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CAG Expansion in Androgen Receptor Gene of Infertile Men in Erbil Governorate

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ABSTRACT

Spermatogenesis and male phenotypic development during puberty are mainly done by androgen and their function is controlled by the gene for androgen receptor (AR). This gene has a region of polymorphism in Exon1 which encode androgen receptor and have various length of CAG trinucleotide repeat which causes the production of polyglutamine chain in different length of the N-terminal region of the AR protein, which reduces producing sperm by disrupting spermatogenesis. The aim is to determine the relation of infertility in male with the AR gene which has more CAG repeats than usual, and the correlation between CAG repetition and hormonal changes. The case-control study was done in the Immunogene center and IVF center in the Kurdistan region of Iraq's maternity teaching hospital. The illustrative sample included 50 men, 30 infertile and 20 fertile over one year starting from March 2021 to March 2022. The result of the recorded analysis of the CAG repetition on fertility showed men with infertility had CAG repeats in their AR gene, ranging from (17-26) repeats, with a mean (21.3 ±0.16). In infertile men, CAG expansion was longer than the fertile men. The sperm's normal morphology and motility in infertile men have negative relation while sperm count and concentration have a positive relation with CAG expansion. The relation of hormones (Testosterone, LH, and FSH) with CAG repetition was statistically not significant. In conclusion,CAG expansion was longer in infertile (case) men compared with fertile (control) men. Polyglutamine effect on increasing sperm abnormal morphology and immotility which is the reason for infertility is statistically not significant and it will not affect hormonal assay in infertile men.

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1. INTRODUCTION

The failure of sexually active and non-contraceptive couples to become pregnant within a year is referred to as male infertility. The decreasing birth rate is one of the most significant social issues affecting modern nations today. Two main factors affect patients with infertility including environmental factors, such as global warming (pollution) and social factors (such as changes in the age at which women marry and the impact of such changes on societal change for women) with the male partner's infertility accounting for about 50% of all cases [1]. Some medical professionals interchangeably use the terms infertility with subfertility[2].Infertility affects more than 186 million people worldwide, with most of them living in poor countries [3]. While aging of the woman is the main factor that reduce fertility [4]. Also, environmental and lifestyle variables are regarded as other factors which have an important role in increasing risk of infertility. According to estimates in the developing world, one in seven weddings and one in every four couples suffer from infertility among reproductive aged couples. Men are shown to be responsible for 20–30% of incidences of infertility, where as they are contribute in 50% of all cases, despite of this, not all parts of the world are adequately represented by these numbers. Male infertility rates ranged from 4.5 to 6%, 9% to 8 to 12% in North America, Australia, and Central and Eastern Europe, respectively, while rates were greatest in Africa and Central/Eastern Europe [5].

It is necessary that each couple be diagnosed for fertility problems and treated together because male and female reasons frequently coexist. There are a number of reversible and irreversible disorders which may affect male fertility.The partner' ages, medicines, surgical histories, exposure to environmental pollutants, genetic problems, and systemic disorders are additional variables that may have an effect on fertility.The main goal of testing a person for infertility is to identify his contributing causes, provide treatment for those that are reversible, decide whether he is a candidate for assisted reproductive methods (ART), and provide counseling for disorders that are both untreatable and irreversible [6]. Male infertility is caused by defect in spermatogenesis, which is necessary for the development and maintenance of male reproduction.The testis's niche or microenvironment, which is essential for maintaining normal spermatogenesis, is composed of somatic cells such as Sertoli and Leydig cells. The control of spermatogenesis is mediated by the actions and processes of both sertoli and Leydig cells [7].

Infertile men may have sperm abnormalities or phenotypic[8].Androgen, which is primarily released by testis, is responsible for male sex differentiation, development, sperm production and sexual behavior. The androgen receptor (AR), is a member of the superfamily of steroid hormone receptors that controls the effects of the testosterone hormone[9].

Cellular processes that react to androgens are critical for the production of spermatogenesis and the male phenotype,genetic modifications and mutations in spermatogenesis-related genes, such as the gene that encodes the androgen receptor (AR), can result in impaired spermatogenesis[10]. The single copy gene that codes for the AR is found on the X chromosome's long arm and mediates the action of androgens. The eighth exon of this gene code for an intracellular transcription factor that is a member of the nuclear receptor superfamily for steroids, this gene which produces polyglutamine has polymorphisms that may be examined including anExon 1 of the N-terminal domain contains a repetition of the CAG trinucleotide. Polyglutamine strands are produced by CAG repeats and are incorporated into the structure of the protein that is being produced[11]. Directly controlling gene transcription is the main mechanism of action for AR,after an androgen binds to its receptor, a conformational change takes place that results translocations into the nucleus, dimerization, and the dissociation of heat shock proteins, the AR dimer attaches to a region of DNA described as a component of the hormonal response, up- or down-regulating the transcription of a certain gene, additionally, AR could possibly function through a non-genomic mechanism that involves the immediate activation of kinase-signaling cascades and the regulation of intracellular levels of calcium [12]. In terms of the direct action, testosterone has an effect by AR, both directly as well as via a metabolite, dihydrotestosterone, which is produced by the

enzyme 5- reductase[12].Several researchers found the number of a repetition of CAG in the analyzed samples byway of sequencing and PCR, and proposed the spermatogenesis may be affected by the CAG polymorphism, while others claimed that,this polymorphism might not have a significant effect in male infertility, however, via sequencing and polymerase chain reaction, research among both fertile and infertile male Egyptians discovered a strong correlation between higher the AR gene's CAG expansion and the production of azoospermia (AS) and oligozoospermia (OS) in infertile males[13]. There is a relation between the AR-CAG repeat polymorphism and male infertility, further investigation into the molecular processes underlying Polymorphism of the male infertility and the AR-CAG repeat risks is necessary, the higher CAG repeat length may alter either the transcription factor's binding region or the AR mRNA sequence's secondary structure [9].

The present research was done to find out the relation of infertility in male with the increased frequency of CAG repetition in the AR gene, and the correlation between CAG repetition and hormonal changes.

2. METHODS AND MATRIALS

This case-control research was done in Immunogen center and IVF center in a maternity teaching hospital Erbil-Kurdistan region-Iraq. The convenience sample included 50 men, 30 infertile and 20 fertile over one year starting from March 2021 to March 2022. The studied population includes young reproductive-age infertile males whose age was between 25-44 years (case) compared with healthy fertile men (control). Inclusion criteria, all young age reproductive men who have had a history of male factor infertility for more than one-year. Infertile men with a history of chronic diseases (chronic renal disease, chronic liver disease, and who are on anti-depressant drugs) were excluded. After taking verbal consent from each participant, we started to collect data.

2.1 Determination of AR gene CAG repeat length

In this study we collected 3ml blood from each participants in a Ethylenediamine tetra acetic acid (EDTA) containing tube and Genomic Deoxyribonuclic acid (DNA) was isolated from peripheral blood using a DNA extraction kit which was (Genomic extraction kit, ROCHE, Germany) utilizing Nano Drop TM One (Thermo Scientific) in accordance with the protocol of manufacturer and the purity of the DNA samples, and then storing them at -20°C. The control samples for the AR gene were screened for CAG expansion in Exon1 of the AR gene. Primer for AR gene Forward: 5'TCCAGAATCTGTTCCAGAGCGTGC-3' and Reverse: 5'-GCTGTGAAGGTTGCTGTTCCTCAT-3'. Polymerase chain reaction (PCR) which prepared manually by using software primer and AR genomic sequence obtained from the NCBI Genomic browse and it was performed to amplify DNA fragments in Exon1 of AR gene and DNA fragments were determined by agarose gel electrophoresis and stained with ethidium bromide.The PCR products were purified and analyzed by using the Sanger sequencing technique a Genetic Analyzer (Applied Biosystems, Hitachi High- Technologies, Tokyo, Japan). The PCR products are sequenced then using (BioEdit.exe)and Mega 6 (http://www.megasoftware.net/) for multiple sequence analysis and alignment.

2.2 Analysis of the semen

After 2 to 7 days without having intercourse, samples of semen were taken for examination. Semen analysis was carried out by WHO recommendations[14]. If a manual analysis was done on a semen sample.

2.3 Hormonal assay

The cubital vein was used to collect five milliliters of venous blood, and the serum was promptly separated and kept at -20 °C at the recruiting center. For hormonal analyses (FSH, LH, testosterone), the laboratory tests were conducted using the collected frozen serums by Cobas e411 immunoassay (Hitachi, Japan).

The Social Sciences were used to analyze the data for statistical analysis (SPSS, version 25). 0.05 as the p-value or less was considered significant. The Chi-square test was employed for comparing expected frequencies between studied groups. A post-hoc test (LSD) was

performed to evaluate each pair of means. the findings of a one-way analysis of variance (ANOVA) were used to analyze more than two means (after doing ANOVA), and molecular genetic analysis was performed by (Bio edit and Mega 6).

3. RESULTS

3.1 Frequency of CAG expansion among case and control groups

To determine if the categorical variables' distributions varied from one another, we used the Chi-square test. Among fertile and infertile males, the prevalence of CAG expansion in the Current research on CAG expansion found in the AR gene of infertile men, ranging from (17-26) repeats, which the mean CAG repeat was (21.3 ±0.16). The CAG repetition number that was seen the most frequently was 25 (16.7%). Studies of controls revealed a total of CAG repeats varying from (21-24) repeats, with a mean of (22.5 ±0.07). The most frequent CAG repetition (21) was reported in (55%) of fertile men. From our research, it is intriguing to discover different repeat sizes' distribution (17-26) in both case and control participants. Almost the same (64%). In the infertile men, the CAG expansion range is longer than the fertile men which are between (17-26) and (21-24) respectively. As shown in (Table1) and (Fig.1).

Table 1: Frequency of CAG expansion in the AR gene among fertile and infertile men

CAG repeat expansion	Fertile men No: (20)		Infertile men No: (30)	
	Number	Percentage	Number	Percentage
17	0	0	1	3.33
18	0	0	2	6.7
19	0	0	4	13.33
20	0	0	3	10
21	11	55	4	13.33
22	3	15	4	13.33
23	3	15	0	0
24	3	15	4	13.33
25	0	0	4	13.33
26	0	0	4	13.33
Mean	21.3		22.5	
SE	0.16		0.07	

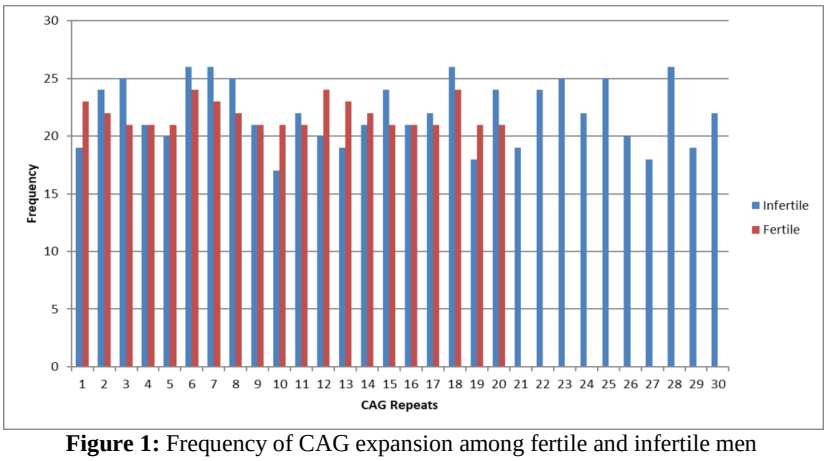

Figure 1: Frequency of CAG expansion among fertile and infertile men

3.2 CAG expansion in infertile men according to age

We compared CAG expansion within the AR gene according to the frequency of repetition with the age of the infertile group (≤20 repetitions, between 21-24 repetitions, and ≥25 repetitions) the mean were 36.40±7.63, 34.47±7.59, and 39.00±7.11 respectively statistically not significant but the repetition was higher in older age (Table 2).

Table 2: CAG expansion in infertile men according to age

	CAG expansion			p.value
	≤20 repetition (No:10)	**21-24 repetition (No:12)**	**≥25 repetition (No:8)**	
Age	36.40±7.63	34.47±7.59	39.00±7.11	0.458

3.3 Association between CAG expansion and seminal parameters in infertile men

The mean values of seminal fluid analysis parameters in terms of volume, concentration, count, motility, and morphology show deferent means according to the subgroups of CAG expansion but are statistically non-significant (p.value>0.05) but we found that the sperm's normal morphology and motility in infertile men was affected by CAG repetition negatively that means they were decreased by increasing time of repetition.

While sperm count and concentration have a positive relation with CAG expansion, that means they were increasing both with increasing CAG repetition shown in (Table 3).

Table 3: Association between CAG expansion and seminal parameters in infertile men

		CAG expansion			p.value
		≤20 repetition (No:10)	**21-24 repetition (No:12)**	**≥25 repetition (No:8)**	
Semen volume (ml)		2.18±0.84	2.54± 1.21	2.038±1.21	0.571
Sperm concentration (10^6/ml)		9.92±19.92	10.20± 10.78	20.38± 28.43	0.459
Sperm count (10^6/ml)		21.81±40.49	32.31± 39.48	32.10±47.07	0.815
Sperm motility (%)	**Motile**	14.20± 19.83	20.17± 19.80	8.13± 9.23	0.339
	Immotile	85.80± 19.83	79.83± 19.80	91.88± 9.23	0.339
Morphology (%)	**Normal**	1.70± 2.06	1.50± 1.38	1.00± 1.19	0.647
	Abnormal	98.30±2.06	98.50± 1.38	99.00± 1.19	0.647

3.4 Frequency of CAG expansion in infertile men according to seminal fluid categories

In infertile men with azoospermia, the most frequent repetition of CAG was seen in the ≤20 repetition subgroup. As we discussed in the table (2) we revealed that sperm count and concentration increase with higher CAG repetition that is why we see the least frequency oligozoospermic members were seen in the highest CAG repetition. In infertile men with normozoospermic, the most frequent CAG repetition was seen (21-24) repetition subgroup.

There was a difference in CAG expansion repetition between Azoospermia, Oligozoospermia, and Normozoospermia but they were statistically non-significant (P=0.604) as shown in (Table 4).

Table 4: Frequency of CAG expansion in infertile men according to seminal fluid categories

	CAG expansion			p.value
	≤20 repetition (No:10)	**21-24 repetition (No:12)**	**≥25 repetition (No:8)**	
Azoospermia (No:10)	4	3	3	
Oligozoospermia (No:12)	5	5	2	0.604
Normozoospermia (No: 8)	1	4	3	

3.5 Association between CAG expansion and hormonal level in infertile men

Table (5) shows thehormonal level in infertile men which include (Testosterone, LH, and FSH). Our study revealed that the hormonal levels not affected by CAG repetition in all three subgroups, p.value was not significant but the level of testosterone was lower in ≤20, 21-24 repetition subgroup compared with ≥25 repetition subgroup.

Table 5: Association between CAG expansion and hormonal level in infertile men

	CAG expansion			p.value
	≤20 repetition (No:10)	**21-24 repetition (No:12)**	**≥25 repetition (No:8)**	
Testosterone (ng/ml)	2.75±1.21	2.98±0.65	3.46±1.37	0.375
LH (mlU/ml)	4.81±3.12	6.25±2.75	6.49±5.41	0.570
FSH (mlU/ml)	8.10±8.32	7.22±5.43	4.73±3.75	0.510

4. DISCUSSION

Reduced intratesticular androgens impair spermatogenesis because androgens are required for proper sperm generation. In addition, the CAG repetition polymorphism which located in Exone1 of AR gene,prostate cancer and spine and bulbar muscle atrophy (SBMA) are linked to repeat lengths greater than 35. Additionally, the AR N-terminal transactivation domain's, a polymorphic CAG repeat that codes for a polyglutamine elongation and ranges in length from 8 to 35, has been associated with male infertility, but the exact molecular mechanisms of how the CAG repeat polymorphism affects male infertility are unknown. It suppose that the secondary structure of AR mRNA sequence or the transcription factors binding site may change by the longer CAG repeat length. [15].

Current findings on the CAG repeats in the AR gene of infertile males revealed, ranging from (17-26) repeats, a mean CAG repetition was (21.3 ±0.16). In the infertile men, the CAG expansion range is longer than the fertile men which are between (17-26) and (21-24) respectively. The current study agrees with a study done on an Iranian population[11],which showed that men with abnormal spermatogenesis had considerably in their AR gene, there are more CAG repeats than men in fertile men. Also, in another study[16], the result indicated that male infertility is correlated with a greater CAG repeat length. In the Caucasian population, CAG repetition length was linked to infertility in men [17]all the above studies were supporting the current study. Male infertility was not linked to CAG repeat length in Egyptian populations [18], which is in contrast to our research, Different ethnic and regional origins, as well as statistical bias selection, may be responsible for this disparity in distribution.

We compared CAG expansion in the AR gene based on the frequency of expansion with the age of the infertile group it was statistically not significant but the repetition higher in older age. A study done in Egypt[19] demonstrates clinical features and hormonal parameters, as well as the properties of the sperm, and the 3 groups' CAG repetition lengths (oligozoospermic, fertile, and asthenospermic) with age which support our study were confirmed by a post hoc analysis that showed no significant differences between the infertile subgroups and the fertile control group.

Frequency of CAG expansion in infertile men with azoospermia the most frequent repetition of CAG was seen in the ≤20 repetition subgroup. In a study [9], increased AR-CAG repeat length had been linked to azoospermia, however, neither severe nor mild oligospermia showed this association which is in contrast to our results. Also, a strong correlation was discovered between the recurrence of the AR-CAG repetition and oligospermia in the cohort study done in Jordon[20]. In infertile men with normozoospermic, the most frequent CAG repetition was seen (21-24) repetition subgroup.

There was a difference in CAG expansion repetition between Azoospermia, Oligozoospermia, and Normozoospermia but they were statistically non-significant (P=0.604). As we revealed

that sperm count and concentration increase with higher CAG repetition that is why we see the least frequency oligozoospermic members were seen in the highest CAG repetition.

In the association between CAG expansion and seminal parameters in infertile men, the mean values of seminal fluid analysis parameters show deferent means for each sperm parameters (volume, concentration, count ,motility and morphology) according to the subgroups of CAG expansion but are statistically non-significant (P value>0.05) but we found that the normal morphology and sperm motility in infertile men was affected by CAG repetition negatively. According to other research, sperm motility might be affected by AR polymorphisms that are within the normal range in a [13], which supports our study that shows increasing in immotile sperms with increasing CAG repetition. Also, another study is done by (D. Milatiner *et.,al*)[21], in agreement with our study found that mean Compared to the normal morphology group, the abnormal morphology group's CAG repeat length was somewhat longer (22.19 6 0.38 versus 21.25 6 0.28 repeats, P = 0.02). There is another investigation, showed that numbers of spermatogonial and sperm morphology may be affected by the lengthy AR-CAG repeats, research has shown[20].

While sperm count and concentration have a positive relation with CAG expansion, that means they were increasing both of them with increasing CAG repetition. Other studies which agreed with the current study found that the length of the 20–23 CAG repetition, which are the most prevalent, is related to semen characteristics, especially sperm concentration and total count[22].A different study done in Egypt [18]and Khuzestan Province, Southwest Iran [23] have not shown that there is a strong association between them, which is in contrast to our results that showed sperm concentration had a positive relation with CAG expansion.

Regarding the association between CAG expansion and hormonal level in infertile men which includes (Testosterone, LH, and FSH) in current study revealed that not affected by CAG repetition in all three subgroups, P.value was not significant but the level of testosterone was lower in ≤20 and 21-24 repetition of CAG subgroup compared with ≥25 repetition subgroup. Other studies have shown a relationship between blood testosterone (T) levels in infertile men and the length of CAG repeats [19] which supports the current study. CAG repetition shows no relationship with LH level [24], which is in line with the current study. In our study level of FSH was not affected by increasing CAG expansion this is supported by a study [25]that demonstrated that there was no link between the levels of FSH and the AR CAG polymorphisms. But the current study in contrast with the research[26], found to be a high correlation between a lower level of men's serum FSH and a longer AR CAG repetition.

5. CONCLUSION

The conclusion of our research findings highlighted that the CAG expansion is longer in infertile (case) men compared with fertile (control) men. Poly glutamine effect on increasing sperm abnormal morphology and immotility which is the reason for infertility but statistically not significant and it will not affect hormonal assay in infertile men.

REFERENCE

[1] T. Miyamoto, A. Tsujimura, Y. Miyagawa, E. Koh, M. Namiki, and K. Sengoku, "Male infertility and its causes in human," *Advances in urology*, vol. 2012, 2011.

[2] M. Vander Borght and C. Wyns, "Fertility and infertility: Definition and epidemiology," *Clinical biochemistry*, vol. 62, pp. 2-10, 2018.

[3] M. C. Inhorn and P. Patrizio, "Infertility around the globe: new thinking on gender, reproductive technologies and global movements in the 21st century," *Human reproduction update*, vol. 21, pp. 411-426, 2015.

[4] R. J. Hart, "Physiological aspects of female fertility: role of the environment, modern lifestyle, and genetics," *Physiological reviews*, vol. 96, pp. 873-909, 2016.

[5] A. Agarwal and D. A. Kumar, "Viscoelastic Assisted Bubble Formation in Pre Descemet's Endothelial Keratoplasty (PDEK)," *The Official Scientific Journal of Delhi Ophthalmological Society*, vol. 26, pp. 37-39, 2015.

[6] K.-W. Shih, P.-Y. Shen, C.-C. Wu, and Y.-N. Kang, "Testicular versus percutaneous epididymal sperm aspiration for patients with obstructive azoospermia: a systematic review and meta-analysis," *Translational andrology and urology*, vol. 8, p. 631, 2019.

[7] R. Zhou, J. Wu, B. Liu, Y. Jiang, W. Chen, J. Li, *et al.*, "The roles and mechanisms of Leydig cells and myoid cells in regulating spermatogenesis," *Cellular and molecular life sciences*, vol. 76, pp. 2681-2695, 2019.
[8] M. C. Louie and M. B. Sevigny, "Steroid hormone receptors as prognostic markers in breast cancer," *American journal of cancer research*, vol. 7, p. 1617, 2017.
[9] B. Pan, R. Li, Y. Chen, Q. Tang, W. Wu, L. Chen, *et al.*, "Genetic association between androgen receptor gene CAG repeat length polymorphism and male infertility: a meta-analysis," *Medicine*, vol. 95, 2016.
[10] K. Bogefors, Y. L. Giwercman, J. Eberhard, O. Stahl, E. Cavallin-Stahl, G. Cohn-Cedermark, *et al.*, "Androgen receptor gene CAG and GGN repeat lengths as predictors of recovery of spermatogenesis following testicular germ cell cancer treatment," *Asian Journal of Andrology*, vol. 19, p. 538, 2017.
[11] S. Sharestani, S. M. Kalantar, N. Ghasemi, and E. F. Yazd, "CAG repeat polymorphism in androgen receptor and infertility: A case-control study," *International Journal of Reproductive BioMedicine*, vol. 19, p. 845, 2021.
[12] G. Tirabassi, A. Cignarelli, S. Perrini, G. Furlani, M. Gallo, F. Pallotti, *et al.*, "Influence of CAG repeat polymorphism on the targets of testosterone action," *International journal of endocrinology*, vol. 2015, 2015.
[13] N. Delli Muti, A. Agarwal, E. Buldreghini, A. Gioia, A. Lenzi, M. Boscaro, *et al.*, "Have androgen receptor gene CAG and GGC repeat polymorphisms an effect on sperm motility in infertile men?," *Andrologia*, vol. 46, pp. 564-569, 2014.
[14] R. Menkveld, "Clinical significance of the low normal sperm morphology value as proposed in the fifth edition of the WHO Laboratory Manual for the Examination and Processing of Human Semen," *Asian journal of andrology*, vol. 12, p. 47, 2010.
[15] C. J. Cortes and A. R. L. Spada, "X-linked spinal and bulbar muscular atrophy: from clinical genetic features and molecular pathology to mechanisms underlying disease toxicity," *Polyglutamine Disorders*, pp. 103-133, 2018.
[16] F. Xiao, A. Lan, Z. Lin, J. Song, Y. Zhang, J. Li, *et al.*, "Impact of CAG repeat length in the androgen receptor gene on male infertility–a meta-analysis," *Reproductive biomedicine online*, vol. 33, pp. 39-49, 2016.
[17] N. Mobasseri, F. Babaei, M. Karimian, and H. Nikzad, "Androgen receptor (AR)-CAG trinucleotide repeat length and idiopathic male infertility: A case-control trial and a meta-analysis," *EXCLI journal*, vol. 17, p. 1167, 2018.
[18] T. Mostafa, L. El-Shahid, A. El Azeem, O. Shaker, H. Gomaa, and H. Abd El Hamid, "Androgen receptor-CAG repeats in infertile Egyptian men," *Andrologia*, vol. 44, pp. 147-151, 2012.
[19] Y. Mosaad, D. Shahin, A. A. M. Elkholy, A. Mosbah, and W. Badawy, "CAG repeat length in androgen receptor gene and male infertility in Egyptian patients," *Andrologia*, vol. 44, pp. 26-33, 2012.
[20] M. S. Al Zoubi, H. Bataineh, M. Rashed, B. Al-Trad, A. A. Aljabali, R. M. Al-Zoubi, *et al.*, "CAG repeats in the androgen receptor gene is associated with oligozoospermia and teratozoospermia in infertile men in Jordan," *Andrologia*, vol. 52, p. e13728, 2020.
[21] D. Milatiner, D. Halle, M. Huerta, E. J. Margalioth, Y. Cohen, A. Ben-Chetrit, *et al.*, "Associations between androgen receptor CAG repeat length and sperm morphology," *Human Reproduction*, vol. 19, pp. 1426-1430, 2004.
[22] H. Wallerand, A. Rémy-Martin, E. Chabannes, L. Bermont, G.-L. Adessi, and H. Bittard, "Relationship between expansion of the CAG repeat in exon 1 of the androgen receptor gene and idiopathic male infertility," *Fertility and sterility*, vol. 76, pp. 769-774, 2001.
[23] S. R. Khatami, H. Galehdari, A. Rasekh, H. Mombeini, and E. Konar, "Assessment of correlation between androgen receptor CAG repeat length and infertility in infertile men living in khuzestan, Iran," *International Journal of Fertility & Sterility*, vol. 9, p. 189, 2015.
[24] C. P. Ryan, T. W. McDade, L. T. Gettler, D. T. Eisenberg, M. Rzhetskaya, M. G. Hayes, *et al.*, "Androgen receptor CAG repeat polymorphism and hypothalamic-pituitary-gonadal function in Filipino young adult males," *American Journal of Human Biology*, vol. 29, p. e22897, 2017.
[25] Y.-M. Ma, K.-J. Wu, L. Ning, J. Zeng, B. Kou, H.-J. Xie, *et al.*, "Relationships among androgen receptor CAG repeat polymorphism, sex hormones and penile length in Han adult men from China: a cross-sectional study," *Asian Journal of Andrology*, vol. 16, p. 478, 2014.
[26] M. Grigorova, M. Punab, T. Kahre, M. Ivandi, N. Tõnisson, O. Poolamets, *et al.*, "The number of CAG and GGN triplet repeats in the Androgen Receptor gene exert combinatorial effect on hormonal and sperm parameters in young men," *Andrology*, vol. 5, pp. 495-504, 2017.