid at position 204 or heterozygous for HRG SNP (C/T) and produce both HRG with proline and serine amino acids. The serine form allows for extra glycosylation in the protein at position of amino acid 202 (11).

The exact bio molecular function is unclear; HRG seems to be an adaptor molecule owing to its unique molecular structure, which allows it to interact with a multitude of different ligands. It is involved in regulation of much biological processes such as apoptosis, angiogenesis, immunological response system, blood coagulation and fibrinolysis. HRG has further

been shown to interact with the ability of different cell types to proliferate, migrate and adhere by either inducing or inhibiting these biological processes depending on the cell type and location (12). HRG might be involved in several of the processes of importance for a pregnancy to occur but the exact role of HRG is not yet fully studied in infertile male and its outcome on ICSI. The aim of the present study is to determine the impact of Histidine rich glycoprotein gene polymorphism HRG (C633T) in infertile male on the outcome of intra cytoplasmic sperm injection.

Subjects and Methods

This is a cohort prospective study including 100 infertile male (oligozoospermia) attended Assisted reproductive unit (ART) in the International Islamic center for Population studies and Research, Al-Azhar University for intra cytoplasmic sperm injection (ICSI) in the period between Januarys to December 2016.

The age of the selected patient is <40 years and BMI <30, with semen parameters of oligozoospermia according to (WHO 2010) (13). Female partners were less than 35 years old and BMI <30 with normal basal hormones and pelvic ultrasound sonography. There was history of pervious ICSI in 6 couples, three females have been pregnant before but had no child's due to previous miscarriage.

The female partners were received the stimulation protocol in the form of mid-luteal long protocol (Triptoline a acetate 0.1 mg aqueous solution of D- Trp6 – GnRH: Ferring, Hoof drop, Netherland) daily from the day 21 of the cycle and for 14 days where E2 level estimates, when it reaches a level less than 50 pg/ml, gonadotropin was started (Gonal F 150 IU pen c.c. for s.c injection (Merk Serono Egypt Pharmaceutical Company) and the dose was modified according to response. E2 measurements were done serially with ultra-sonic monitoring during folliculometry. Women were scheduled for oocytes retrieval when at least five follicles reached 18 mm size at this time human chorionic gonadotropin (HCG) injection 10,000 IU was given. Transvaginal ultrasonography guided oocyte retrieval was then planned 36 hours after HCG. The retrieved

oocytes were then incubated for 1 hour in global fertilization media and then, depending on maturity of oocytes, ICSI was performed. Denudation of oocytes was carried out chemically and mechanically before ICSI was performed. Oocytes were incubated overnight in global total media in a triple gas incubator (Labotect) and observed after 16-18 hours post injection for fertilization. The fertilized oocytes were then transferred into a cleavage medium and incubated. Embryos were observed on day 2 and transfer was scheduled according to quality of embryos. Nearly from two to three embryos on day 3 (8 cell stage grade A) were selected and transferred to each female partner, according to standardized clinical morphological protocol (Alpha scientist in reproductive medicine and ESHRE) special interest group of embryology, 2001. All poor quality embryos were unfit for transfer during early development.

Exclusion criteria.

- Obese, azoospermic or normospermic men.
- Male with previous history of genito-urinary operations, inguinal-hernia or varicocele.
- Male with chromosomal abnormality.
- Female factor infertility e.g. tubal factors, polycystic ovary, ovulatory dysfunction or endometriosis.
- Known risk factors for miscarriage or implantation failure e.g. systemic lupus erythematous, thrombophilia, diabetes mellitus, major chromosomal aberration, hypothyroidism, abnormal uterine cavity or fibroid tumor.

Blood samples for female partners were taken at the 3 day of the cycle in plain tubes for the assessment of basal hormones (FSH, LH, Estradiol and Prolactin) and Thyroid stimulating hormone (TSH), which were measured by enzyme linked fluorescence assay (ELFA) technique (BioMerieux, France) assay kit using Vidas apparatus.

Chemical pregnancy test for female partners (B-HCG) was done two weeks after embryo transfer.

Semen samples were obtained by masturbation at ICSI day.

Blood samples of infertile males were collected in plain and EDTA tubes during preparation for ICSI.

Serum samples were used for measurement of FSH, LH and Total testosterone hormones by ELFA technique (BioMerieux, France) assay kit using Vidas apparatus.

EDTA blood samples were used for DNA extraction, in 10 ml conical centrifuge tubes 1 ml of ficoll – paque was added then 2 ml of blood samples were added slowly to the wall of centrifuge tubes, the tubes are centrifuged at 1600 for 15 minute. After centrifugation, the supernatant plasma was discard and the buffy coat layer (under the plasma and above ficoll and red blood cell layers) was aspirated into eppindurf tubes and stored at -80 C° till the time of genotyping.

DNA Genotyping:

Genotyping for Histidine rich glycoprotein (C633T) polymorphism rs (9898) was performed by real time polymerase chain reaction based restriction fragment length polymorphic assay. According to manufacturer's instruction (Taqman Genotyping assay, Applied Biosystem Inc., Foster City, CA, USA), polymerase chain reactions were performed in 96 well plates in total volume of 25 ml for each well. Each reaction consisted of 1x Taqman Universal PCR master mix formed of the following:

- 1- Ampli Taq Gold DNA polymerase, in which enzyme is purified to reduce bacterial DNA introduced from host organism and the enzyme clears only probes that are hybridized to the target.
- 2- Rox passive reference: provides internal reference to which reporter – dye signal can be normalized during data analysis (normalization is necessary to correct for fluorescence fluctuations due to change in concentration volume).
- 3- PCR buffer component optimized for high end point fluorescence clusters.

- 4- Deoxy nucleotide triphosphate (d NTPs).

1X Single nucleotide polymorphism genotyping assay formed of:

1-Two primers forward and reverse primer to amplify polymorphic sequence of interest.

2-Two Taqman MGB probes formed of specific oligonucleotides which are labeled with:

- a- VIC dye is linked to the 5' end of allele 1 probe.
- b- 6 FAM dye is linked to the 5' end of allele 2 probe.

Each Taqman MGB probe anneals specifically to a complementary sequence, if present, between the forward and reverse primer sites.

3-A minor groove binder (MGB) which increases the melting temperature (T_m) without increasing probe length, thereby allowing the design shorter probes (for accurate allelic discrimination).

3-A non-fluorescent quencher (NFQ) at the 3' end of the probe.

Cycling conditions:

a- 10 minute at 95 C° for enzyme activation.

b- 40 cycle of 15 second at 92 C° for denaturation and 1 minute at 60 C° for annealing and elongation (real time fluorescence was performed). Sequence detection system software (Applied Biosystems) was used to plot fluorescence (R_n) values based on the signals from each well-the plotted fluorescence signals indicated which alleles were present in each sample.

Statistical analysis

All statistical analysis was performed using the statistical package for social science (SPSS) 20.0 for windows software pack (SPSS, Chicago, IL). Differences in proportion of infertile men between different HRG C633T SNP groups demographic and laboratory data were analyzed using chi-square test and Mann-Whitney U test. P value was considered significant if less than 0.05.

Results

The patients were classified according to HRG (C633T) genotyping into (C/C) homozygous, (C/T) heterozygous and (T/T) homozygous, and

according to success of ICSI into, successful ICSI with positive pregnancy test of female partner and failed ICSI with negative pregnancy test of female partner.

Table 1: ICSI outcome according to HRG (C633T) genotypes ininfertile males

HRG Genotype	Successful ICSI		Failed ICSI		P value
	Number=46	%	Number =54	%	
C/C	16(34.78 %)		20(37.03 %)		p>0.05
C/T	26(56.52 %)		18(33.33%)		p 0.05
T/T	4(8.69 %)		16(29.62%)		p 0.05

Table (1) showed that 56, 52 % of infertile males with succeeded ICSI have C/T genotype, 34.78% of them have C/C genotype and 8.69% of them have T/T genotype. While 33.33% of infertile male with failed ICSI have C/T genotype, 37.03 % of them have T/T genotype and 29.62% of them have C/C genotype. This indicated that most patient enrolled in this study have C/T mutation

(44%) while the lowest percentage of infertile males have T/T Mutation (20 %). There was significant increase of C/T mutation (56.52%) in infertile males with successful ICSI compared to failed ICSI (33.33%) p 0.05. While there was significant decrease of T/T mutation (8.69%) in infertile males with successful ICSI compared to failed ICSI (29.26%) p 0.05.

Table 2: Demographic data according to HRG single nucleotide polymorphism genotypes.

	C/T Mean± SD	C/C Mean± SD	T/T Mean± SD	P value
Age(year)	38.1±3.1	37.9 ±3.6	39.1±3.8	p>0.05
BMI(weight /length ²)	24.1±1.23	25.2±1.5	25.7±1.7	p>0.05
Smoking(%)	5(11.36%)	4(11.11%)	2(10.0%)	p>0.05
Infertility duration (year)	3.50 ± 0.2	3.41 ± 0.4	3.49 ± 0.6	p>0.05
Age of partner (year)	32.3 ± 2.2	33.5 ± 2.7	31.8 ± 1.9	p>0.05
Number of embryos transferred	2.47± 0.21	2.53±0.12	2.50± 0.3	p>0.05

Table (2) showed that there were no significant differences between HRG (C633T) genotypes as regard demographic data.

Table 3: Semen parameters of oligozoospermic infertile men according to HRG (C633T) genotypes.

	C/T Mean± SD	C/C Mean± SD	T/T Mean± SD	P value
Volume (ml)	2.6 ± 0.164	2.8 ± 0.231	2.1 ± 0.192	p>0.05
Total sperm count (10 ⁶)	23.4 ± 2.32	18.5 ± 2.82	16.2 ± 3.13	p<0.05
Sperm concentration (10 ⁶ /ml)	10.2 ± 0.47	8.3 ± 0.96	7.4 ± 0.75	p<0.05
Total motility (%)	35.7 ± 0.2	22.5 ± 3.5	18.3 ± 3.22	p<0.05
Abnormal forms (%)	96.9 ± 8.1	97.1 ± 7.3	97.8 ± 9.2	p>0.05

In table (3): There were significant differences between HRG (C633T) genotype C/T compared to C/C and T/T genotypes regarding total sperm

count, sperm concentration and total motility $p < 0.05$.

Table 4: Hormonal profile of oligozoospermic infertile men according to HRG genotypes.

	C/T Mean± SD	C/C Mean± SD	T/T Mean± SD	P value
FSH (mIU/ml)	7.2 ± 0.83	8.3 ± 0.9	7.1 ± 0.78	$p > 0.05$
LH (mIU/ml)	7.1 ± 0.55	7.3 ± 0.6	8.5 ± 0.68	$p > 0.05$
Total Testosterone (mg/ml)	5.76 ± 0.43	6.2 ± 0.52	6.61 ± 0.7	$p > 0.05$

Table (4): showed that there were no significant differences between HRG (C633T) genotypes regarding hormonal profile (FSH, LH and Total Testosterone) $p > 0.05$.

Discussion

Infertility troubles millions of people worldwide and as many as about 20% of these men and women never even get to know why cannot get pregnant.

Further knowledge of the etiology and mechanisms behind this barrier to reproduction is essential for understanding and treating infertility. Male autosomal chromosome variation i.e. differences in size or staining of chromosome segments, has been negatively associated with fertilization rate and clinical pregnancy rate following IVF, whereas such findings in women has no influence (14).

It is well known that both autosomal and sex chromosome genes are involved in complex regulation of spermatogenesis (15).

With the rapid DNA sequencing technologies, it is anticipated that genome wide analyses might help to identify hidden genetic factors behind the idiopathic male infertility. Male infertility may be related to various genetic polymorphisms and their combinations and not solely on a single one. Perhaps in the future, a panel of polymorphisms may guide the clinicians and patients to the best treatment alternatives of infertility (16).

Although HRG has not been widely studied in context of male infertility and ICSI outcome, but mostly in tumors and carcinogenesis. Little is

known about the exact role of HRG in infertility, embryo development, implantation and placentation.

Most previous studies focused on female factors as field of research concerning recurrent miscarriage or implantation failure, rare studies were done to explore the role of male partner as a cause of implantation failure. This study is one of the fewest studies to explore the potential role of male genotype HRG (C633T) single nucleotide polymorphism (SNP) in implantation after ICSI and its outcome. Once infertility is established there is help in the form of IVF/ICSI but halve of couples remain childless even after treatment. In these patients HRG (C633T) might be of importance. Apparently, the exact role of HRG in reproduction remains to be investigated as its exact bimolecular function is unclear.

In this study single nucleotide polymorphisms of HRG (C633T) rs (9898) were analyzed for all infertile patients. Results showed that 56.52% of infertile males with successful ICSI have C/T genotype 34.78% have C/C genotype and 8.69% of them have T/T genotype, while 33.33% of infertile males with failed ICSI have C/T genotype, 37.03% have C/C genotype and 29.62% of them have T/T genotype. There was significant increase of C/T mutation (56.52%) in infertile males with successful ICSI compared to 33.33% in failed ICSI $p < 0.05$. While there was significant decrease of T/T mutation 8.69% in infertile males with successful ICSI compared to 29.62% in failed ICSI $p < 0.05$. This indicated that most patients enrolled in this study have C/T mutation 44%, 36% have C/C mutation, while the lowest percentage of infertile males have T/T mutation 20%.

The increased percentage of C/T heterozygous mutation in our study 56.52% in infertile males with successes ICSI is in agreement with theory of heterozygote advantage, according to which heterozygous carriers present a selective advantage in viability and reproductive fitness over homozygous in natural populations (17).

In a European population the allele frequency of HRG (C633T) genotype is 0.67 for the C-allele (proline) and 0.33 for the T-allele (serine) and the genotype frequency is 45%, 44% and 11% for the C/C, C/T and T/T genotypes respectively, in other parts of the world i.e. populations from Africa and South Asia, the T-allele is often the major allele are in the range of 0.5-0.7 (http://www.ensembl.org/homo-sapiens,rs9898,population_genetics).

In this study the distribution of HRG genotypes was similar to that reported in European population; it was 44%, 36% and 20% for the C/T, C/C and T/T genotypes respectively. Our results agree with Lindgren *et al* (18) study which reported that C/T SNP was not associated with pregnancy rate in infertile women, however in infertile male HRG had an impact on the pregnancy rate after IVF treatment. They found that most successful pregnancies were couples with male heterozygous C/T mutation where 52% achieved pregnancy, whereas homozygous T/T had the lowest chance of successful IVF treatment 14.39%. Also, they clarified that men diagnosed with male factor infertility (as a single factor) were more often homozygous T/T as compared to normal fertile men. In addition, male heterozygous C/T was superior in terms of IVF treatment outcome and was also associated with the lowest number of total treatment compared with homozygous carrier.

Laanpere *et al* (19) investigated infertile women undergoing IVF treatment and the importance of several different SNPs in the folate pathway. They reported positive association between heterozygous carriers and pregnancy rate among other outcome parameters.

Different possible mechanisms were suggested in previous studies to demonstrate benefit of HRG

heterozygous C/T over homozygous C/C or T/T infertile males as a possible factor for successful implantation after ICSI, when the two HRG proteins created by polymorphism could possess functions somewhat different from each other, and the heterozygous men could benefit from having both allele. Heterozygous C/T having both proteins containing proline and serine from two alleles, which are important for properly regulated angiogenesis as a vital process for an adequate placentation (19).

Previous studies have suggested that an imbalance endometrial angiogenesis and a too rapidly formed vascular network may result in failed implantation and first-trimester miscarriage. It is well known that development of a normal well-functioning vascular in the uterus and placenta needs cooperation between different cell types and various growth factors, in the processes of implantation, embryo development and placentation (20).

First mechanism; HRG containing proline at amino acid 204 is important for protein structures and contributes to exceptional conformational rigidity due to its unique cyclical formation with a secondary amine. The variant protein (has serine at amino acid 204) could therefore have a more loose protein structure, in addition it allows for extra glycosylation at position 202 in protein which is very close to a potential inter-domain disulphide bridge (interfere with disulphide bridge formation) both factors lead to a decrease protein stability and alternation in ligand interaction, so the heterozygous C/T HRG could benefit from having both (11).

Second; HRG containing proline the disulphide bridge connects to the region in protein that when released this fragment from this region exerts anti-angiogenic effect on endothelial cells that is responsible for most of anti-angiogenic effect that HRG exerts on growth stimulated endothelial cells. It was done by inhibiting vascular endothelial growth factor and fibroblast growth factor mediated angiogenesis of endothelial cells in vitro (21).

In the variant protein (serine at amino acid 204) HRG stability of disulphide band is altered, thereby disabling proteolytic release of anti-angiogenic fragment (reducing their anti-angiogenic effect). HRG heterozygous has been benefit from both pro and anti-angiogenic properties owing to its multi domain structure and the activity of proteolytically released fragment(22).

Third; HRG containing proline can act in favor of angiogenesis that doesn't occur in serine containing variant as HRG containing proline binds to thrombospondins (TSPs) inhibiting its anti-angiogenic activity (indirect proangiogenic effect). Thrombospondins are potent inhibitors of angiogenesis that, when bind their receptor CD 36 inhibits the response of a number of different growth factors, including fibroblast growth factor and vascular endothelial growth factor (23). HRG contains two CD 36 homology domains that bind to TSP with high affinity, thereby blocking its anti-angiogenic activity. Substitution from proline to serine is situated in CD36 homology domain and this might lead to a disrupted interaction between CD36, TSP and HRG abolishing its indirect pro angiogenic effect (24).

HRG further acts in favor of angiogenesis by binding plasminogen or plasmin to the surface of cells, potentiating directed endothelial cell migration and invasion (25).

The pro and anti- angiogenic effects mentioned above in the C/T heterozygous HRG could be lacking in the less common variant of protein and so possibly contribute to the lower pregnancy rates in infertile male homozygous carriers of HRG T/T. Embryos homozygous for HRG T/T might have an impaired regulation of angiogenesis leading to a defect in implantation and placentation that eventually leads to expulsion of embryo.

It is well known that HRG interacts with components of the immune system; it prevents the formation of immune complex and enhances immune complex stability and clearance by binding to IgG (26).

Moreover, HRG is antimicrobial under acidic conditions or when Zinc is present making it effective against gram positive and negative bacteria (27).

HRG has anticoagulant ability which was demonstrated in a HRG knock-out mice model, where HRG deficient mice had a shorted activated partial thromboplastin time and lower thrombin generation (28).

It was recently discovered that human embryos, already as 4-cell stage, produce HRG and this production is continued up till the blastocyst stage. HRG is found in almost all cells of the inner cell mass and the outer layer of tropho-ectoderm cells that together with maternal tissues will give rise to the placenta, furthermore embryos secrete HRG into the surrounding media during culture (29).

In this study there were significant differences between HRG (C633T) genotype C/T heterozygous compared to homozygous C/C and T/T regarding total sperm count, sperm concentration and total motility $P < 0,05$. HRG is detected in tests, prostate and seminal vesicles of the human reproductive tract and the antibody based human protein Atlas demonstrate low to moderate staining for HRG in the testis (Seminiferous ducts and Leydig cells) and prostate but no expression in cells of the epididymis and seminal vesicles (30).

Also in our study of HRG, homozygous (T/T) SNP carriers had overall lower semen test results, since HRG seems to be present in the testis, this finding suggests an influence from the polymorphism on spermatogenesis.

Normal spermatogenesis requires cross talk between somatic and germ cells, relevant endocrine signaling and adequate blood flow, the human testis has a highly developed micro vasculature (31). It is known that transcripts and proteins of importance for early embryogenesis accompany the male DNA and are introduced to the oocyte through sperm. Also, the microenvironment in which the spermatozoa are produced affects their reproductive capacity (32).

HRG may act as regulator of angiogenesis during spermatogenesis and maturation of spermatozoa. HRG also influences VEGF, another key player in angiogenesis. The major sources of VEGF in the male reproductive tract are the prostate and seminal vesicles, but Leydig and Sertoli cells also produce VEGF to some extent (33).

While the function of VEGF in the male genital tract is unclear, it appears to influence the testicular microvasculature and the composition of seminal plasma. HRG C/T SNP heterozygous carriers might have much better semen parameters compared to other genotypes due to a more balanced regulation of angiogenesis during spermatogenesis through different pro and/or anti-angiogenic properties depending on HRG genotype, and this regulation is lacking in the homozygous HRG men.

Conclusion and Recommendations

In this study the distribution of HRG (C633T) genotypes was similar to that reported in European population , the heterozygous C/T infertile males have high success rate after ICSI treatment while homozygous T/T have the lowest success rate. We advise for other studies with higher population numbers due to low number of studied infertile males in our study. Other studies comparing HRG (C633T) genotypes in infertile males with normal fertile males are recommended. We advise for use of HRG Genotype in infertile male for genetic counseling before ICSI treatment.

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