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ROLE OF HUMAN ADENOSINE DEAMINASE IN SEMINAL FLUID

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Abstract: The purpose of this study was to determine the Activities of Human Adenosine deaminase (ADA), in fertile and Oligospermia men. Activities of ADA were measured in 20 fertile men and 20 Oligospermia men. There was a significant difference in the ADA Activities between fertile and infertile individuals. The Activity of ADA in Oligospermia men (32.91 ± 11.83 IU/ml) was higher than that in fertile (15.12 ± 3.44 IU/ml) individuals. This alteration in ADA activity can lead to reduced adenosine levels, which may be involved in disturbing the fertility process. In conclusions Adenosine deaminase activity was significantly higher in Oligospermia men individuals than normal men in this study. Previous reports in this topic are contentious therefore, ADA activity could be regulated in different population by immunologic and genetic factors. **The Aim of the present work:** The aim of the present study was to investigate the activity of Human Adenosine deaminase in Seminal fluid of normal men and Oligospermia men. and the relationship of this enzyme on the age, number and activity of sperms in normal men and in Oligospermia men.

Keywords: Adenosine deaminase, Oligospermia, Infertility, Fertility.

Introduction: One of the most serious social problems facing developed countries today is the declining birth rate, although it is generally not well recognized that the number of infertile couples is on the rise in these countries. While both social (i.e., social progress for women and the resulting increase in the age at which women marry) and environmental (i.e., pollution and global warming) factors are behind part of the increase in the number of patients with infertility, infertility in the male partner contributes to approximately half of all cases. To date, various techniques, such as in vitro fertilization (particularly, intracytoplasmic sperm injection or ICSI) and so-called TESE-ICSI involving the harvesting of sperm from the testes, have been developed for male infertility. Although these methods are steadily producing results, no technique has proven effective for patients with nonobstructive azoospermia, in which there is an absence of mature sperm in the testes. Evidence suggests that many patients with azoospermia have a genetic predisposition to the condition, although the cause has not been elucidated in the vast majority of cases [1]. Conversely, studies using knockout mouse models have recently linked many genes to spermatogenesis, the mechanisms of which are currently being clarified. These animal findings have yet to be shown applicable to most human cases. This is because identifying the affected genes in humans requires a retrograde genetic approach and because the knockout mouse phenotype is not always faithfully reproduced in humans. This paper discusses the

environmental factors considered likely to be involved in male infertility and the genes that have been clearly shown to be involved in male infertility in humans, including our recent research findings.

Fertility: is the capacity to establish a clinical pregnancy. The term infertility is used by some clinicians interchangeably with sub fertility. Formal definitions are, however, very important for appropriate management of reproductive disorders. Worldwide > 186 million people suffer from infertility, the majority being residents of developing countries. While the most powerful negative predictive factor of fertility is increasing women's age at conception, other factors including lifestyle and environmental factors are believed to play an increasing role. Factors influencing fertility will be presented as gender specific or not.

Male fertility: Some research suggests that older males have decreased semen volume, sperm motility, and impaired sperm morphology. In studies that controlled for female age [2], comparisons between men under 30 and men over 50 found relative decreases in pregnancy rates between 23% and 38%. Sperm count [2] declines with age, with men aged 50–80 years producing sperm at an average rate of 75% compared with men aged 20–50 years and larger differences exist in the number of seminiferous tubules in the testes containing mature sperm [2]:

- In males 20–39 years old, 90% of the seminiferous tubules contain mature sperm.
- In males 40–69 years old, 50% of the seminiferous tubules contain mature sperm.
- In males 80 years old and older, 10% of the seminiferous tubules contain mature sperm.

Decline in male fertility is influenced by many factors, including lifestyle, environment and psychological factors. Some research suggests increased risks for health

problems for children of older fathers, but no clear association has been proven. Australian researchers have found evidence to suggest obesity may cause subtle damage to sperm and prevent a healthy pregnancy. They reported fertilization was 40% less successful when the father was overweight. The American Fertility Society recommends an age limit for sperm donors of 50 years or less, and many fertility clinics in the United Kingdom will not accept donations from men over 40 or 45 years of age.

Oligospermia: Terms oligospermia, and low sperm count refer to semen with a low concentration of sperm and is a common finding in male infertility. Often semen with a decreased sperm concentration may also show significant abnormalities in sperm morphology and motility (technically oligoasthenoteratozoospermia). There has been interest in replacing the descriptive terms used in semen analysis with more quantitative information.

Diagnosis: The diagnosis of oligozoospermia is based on one low count in a semen analysis performed on two occasions. For many decades sperm concentrations of less than 20 million sperm/mL were considered low or oligospermic, recently, however, the WHO reassessed sperm criteria and established a lower reference point, less than 15 million sperm/mL, consistent with the 5th percentile for fertile men [3]. Sperm concentrations fluctuate and oligospermia may be temporary or permanent. Sources usually classify oligospermia in 3 classes [4]: Mild: concentrations 10 million – 15 million sperm/Ml, Moderate: concentrations 5 million – 10 million sperm/mL, Severe: concentrations less than 5 million sperm/mL, The diagnosis of oligozoospermia requires a work-up via semen analysis.

Causes: There are many causes for oligospermia including [5]: **Pre-testicular causes:** Pre-testicular factors refer to conditions that impede adequate support of the testes and include situations of poor hormonal support and poor general health including: Hypogonadism due to various causes, Drugs, alcohol, smoking, Strenuous riding (bicycle riding [6] horseback riding), Medications, including androgens.

Post-testicular causes: Post-testicular factors decrease male fertility due to conditions that affect the male genital system after testicular sperm production and include defects of the genital tract as well as problems in ejaculation: Vas deferens obstruction, Lack of Vas deferens, often related to genetic markers for cystic fibrosis, Infection, e.g. prostatitis, Ejaculatory duct obstruction

Testicular factors: Testicular factors refer to conditions where the testes produce semen of poor quality despite adequate hormonal support and include:

Age: A 2001 review suggested older men have decreased pregnancy rates, increased time to pregnancy and increased infertility at a given point in time. When controlling for the age of the female partner, comparisons between men under 30 and men over 50 found relative decreases in pregnancy rates between 23% and 38%.

Cryptorchidism: Many men who were born with undescended testes have reduced fertility, even after orchiopexy in infancy. The reduction with unilateral cryptorchidism is subtle, with a reported infertility rate of about 10%, compared with about 6% reported by the same study for the general population of adult men. The fertility reduction after orchiopexy for bilateral cryptorchidism is more marked, about 38%, or six times that of the general population. The basis for the universal recommendation for early surgery is research showing degeneration of spermatogenic tissue and reduced spermatogonia counts after the second year of life in undescended testes. The degree to which this is prevented or improved by early orchiopexy is still uncertain.

Varicocele: A varicocele is an abnormal enlargement of the pampiniform venous plexus in the scrotum. This plexus of veins drains blood from the testicles back to the heart. The vessels originate in the abdomen and course down through the inguinal canal as part of the spermatic cord on their way to the testis. Varicoceles occur in around 15% to 20% of all men. The incidence of varicocele increase with age. is an accumulation of serous, fluid in a body cavity. A hydrocele testis is the accumulation of fluids around a testicle. It is often caused by peritoneum wrapped around the testicle, called the tunica vaginalis. Provided there is no hernia present, it goes away without treatment in the first year.

Mumps: is a viral disease caused by the mumps virus. Initial symptoms are non-specific and include fever, headache, malaise, muscle pain, and loss of appetite. These symptoms are usually followed by painful swelling of the parotid glands, called parotitis, which is the most common symptom of infection. Symptoms typically occur 16 to 18 days after exposure to the virus and resolve within two weeks. About one third of infections are asymptomatic. Complications include deafness and a wide range of inflammatory conditions, of which inflammation of the testes, breasts, ovaries, pancreas, meninges, and brain are the most common. Testicular inflammation may result in reduced fertility and, rarely, sterility.

Malaria: The effect of malaria on semen in an infected male is a myth. Decreased semen quality in malarial infected male has been reported. Severe oligozoospermia, necrozoospermia or azoospermia can be observed. It is proposed that malaria or its treatment might be the etiologies.

Idiopathic oligospermia (oligoasthenoteratozoospermia): In about 30% of infertile men no causative factor is found for their decrease in sperm concentration or quality by common clinical, instrumental, or laboratory means, and the condition is termed "idiopathic". A number of factors may be involved in the genesis of this condition, including age, infectious agents, Y- chromosome microdeletions, mitochondrial changes, environmental pollutants, and "subtle" hormonal changes. A review in 2013 came to the result that oligospermia and azoospermia are significantly associated with being overweight (odds ratio 1.1), obese (odds ratio 1.3) and morbidly obese (odds ratio 2.0), but the cause of this is unknown. It found no significant relation between oligospermia and being underweight.

DNA damage: The human breast cancer susceptibility gene 2 (BRCA2) is employed in homologous recombinational repair of DNA damages during meiosis. A common single-nucleotide polymorphism of BRCA2 is associated with severe oligospermia. Men with mild oligospermia (semen concentration of 15 million to 20 million sperm/mL) were studied for an association of sperm DNA damage with life style factors [7]. A significant association was found between sperm DNA damage and factors such as age, obesity and occupational stress.

Infertility: Infertility is described as the lack of conception after one year of unprotected intercourse [8]. However, the amount of time after which treatment to a couple is administered varies considerably. It might be greatly reduced in the case of an obvious infertility problem, such as blockage of the fallopian tubes. Considering that there is an approximate 15-20010 chance per month of achieving pregnancy, and that about 73- 80010 of couples achieve pregnancy before six months of trying, it is justified that an infertility evaluation and treatment are initiated after six months rather than one year of trying. Infertility per se is not very common, affecting about 5-8% of the population in developed countries. What is more common, however, is the loss of early pregnancies. Various studies were carried out on women who ever experienced an early abortion, and certain trends in the results of these studies persisted from month to month. It was in fact observed that in anyone month under normal conditions:

- 15% of oocytes fail to undergo fertilization
- 10-15% of oocytes become fertilized and cleave, but fail to implant
- Only 42% of implanted ova succeed in suppressing the following menstrual period
- A further 24% lose their foetus after week four of gestation.

New reproductive technologies are providing approximately 80010 of infertile couples who seek treatment with offspring which is genetically related to both parents, whereas another 10-15% is also provided with pregnancy, however using donated gametes (i.e. offspring would be related to only one of the parents). It is important to stress that infertility is not only a medical problem, but it also involves many psychological, legal, moral and social issues, and hence administering the proper treatment becomes even more difficult. This in turn explains why, in a survey performed in the United States in 1995, it was discovered that despite of the availability of treatment only 21 % of infertile couples ever sought 63 medical treatments. It is hence not difficult to conclude that infertility has a devastating emotional and psychological impact on any couple concerned.

Male Infertility: In approximately 30% of infertile couples, the infertility cause is male factor. In most of these cases, the problem is oligospermia, i.e., the semen would contain few spermatozoa or the sperm is of poor quality or motility. Azoospermia i.e., no sperm production, is far less common. In those cases, where azoospermia is accompanied by normal gonadotropins, an obstructed outflow tract or congenital absence of the vas deferens are likely to be the cause for the absence of sperm in the semen [8]. Certain anabolic steroids and other oral hormonal agents can cause oligospermia and sometimes even azoospermia. Medications which can affect spermatogenesis include cimetidine, spironolactone, isoniazid, calcium channel blockers and chemotherapeutic drugs all of which must be taken into consideration when performing an infertility evaluation. Sometimes, a male may have a normal number of spermatozoa but is still infertile. In such cases, approximately half of the spermatozoa are found to have abnormal morphology, such as, sperm with two heads, sperm with abnormally shaped heads, or even sperm with two tails. Furthermore, in some cases the sperm may appear morphologically normal but its motility is entirely or relatively impaired [9]. Another possible cause for male infertility is the varicocele, which is a dilated scrotal vein present in 25% of infertile males [8]. Although the exact link between varicoceles and male infertility is not known, the most acceptable theory is that the dilated testicular vein raises the temperature of the testes above the optimal temperature thereby affecting spermatogenesis. (The optimal temperature for

spermatogenesis is (-16.67) - (-16.11) °C lower than core body temperature). Male infertility may also be due to genetic defects, such as Kleinfelter's syndrome, which may be detected by an elevated FSH level. However, an abnormal FSH level in males may also be due to sertoli only cell syndrome, previous mumps orchitis or prior cancer treatment. In cases of Kallmann's syndrome and hypothalamic dysfunction, both characterized by decreased or undetectable levels of FSH and LH. the infertility may be treated with FSH and Human Chorionic Gonadotropin (hCG), or with GnRH [10]. It is important to stress that IVF is not the solution to all infertility problems. Depending on the nature of the cause of infertility, the appropriate treatment must then be given. Therefore, an accurate and complete infertility evaluation is absolutely necessary in order to have a better understanding of the problems that are preventing the couple concerned from conceiving.

Infertility and genetics: Advances in molecular biology have resulted in determinant genetic causes for reproductive disorders in both men and women. However, this represents a very small group of patients. There are some cases with a well-defined pattern of purely gene alterations and others with multiple gene involvement. Abnormal developmental and functional forms have been described up to the present, which may be caused by alterations at different levels of the hypothalamic–pituitary–ovarian axis.

Genetics and male infertility: Genetically speaking, male infertility may be classified as being due to a single gene defect, or to a numerical structural chromosomal defect. If the defect affects hormone production, a pre-testicular defect is involved. If the gene failure affects spermatogenesis, a testicular factor should be involved. On the other hand, when the defect affects the

transport of spermatozoa, the cause is related to a post-testicular factor. If the gene or genetic disorder affects the spermatozoon, a spermatic factor should be involved. Single gene defects that generate male sterility are due to mutations in one allele or in two alleles at the same locus. The inheritance of the defect may be dominant or recessive in nature. These genes will be considered briefly.

Table 1.1 Describes the most frequent forms of male sterility arising from a single gene defect.

Pathology	Frequency	Phenotype	Heredity/genotype
Kallman's syndrome	1 in 30 000	Anosmia Delayed puberty Azoospermia or oligozoospermia	X-linked recessive
Cystic fibrosis	1 in 2500	Respiratory infections Pancreatic insufficiency Poor Wolffian duct development Obstructive azoospermia	Autosomic recessive CFTR gene, chromosome 7
Immotilecilia syndrome	1 in 30 000	Blonchiectasias	Autosomal recessive
		Situs inversus Immotile spermatozoa	Chromosome 19
Myotonic dystrophy	1 in 8000	Muscle weakening	Autosomal dominant
		Testicular atrophy	Chromosome 19
Androgenic insensitization	1 in 60 000	Feminizing testicle	X-linked recessive
Renal polycystic disease	1 in 800	Multiple kidney liver, epididymis cysts	Autosomal dominant
Usher syndrome	1 in 30 000	Deafness	Autosomal

			recessive
		Retinitis Spermatic axonemal defects	
Usher syndrome	1 in 30 000	Deafness Retinitis Spermatic axonemal defects	Autosomal recessive

Causes of Infertility: The causes of infertility are various and in some instances, a combination of factors may exist. Identifying the cause of infertility is the main key which will then lead to the administration of the appropriate treatment. This in turn points out the necessity of an accurate infertility evaluation:

Pre-testicular factors: These are conditions that impede adequate support of the testes and include situations of poor hormonal support and poor general health including hypogonadism; drugs such as cimetidine and spironolactone that decrease follicle stimulating hormone (FSH) levels, and nitrofurantoin that decreases sperm motility; adopted life style (chronic alcoholism, marijuana, cigarette smoking); and strenuous activities such as strenuous bicycle riding [11].

Testicular factors: These are factors that affects quality and quantity of semen produced by the testes and include age, genetic defects of the Y-chromosome (Klinefelter syndrome), neoplasm

e.g. seminoma, cryptorchidism, varicocele which account for 14% [12], mumps viral infection and may be idiopathic which accounts for 30 % of male infertility [13]. Also, there are defects in USP26 in some cases [14].

Post-testicular factors: Post-testicular factors decrease male fertility due to conditions that affect the male genital system after testicular sperm production and include defects of the genital tract as well as problems in ejaculation: e.g. impotence, Vas deferens obstruction, lack of Vas deferens, infection e.g. prostitis, ejaculatory duct obstuction and hypospadias.

Hypothalamic-pituitary factors: Conditions that affect the hypothalamopituitary-axis will eventually affect the gonadotrophin releasing hormone (GRh) and hence the levels of follicle stimulating hormone, luteinizing hormone and prolactin hormone. These conditions include Kallmann syndrome (isolated gonadotropin deficiency), hyperprolactinemia and hypopituitarism. Hyperprolactinemia may be due to diseases affecting the hypothalamus and pituitary gland or secondary to disease of other organs such as the liver, kidneys and thyroid [15] Hyperprolactinemia may cause hypogonadism, erectile dysfunction, decreased libido, gynecomastia and infertility.

Environmental factors: The etiological importance of environmental factors in infertility has been stressed. The implication of toxins such as glues, volatile organic solvents, silicones, physical agents, chemical dusts and pesticides in infertility had been established [16]. Radiations and excessive heat to the genitalia have damaging effect on the testicles. Hence individuals having direct contact with or exposure to such chemicals have high chances of having primary or secondary infertility as the case may be. Estrogen like hormone disrupting chemicals such as phthalates are of particular concern for infertility in men and for effects on offspring of women. Exposure to phthalates can occur via dietary consumption, dermal absorption or inhalation and has been linked with impaired spermatogenesis and increased sperm DNA damage [17]. The mechanism for this is probably due to increase in the generation of reactive oxygen specie (ROS) within the testis and a concomitant decrease in antioxidant levels, culminating in impaired spermatogenesis as observed in rats.

Life style and infertility: The contribution of tobacco smoking and alcohol intake to infertility had been established. Tobacco smoking was observed to damage sperm DNA [18]. Though some of the damage is irreversible, but stopping smoking can prevent further damage [19]. Smokers are 60% more likely to be

infertile than non-smokers. Smoking reduces the chances of IVF producing a live birth by 34% and increases the risk of an IVF pregnancy miscarrying by 30% [20]. Smokers have decreased levels of antioxidants such as Vitamin E and Vitamin C, placing their spermatozoa at additional risk of oxidative damage.

Sexually transmitted disease (STD) and infertility: STDs are diseases transmitted through sexual activity with an infected partner caused by viruses, bacteria, or parasitic microorganisms. STDs are a leading cause of infertility. They are often asymptomatic but may display few symptoms, with the risk of failing to seek proper treatment in time to prevent decreased fertility [19]. Some of the identified STDs (such as syphilis, trichomoniasis, chancroid, chlamydia, gonorrhea, herpes simplex virus, human papilloma virus, lymphogranuloma venerum) are treatable while many are not and may eventually lead to death.

Urinary Tract Infection and infertility: The urinary tract has a relative anatomical association with the reproductive tract. Escherichia coli and Staphylococcus aureus are reputed to be the most prevalent Gram negative and Gram positive organisms implicated in UTI respectively.

Structural obstruction and infertility: Vas deferens and ejaculatory duct obstruction could be an important cause of infertility as they affect ejaculation. Hypospadia may also be of etiological importance. Obstructive azoospermia may result from previous vasectomy; epididymal, vassal, or ejaculatory duct pathology relating to genitourinary infection; iatrogenic injury during inguinal or scrotal surgery and congenital anomalies.

Spermatozoa and infertility: Azoospermia (low sperm counts), abnormal spermatozoa morphology (shape) and low sperm motility are usually asymptomatic conditions to most males but of great etiological importance. Most cases of low sperm counts are "idiopathic" but may be associated with varicocele and chronic testicular diseases. The quality and the total number of spermatozoa are a reflection of the testicular state, plasma testosterone level and the patency of the post-testicular duct system. The total seminal fluid volume is contributed by the various accessory glands and is thus a function of the secretory activity of the accessory glands. The quality of the spermatozoa (count, vitality, motility and morphology) and the composition of seminal fluid are also important index of sperm function. It is well recognized that sperm DNA can be damaged oxidatively by oxidative stress [21] and non-oxidatively by mechanisms such as aberrant apoptosis and incomplete sperm protamination [22].

Chemotherapy and infertility: Studies have shown that the antral follicle count decreases after the third series of chemotherapy, whereas follicle stimulating hormone (FSH) reaches menopausal levels after the fourth series inhibin B and anti Mullerian hormone levels decrease following chemotherapy. Drugs with high risk of infertility include procarbazine, cyclophosphamide, ifosfamide, busulfan, melphalan, chlorambucil and chlormethine drugs like doxorubicin, cisplatin and carboplatin have medium risk while therapies with plant derivatives (such as vincristine and vinblastine), antibiotics (such as bleomycin and dactinomycin) and antimetabolites (such as methotrexate, mercaptopurine and 5-fluoruracil) have low risk of gonadotoxicity [23].

Microorganisms and infertility: Microbial infections have been reported to reduce sperm viability. Staphylococcus aureus is the most prevalent Gram positive organism, while Escherichia coli is the most prevalent Gram negative organism isolated in the semen of males with primary infertility [24]. Chronic epididymitis secondary to Chlamydia trachomatis infection had been shown to blockage of the epididymis and thus obstructive azoospermia [25]. However, Ureaplasma urealyticum infections induces leukocytospermia and consequently lead to sperm damage, decrease sperm counts and invariably impaired sperm motility [26]. Herpes simplex virus (HSV) was reported to have been found in the semen of some infertile men and was related to low sperm count and poor motility. Mumps viral infections in adolescent

and adult males carry about 30% risk of developing orchitis or epididymitis, which can result in testicular atrophy and sterility.

Adenosine deaminase: ADA (EC 3.5.4.4), the key enzyme involved in the degradative pathway for adenosine to inosine, is generally described as a small (40 kDa), monomeric enzyme of 363 amino acid residues. In humans, different ADA isoforms has been identified and designated as adenosine deaminase-1 (ADA-1), adenosine deaminase-2 (ADA-2) and adenosine deaminase-3 (ADA-3). ADA-1, usually quoted as ADA, may exist either as a low molecular weight form, or as a high molecular complex with the ADA-binding protein CD26. ADA-2 is a 114-kDa homodimer, which is characterized by a complex multi domain architecture, and displays high similarity with the enzymatic mechanism of ADA-1. ADA-2 has been detected only in eukaryotes and multicellular organisms, and it has been classified as an ADA growth factor (ADGF), due to its role in tissue development. ADA-2 has been mainly localized in the extracellular space and, at variance from ADA-1, it presents a peculiar 80-100 amino acid sequence at the N-terminus, accounting for its growth factor activity. Scarce information is currently available about the recently discovered ADA-3 or ADA-like protein (ADAL) [27]. X-ray crystallographic analysis of ADA revealed that this protein adopts a triose phosphate isomerase (TIM)-barrel conformation, folding up in a typical β/α-barrel made of eight central β strands and eight peripheral α helices. Five additional α-helices break off the regularity of barrel [28]. The active site of the enzyme is located and deeply buried in the C-terminal side of the barrel. It appears as a funnel-shaped pocket, with a catalytic zinc ion tightly sequestered in its deepest part (Fig. 1). The zinc ion plays a key role in the enzymatic mechanism, allowing the addition-elimination, SN2-like, reaction catalyzed by ADA [28]. Behaving like a strong electrophile, it polarizes and activates a water molecule which, in turn, may transfer stereospecifically its hydroxyl group to the C6 position of adenosine, to yield the tetrahedral transition state intermediate. The subsequent loss of ammonia from the intermediate generates the inosine product. The zinc ion plays also a relevant structural role, as its removal leads to structural changes, which propagate throughout the ADA, decreasing significantly its stability [29].

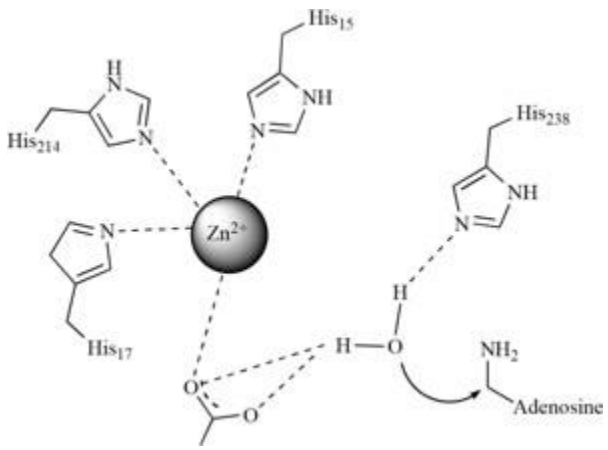

Fig. 1-1 Active site pocket of adenosine deaminase (ADA). Adenosine exerts its modulating actions by interaction with four G protein-coupled receptors, currently classified as A1, A2A, A2B and A3 [30]. The functional relevance of adenosine receptor subtypes is closely correlated with their tissue density, as well as with endogenous adenosine levels in the microenvironment of their biophase [31]. In this regard, the evaluation of tissue expression and function of the enzymes deputed to adenosine synthesis or metabolism appears of extreme interest for understanding the role played by adenosine pathways in the pathophysiology of various diseases. The extracellular concentration represents a crucial determinant for the ability of adenosine to regulate a number of biological functions. The levels reached by adenosine in the biophase of its receptors are strictly regulated by dynamic mechanisms, such as intracellular and extracellular

biosynthesis, transport and metabolism, and they are tightly linked to the energy status available in tissues [32]. Accordingly, several lines of evidence indicate that extracellular adenosine levels are subjected to marked variations in the presence of different adverse conditions [33]. Pathological events, such as hypoxia or inflammation, are associated with marked rearrangements of both synthetic and catabolic enzymes, all concurring in increasing adenosine levels at an early stage of the abnormal condition [34]. In particular, an enhanced adenosine production results from ATP phosphohydrolysis, arising from the amplification of ecto-apyrase (designated also as CD39) and 5'-nucleotidase (designated also as CD73) enzyme activities. In parallel, the suppression of adenosine kinase and nucleoside transporter functions can lead to a sustained adenosine production aimed at preserving tissue integrity [35]. Such an increment of adenosine levels is aimed at increasing organ blood flow by vasodilation, thus ensuring a readjustment of energy supply to demand ratio and limiting the intensity of acute immune inflammatory responses [36].

Role Adenosine deaminase in the immune system: Increasing interest on the regulatory role played by ADA in the activity of the immune system stems from the evidence that a congenital defect of this enzyme represents the main cause of an impaired T and B cell function, as typically observed in the 20-30% of severe combined immunodeficiency disease (SCID) cases. Since those observations, considerable research efforts, attempting to clarify the involvement of purine metabolism in immune cell activity, have pointed out a crucial role of ADA in both development and function of the immune system [37]. In particular, ADA has been found to participate to the regulation of several immune cell types, including macrophages, lymphocytes, neutrophils and dendritic cells

Table 1.2 Describes the most Role Adenosine deaminase in the immune system

Macrophages	Regulation of monocyte maturation and their differentiation into macrophages by ADA [38] Marked increase in ADA activity in stimulated macrophages [39] Correlation between superoxide production and intracellular ADA activity during phagocytosis [40] Increased ADA activity in plasma membranes from murine macrophages and an enhanced enzyme activity in proximity of phagocytic vacuoles [41] Release of ADA from macrophages or monocytes into their surrounding environment [42]
Lymphocytes	Regulation of monocyte maturation and their differentiation into macrophages by ADA [38] Marked increase in ADA activity in stimulated macrophages [39] Correlation between superoxide production and intracellular ADA activity during phagocytosis [40] Increased ADA activity in plasma membranes from murine macrophages and an enhanced enzyme activity in proximity of phagocytic vacuoles [41] Release of ADA from macrophages or monocytes into their surrounding environment [42]
Neutrophils	Increased activity and/or expression of ADA facilitates neutrophil responsiveness to chemotactic stimuli [43] Enhancement of the release of toxic oxygen radicals from neutrophils [44]
Dendritic Cells	Marked ADA activity during inflammation [45]

Adenosine deaminase in seminal fluid: ADA activity has been detected in whole semen, seminal plasma, and sperm cells [46]. It has been reported that inhibition of ADA1 modulates sperm fertilizing ability [47]. Further studies have led to the discovery of ADA1 on mammalian spermatozoa surface (ecto-ADA) and also confirmed the presence of both dipeptidyl peptidase IV (CD26) and adenosine A1 receptor (A1R) as ecto-ADA complexing proteins on spermatozoa [48]. It has been demonstrated that addition of ADA to sperm suspension changes adenylate cyclase activity by decreasing adenosine concentration [49], so extracellular adenosine concentration has to be regulated by ecto-ADA bounded to the cell surface. Previously, has shown a higher activity of plasma ADA in infertile men compared with fertile subjects [50]. In addition, researches have revealed non-enzymatic roles for ecto- ADA such as interaction and/or fusion between prostasomes and spermatozoa [51]. ADA1 encoded by gene located on long arm of chromosome 20 consists of 12 exons and 11 introns. The amino acid sequence of ADA1 is highly conserved among species [52]. Many single nucleotide polymorphism have been observed in ADA1 gene, but the most important polymorphism that affects enzyme function is G22 A in exon 1, resulting from substitution of asparagine (Asn) instead of aspartic acid (Asp) at the eighth codon [53]. Studies have shown that the activity of this enzyme is related to the G22 A genotypes [54]. On the basis of role of ADA in regulating adenosine concentration, which may impact sperm capacitation, motility, and function, any changes in the structure and activity of this enzyme can affect male fertility [51]. To our knowledge, there is no reported study addressing the impact of ADA polymorphism in infertile men. Thus, in this study we aimed to evaluate the frequency distribution of ADA1 G22 A alleles and genotypes in fertile and infertile men. In addition, the correlation of different G22 A genotypes and activity of ADA isoenzymes were investigated in this population.

MATERIALS AND METHODS: Samples collection of the study: The Samples in this study were divided into two parts: **Patients (Oligospermia):** Samples were collected from patients coming to laboratories in Fallujah city during the period from 5 October to 15 December, 2021 AD, and their ages ranged between (27-41 years), and the number of samples was 20. Patients were selected based on the number of sperms in the semen of these persons, as all of these persons had a sperm count of less than 20 million sperms/mL of semen. The physiological and health status of the patients was confirmed, and a special form was adopted for this purpose. **The control group (Normal):** This group included 20 normal persons, their ages ranged between (20-25 years), and the number of sperms in the semen of these persons ranged between (35-83 million.sperm/mL). The physiological condition was confirmed and health of volunteers and adopted a special form for this purpose. **Semen analysis methods:** Were collected and analyzed Forms based on actions recommended by the [55] World Health Organization Semen samples were collected in the laboratory by masturbation (after abstaining from sexual intercourse) Intercourse (for 3-5 days, semen collection) In clean, sterile plastic containers. **Preliminary microscopic examination:** It included a physical examination Appearance test (number pH, volume, volume Consistency (according to procedures recommended by (W.H.O) World Health Organization [55]. **Initial microscopic examination:** Examination included Primary microscopic estimation of potency, total number and shape of sperms differential according to the WHO method [55]. **Activity of adenosine deaminase:** The activity of the enzyme was measured for adenosine deaminase in the serum. The enzyme activity was measured on the basis of the Berthelot reaction that included the formation of a colored complex indophenol in the presence of phenol with sodium hypochlorite and ammonia in the presence of nitroprusside as a catalyst. The intensity of the color was measured at a wavelength (628 nm) using a spectrophotometer.

Statistical analysis: The study effect was analyzed Statistically, by analysis of variance using Spss 20 program to show the difference between more than one Two groups.

Preparation of solutions: Phosphate buffer solution**:** The buffer solution was prepared by dissolving (4.73 g) of monosodium phosphate (NaH2PO4.2H2O) with (5.62 g) of disodium phosphate (Na2HPO4.12H2O)

in a liter of ammonia-free distilled water and adjusting the pH to pH = 6.5, then keeping the solution in temperature (4°C) until use.

Substrate buffer solution (ADA): (15 mL) of the phosphate buffer solution was added to (140 mg) of the base material (ADA) in a volumetric flask (25 mL), the mixture was placed in a water bath at a temperature of (70 °C) for two to four minutes, the solution was cooled, then the volume was completed with the phosphate buffer solution to (25 ml) This solution must be prepared immediately.

Stock solution of ammonium sulfate: Dissolve (1.982 g) of anhydrous ammonium sulfate in (250 mL) of ammonia-free distilled water and complete the volume to a liter of the same water in a volumetric flask and keep at (4°C) until use.

Standard ammonium sulfate solution: Dilute (0.5 mL) of ammonium sulfate buffer solution in (25 mL) of phosphate buffer solution and complete the volume to 100 mL of the same solution.

Phenol nitroprusside solution: Dissolve 10 g of phenol with 50 mg of sodium nitroprusside in 500 mL of ammonia-free distilled water, then add the volume to a liter in the same water and keep at 4°C until use.

Basic sodium hypochlorite solution: Mix 125 mL of (N1) sodium hydroxide with 16.4 mL of Clorox containing NaOCl 5% w/v and supplement the volume with ammonia-free distilled water to a liter in a volumetric flask. Measure method of activity of adenosine deaminase enzyme

Table 2.1 Sequence of adding solutions to measure enzyme activity, step One

Test tube	Efficient	Standard	Adjust	Sample
Phosphate buffer solution	1 mL			
Adenosine buffer solution			1 mL	1 mL
Standard ammonium sulfate solution		1 mL		
Sample				0.05 mL
Distilled water	0.05 mL	0.05 mL		

Shake the tubes well, then close them tightly, and place them in a water bath for 60 minutes at a temperature of 37°C, and then add

Table 2.2 Sequence of adding solutions to measure enzyme activity, step Two

Test tube	Efficient	Standard	Adjust	Sample
Phenol nitroprusside solution	3 mL	3 mL	3 mL	3 mL
Sample			0.05 mL	
Basic Sodium hypochlorite solution	3 mL	3 mL	3 mL	**3** mL

After adding the phenol solution and the basic sodium hypochlorite solution, the tubes are returned well and then placed in a water bath for 30 minutes and at a temperature of 37°C. The absorbance is measured at a wavelength 628 nm using a spectrophotometer.

Calculations to find the Activity of Adenosine deaminase enzyme

$$\text{Activity}_{(IU/ml)} = \frac{A-B}{C} \times \frac{1}{T} \times \frac{1}{Vs} \times \frac{Vi}{Vt} \times 1000$$

Where:

A= Sample absorbance – Adjust absorbance

B= Adjust absorbance – Efficient absorbance **C=** Standard absorbance – Adjust absorbance

T= Incubator time

Vs= Sample volume

Vi= Sample of volume in incubator period

Vt= The volume of the total solution

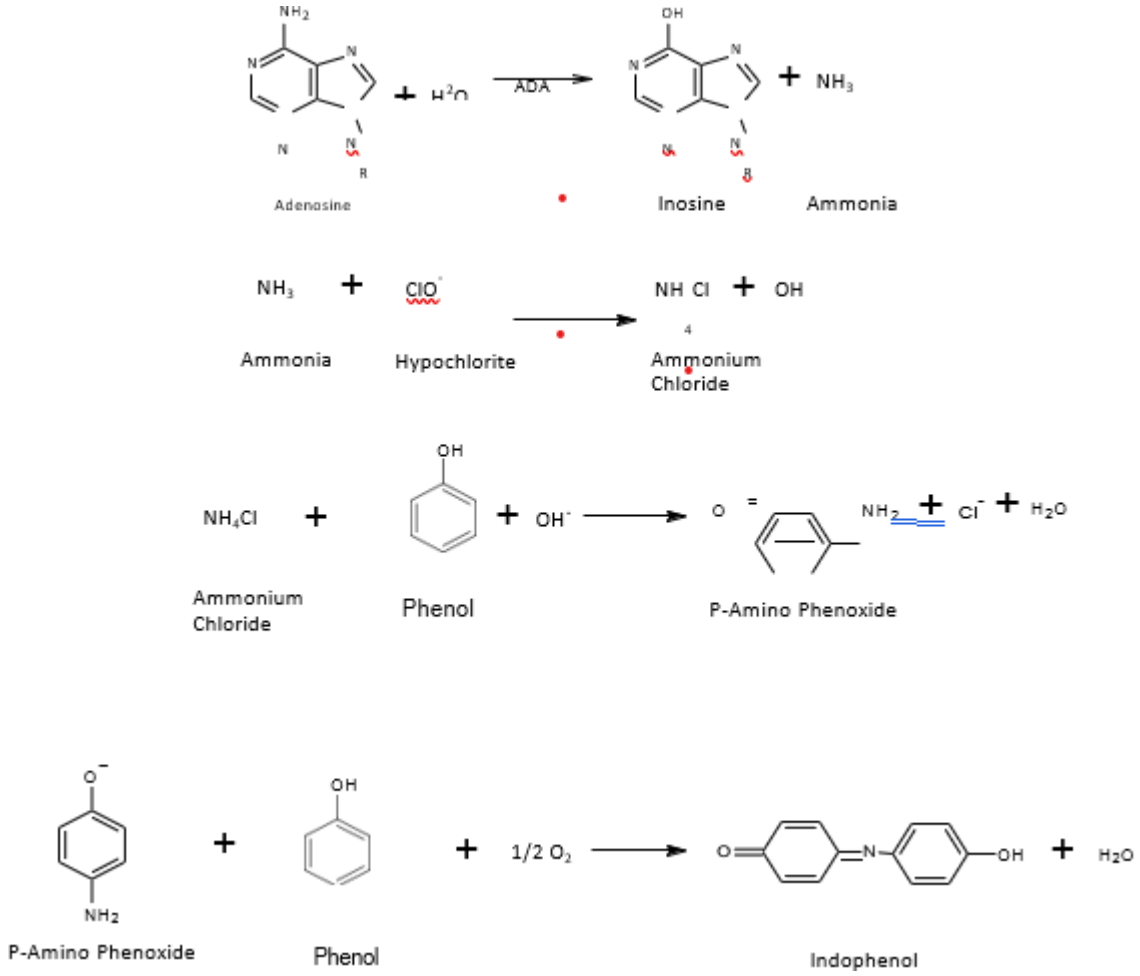

Fig. 2-1 The basis of the Berthelot reaction of Adenosine Deaminase

Result: The mean ages of normal and oligospermia men were (32.7 ± 4.37) and (26.8 ± 5.2) years respectively. The two groups are given in Table 3 presents semen parameters of the oligospermia and normal groups. Adenosine deaminase activity is shown in Table 3. There were significant differences in the ADA Activity between normal and oligospermia groups. The Activity of ADA of oligospermia men was higher than that of the normal men individuals.

We did observe significant correlation between ADA Activity with Age and count of sperm parameters. As the relationship between Activity of Adenosine deaminase and age is direct, as the age increases, the Activity Adenosine deaminase increases and vice versa, while the relationship between the Activity Adenosine deaminase and Count of sperm and is inverse, as the higher the Count of sperm, the lower the Activity Adenosine deaminase. We did not observe significant correlation between ADA activity with other Parameter.

Table 3.1 Describes the Parameter and Activity Adenosine deaminase in seminal fluid			
Parameter	Oligospermia group Mean ± SD N=20	Control group Mean ± SD N=20	p- value
Age (Year)	32.7 ± 4.37	26.8 ± 5.2	N.S
BMI (Kg/m2)	27.33 ± 1.35	24.53 ± 2.11	N.S
Count (1000sperm/ml)	12.80 ± 2.85	50.95 ± 20.89	0.00
Activity of ADA(IU/ml) in Seminal Fluid	32.91 ± 11.83	15.12 ± 3.44	0.00

Discussion

In the present study, we determined the Activity Adenosine deaminase in seminal fluid of normal men and oligospermia men. Significant differences were found between activities of ADA between normal men and oligospermia men individuals. Activity of ADA higher in oligospermia men than in the normal men group. ADA converts adenosine to inosine; therefore, increased ADA activity in infertile individuals can lead to reduced adenosine levels. It is generally accepted that adenosine plays an important role in the capacitation and fertilization process [56]. There is evidence revealing the effect of inhibition of enzymes involved in adenosine metabolism on sperm fertilization and capacitation. Inhibition of adenosine deaminase activity leads to increased adenosine level that improves and accelerates sperm fertilization [57]. Our results indicate increased ADA activity in Oligospermia men, which can result in reduction of adenosine availability. Our findings suggest that increased ADA activity and decreased adenosine level may be involved in reducing the fertile rate in Oligospermia individuals. The reasons of elevated ADA activity in Oligospermia men are not clear. As the major source of ADA in serum is likely to be the monocyte–macrophage system [58], it is possible that higher numbers of monocytes in Oligospermia men than in fertile men may be one reason for increased ADA activity in Oligospermia individuals. Another reason for different activity of ADA in fertile and Oligospermia men may be genetic variation in this enzyme. In humans, ADA shows a polymorphism due to G to A transition at nucleotide 22 of exon 1, which results in two isoforms, ADA1 and ADA2. Activity of ADA1 is higher than that of ADA2 [59]. Some studies have reported higher frequency of ADA1 isoenzyme in couples with sterility, which is in agreement with our findings [60]. On the other hand, higher activity of ADA in Oligospermia compared with that of fertile men may result from genetic variation in ADA. It seems that activity of and genetic variation in ADA have an important role in human reproduction. Recently, [60] suggested that ADA polymorphism may be a genetic marker for prediction of human reproduction. Further studies need to clear the role of ADA in male infertility. Considering our study results and lack of appropriate research about this polymorphism and male Fertility, study of ADA isoenzymes activity in seminal fluid with evaluation of G22A polymorphism in fertile and infertile men seems necessary. Furthermore, there is a need to assess impact of this polymorphism on binding of ecto-ADA to its cell surface receptors. Moreover, we found high activity of ADA in Oligospermia male and it maybe decrease adenosine concentration around spermatozoa. So, selective adding of adenosine to sperm environment in Oligospermia men with high ADA activity, might improve fertilization and pregnancy rates. As, previous studies have shown significant improvements in sperm motility, number of recoverable sperms, fertilization rate, number of replaceable embryos and pregnancy rate following sperm incubation in analogue of adenosine.

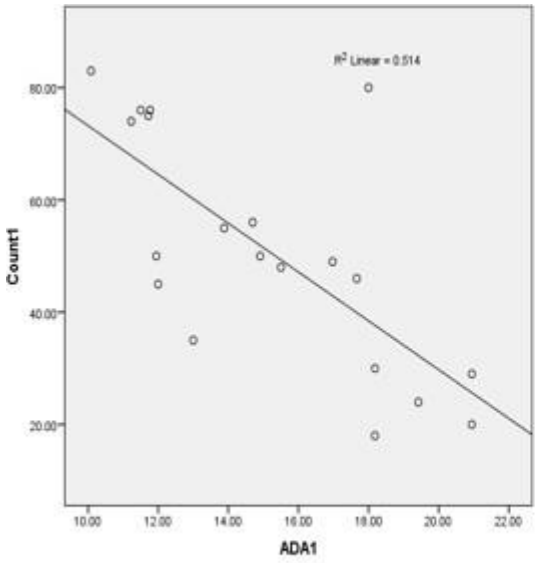

Fig. 3-1 Describes the relationship between sperm count and ADA

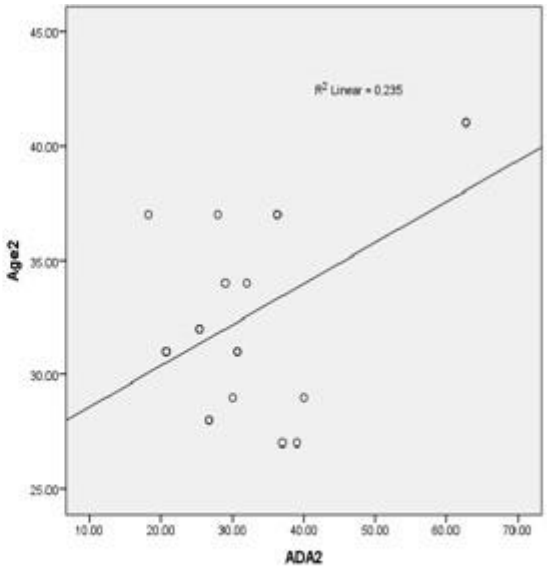

Fig. 3-2 Describes the relationship between Age and ADA activity

The seminal ADA levels showed a negative correlation with count of sperm (**Fig.** 3-1) and a positive correlation with age (**Fig.** 3-2) when correlation analysis was applied to the total sample (n = 40). The above-mentioned correlation may suggest the possibility of an inflammation in the genital tract that is adversely affecting the quality of sperms

Conclusion

Our results showed an increase in ADA activity in Oligospermia than in fertile men. This alteration in ADA activity can lead to reduced adenosine levels, which may be involved in disturbing the Fertility process. Previous reports in this topic are contentious therefore, ADA activity could be regulated in different population by immunologic and genetic factors.

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