BIOCHEMICAL EFFECT OF LIPOPROTEIN MODULATORS IN INFERTILE PATIENTS (POLYMORPHISM OF ESTROGEN RECEPTOR BETA)

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A B S T R A C T

Male infertility is a multifactorial condition with a strong genetic component. In the last decade a large number of investigations focused on the identification of gene variants affecting spermatogenesis in human. Polymorphisms of the estrogen receptor (ER) genes, have been implicated in male infertility, however, comprehensive data are lacking. We investigated the association between the ER-β gene (ESR2) RsaI and Alul polymorphisms and the idiopathic male infertility in Egyptian males. Allel specific PCR method and restriction fragment length polymorphism (RFLP) were used to detect the ER-β gene polymorphisms in 24 infertile men and 34 age-matched healthy controls. Reproductive hormones were measured and at least two semen analyses were performed in each subject. Compared with the controls, the frequency of the heterozygous Rsa1 AG genotype was almost three times higher in infertile men (16.6 vs. 5.8 %; P = 0.17). The heterozygous Rsa1 AG genotype was associated with an reduction in LH concentration compared with the wild-typ1e Rsa1 GG genotype in both controls and infertile men. Our results further suggest a possible role of ER-β polymorphisms on male infertility. Further studies are needed to replicate our findings as well as to better elucidate the biological mechanisms of the modulation of ER-β on male infertility.

Keywords: polymorphism, idiopathic infertility, estrogen receptor beta, genotype, homozygous, heterozygous.

(BVMJ-24(1): 343-358, 2013**)**

1. INTRODUCTION

In almost 50% of infertile couples, the problem is related to the male; the cause has not been determined in at least 30% of these cases, and are thus considered as idiopathic infertility (43). Estrogen biology is exceedingly complex and important in the development and function of numerous tissues and physiological phenomena (34, 36, 41). There is much evidence to suggest that estrogen and estrogen receptors (ERs) play very important roles during male reproduction (17, 27, 37, 42). In the male testes, estrogens are synthesized from testosterone via the action of aromatase cytochrome P450. Some studies have shown that aromatase deficiencies cause progressive infertility in adult mice, resulting in reduced sperm production and motility (11, 12, 48). However, although many studies have demonstrated a strong association between ER inactivation and abnormal spermatogenesis, the specific role of ER gene mutations or polymorphisms in male infertility is still novel. It has been hypothesized that changes in the androgen-estrogen balance in utero could lead to impairment of embryonic programming including the development of male reproductive organs during fetal life. The hormonal balance could, for example, be

disturbed by increased exposure to estrogenic or antiandrogenic endocrine disruptors (55, 58). Any alteration in the genes involved in androgen–estrogen action or metabolism may disturb the androgen–estrogen balance. It is well known that estrogenic activity is mediated by at least two functional isoforms of the ER, ERα and ERβ (24, 41). Animal models revealed that knockout (ERαKO) and double knockout (ERα/βKO) mice are infertile from puberty, and presented atrophy of the testes and seminiferous tubule dysmorphogenesis, which could lead to decreased spermatogenesis and sperm motility (23). Other studies have identified silent polymorphisms in the human ERα, which may be associated with male infertility .xbaI polymorphism (rs9340799) was associated with either azoospermia or idiopathic severe oligospermia in men (30). Other ERα mutations may interfere with human spermatogenesis (25, 64). ERα is known to be strongly expressed in the epididymis, efferent ductules and Leydig cells, whereas ERβ is predominantly expressed in germ cells, particularly in the primary spermatocytes and round spermatids of the human testis (18, 45, 51, 52). These data suggest that ERβ may also play an important role in male infertility. Noticeably, the male-infertility rate has significantly increased in our reproductive center over the past decade, as well as in other countries (8). A DNA polymorphism is defined when a single nucleotide—A, T, C, or G—in the genome differs between members of a biological species، these single nucleotide polymorphisms (SNPs) may occurs within the coding sequences of genes ،noncoding regions of genes ،or in the intergenic regions . Changes in the regulatory parts of the gene could affect the degree of expression of the gene ،and thus the levels of the protein (29). Polymorphic variants of both ERα and ERβ genes have been identified in recent years, and studied for possible association with reproductive outcomes (15, 20, 25, 33, 50, 61, 65, 68,). Estrogen signaling in the cell is mediated by estrogen receptors (ERs), of which at least two subtypes exist, ERα and ERβ. Two silent polymorphisms in ERα have been associated with azoospermia or severe oligozoospermia (30, 64). Recently several sequence variants of the ERβ gene have been described (49) including two silent G⇒ A polymorphisms, RsaI and AluI. Both polymorphisms have been overrepresented in ovulatory dysfunctions (63). However, studies on genetic variants of ERβ with respect to male infertility are still lacking. Such information might add to our knowledge regarding the role of estrogens in the physiology and pathophysiology of male reproductive systems.

2. PATIENTS AND METHODS

2.1. *Infertile men:*

Twenty-four Egyptian men from infertile couples were included in the study. All men presented with sperm count lower than 2 million in all (at least two) ejaculates, delivered for examination. Although the fertility of their female partners was not explored. Men with known genetic causes of infertility. As well as those with a history of cryptorchidism were excluded.

2.2. *Controls.*

Normal controls served a group of thirty four Egyptian men without genital abnormalities and with sperm count higher than 20 millions/ ml.

2.3. *Hormone analysis*

Circulating levels of FSH, LH, testosterone, and estradiol were measured by the electrochemiluminescence immunoassay (ELICIA) which is intended for use on Elcsys and cobas immunoassay analyzer.

2.4. *Allele-specific PCR*

In all subjects, allele-specific PCR was performed to detect the RsaI and AluI

variants of ERβ. For each polymorphism two reactions per subject were run, using a specific primer for either the polymorphic A variant or for the wild-type G variant, together with an upstream and a downstream primer. PCR conditions were established to generate both a control fragment and a shorter, allele-specific band in the presence of the variant and only the control fragment in its absence.

Allele-specific PCR of the RsaI polymorphism was performed in a total volume of 25 µl containing 25 ng genomic DNA, 45 mmol/liter KCl, 10 mmol/liter Tris HCl (pH 9.1), 0.1% Tween 20, 0.2 mmol/liter deoxynucleotide triphosphate, 1.5 mmol/liter MgCl2, 1 U Dynazyme Taq polymerase (Qiagen, USA), and 0.5 µmol/liter of each of the primers RsaI forward (Fw), RsaI reverse (Rev), and either RsaI RevA or RsaI RevG. Primer sequences are presented in Table 1.

Amplification was performed for 35 cycles; each cycle including denaturation for 1 min at 96 C, primer annealing for 30 sec at 58 C, and primer extension for 3 min at 72 C, with an initial denaturation step for 3 min at 96 C, and a final extension step for 7 min at 72 C. For the AluI polymorphism, an annealing temperature of 54 C for 30 sec was used. Other conditions were the same as for the RsaI reaction. The sequences of the primers are presented in Table 1.

2.5. *Analysis of restriction fragment length polymorphisms*

Both the RsaI and AluI polymorphisms are restriction fragment length polymorphisms, and digestion with the respective restriction enzymes was performed according to the manufacturer (real biogene , Canada) to verify the results from the allele-specific PCR. In the RsaI polymorphism, a G to A nucleotide exchange at nucleotide 1082 in exon 5 created a recognition site for RsaI, and in the AluI polymorphism an exchange of G to A at nucleotide 1730 in the noncoding end of exon 8 introduced a recognition site for AluI (nucleotide numbering according to GenBank accession no. AB006590).

In both positions a G nucleotide was considered the wild-type sequence and was not digestible by RsaI or AluI. RsaI digestion produced one uncleaved band of 409 bp in subjects with the homozygous wild-type GG genotype, two bands of 110 and 299 bp in homozygous polymorphic AA subjects, and all three bands in heterozygous AG carriers. AluI digestion yielded one band of 405 bp in the uncleaved homozygous wild-type GG polymorphism, two bands of 163 and 242 bp in the homozygous polymorphic AA polymorphism, and all three bands in heterozygous AG subjects. We did not compare the hormone levels between the two groups but instead compared the hormone concentrations between the genotypes within each group.

2.6. *Semen analysis*

The ejaculate was obtained by masturbation after a minimum 48 h of sexual abstinence. Semen analysis includes physical examination (color, PH and viscosity) and microscopic examination (sperm count, motility and morphology).

2.7. *Statistical analysis*

The distributions of ERβ polymorphisms were compared between the patient groups and controls using Fisher's exact test. Median and mean for hormone parameter in control and infertile men and also for hormone levels in the different genotypes, in two groups, separately, were compared by Mann-Whitney U test applying the SPSS statistical software (version 11). $P < 0.05$ was considered statistically significant.

3. RESULTS

The RsaI AA genotype was not found in control or infertile men subject. When analyzing the distribution of the RsaI polymorphism, we found that the infertile

Table 1. Primer sequences

Primer	Sequences (5'-3')	Fragment length (bp)
Rsa1 Fw	ACT TGC CAT TCT GTC TCT ACA	
Rsa1 Rev	CAC AGG ACC CTG AAT CCT	409 (control)
Rsa1 RevA	AGC TCT CCA AGA GCC GT	127 (A-Variant)
Rsa1 RevG	AGC TCT CCA AGA GCC GC	127 (G-Varaint)
Alu1 Fw	TTT TTG TCC CCA TAG TAA CA	
Alu1 Rev	CCT CTG CTA ACA AGG GAA A	405 (control)
Alu1 RevA	GAG TTC ACG CTT CAG CT	258 (A-variant)
Alu1 RevG	GAG TTC ACG CTT CAG CC	258 (G-variant)

Table 2. Distribution of the Rsa1 and Alu1 genotype AA, AG, GG.

Study group	Rsa1(AA)	Rsa1(AG)	Rsa1(GG)	Alu1(AA	Alu1(AG)	Alu1(GG)
Control (n = 34)	0 (0)	2 (5.8)	32 (94.1)	9 (26.4)	11 (32.3)	14 (38.2)
Infertile men (n=24)	0 (0)	4 (16.6)*	20 (83.3)	6 (25)	8 (33.3)	10 (33.3)

For two groups, the numbers of subjects and the proportion (in brackets) presenting with the specific genotype are presented.* Heterozygous polymorphic genotype AG vs. homozygous wild-type genotype GG, P = 0.17, compared with controls (Fisher's exact test).

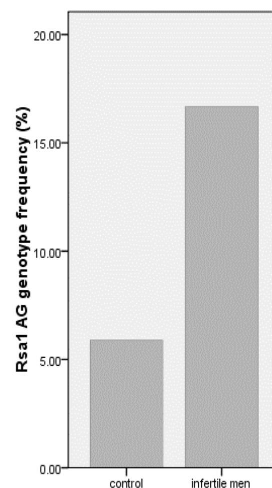

Fig. 1. proportion of Rsa1 AG genotype in control and infertile men

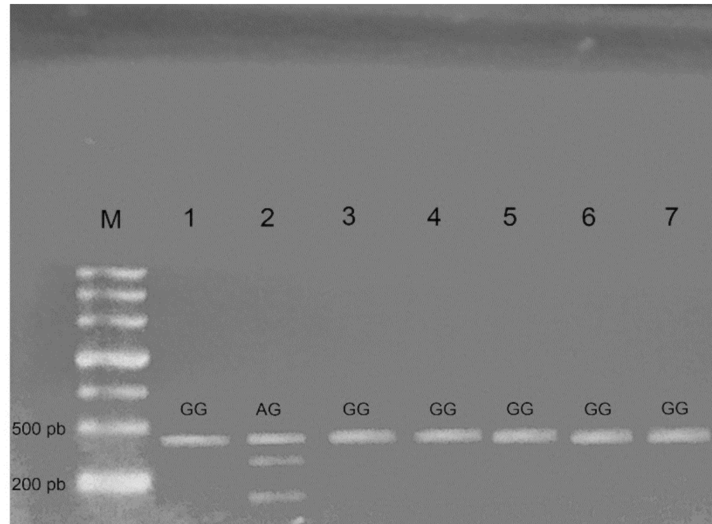

Fig. 2. PCR-RFLP results for Rsa1. M: marker(Gel pilot wide range), 200–4500 bp; 1–7: different samples. Wild-type GG genotype with one band of 409 bp, as in samples 1 and (3-7); homozygous polymorphic AA genotype was not found in any sample for Rsa1; heterozygous AG carriers with three bands, 110, 299 and 409 bp, as in samples 3. PCR-RFLP, polymerase chain reaction (PCR)-restriction fragment length polymorphism

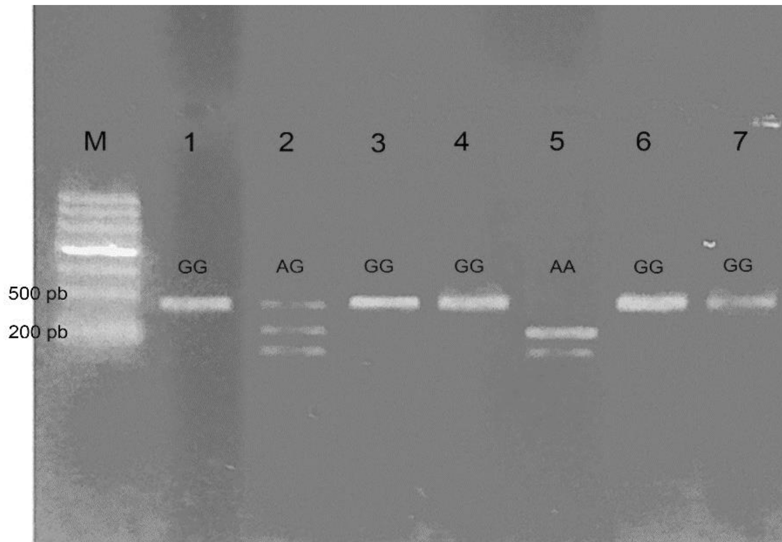

Fig. 3. PCR-RFLP results for Alu1. M: marker(Gel pilot wide range), 200–4500 bp; 1–7: different samples. Wild-type GG genotype with one band of 405 bp, as in samples 1 ,3,4,6 and 7 ; homozygous polymorphic AA genotype with two band , 163, 242 as in sample 5 ; heterozygous AG carriers with three bands, 163, 242 and 405 bp, as in samples 3. PCR-RFLP, polymerase chain reaction (PCR)-restriction fragment length polymorphism.

Table 3. Serum hormone parameter in infertile men and control groups.

Hormone	n	Infertile men	n	Control
FSH(MIU/ml)	2		34	
Median	4	18.07*		4.48
Mean		18.05		5.54
SD		11.32		3.47
T(ng/ml)	2		34	
Median	4	4.25*		6.65
Mean		4.04		6.64
SD		2.05		2.12
LH(MIU/ml)	2		34	
Median	4	4.99		5.15
Mean		5.68		5.02
SD		2.98		1.33
E2(pg/ml)	2		34	
Median	4	22.6		25.25
Mean		23.54		26.03
SD		10		9.34

TABLE 4. Hormone concentration in Rsa1 genotypes.

Hormone	N	Control Rsa1 AG	n	Control Rsa1 GG	n	Infertile men Rsa1 AG	n	Infertile men Rsa1 GG
FSH(MIU/ml)	2	5 (1.6-8.39) 5.02 4.76	32	4.48 (1.42-14.58) 5.58 3.48	4	16.65 (1.9-24.1) 14.82 9.98	20	18.07 (2.8-47.7) 18.69 1.16
T(ng/ml)	2	5.55 (3.9-7.2) 5.55 2.33	32	6.65 (3.4-13.2) 6.7 2.13	4	3.7 (1.7-4.8) 3.47 1.52	20	4.25 (1.12-11.1) 4.16 2.15
LH(MIU/ml)	2	3.14 (2.97-3.32) 3.14 0.24	32	5.19 (2.62-7.7) 5.14 1.28	4	2.85* (1.9-5.6) 3.3 1.61	20	5.47 (1.3-15.3) 6.16 2.99
E2(pg/ml)	2	26.25 (25-27.5) 26.25 1.76	32	25.2 (14.1-63.3) 26.02 9.63	4	20.6 (18.1-23.2) 20.65 2.35	20	25 (7.2-46) 24.12 1.08

`Fig. 4, 5. Box plots showing median and interquartile range of the LH levels according to Rsa1 in control and infertile men .The median LH level was significantly lower in Rsa1 AG ,compared with Rsa1 GG.

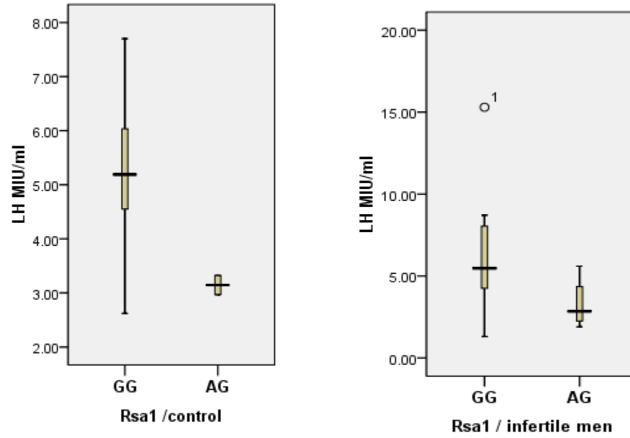

TABLE 5. Hormone concentration in Alu1 control genotypes.

Hormone	n	Control Au1 AA	n	Control Alu1 AG	n	Control Alu1 GG
FSH(MIU/	9		1		14	
ml)		5.37	1	4.64		3.97
Median		(1.66-		(1.42-		(1.65-
(range)		14.1)		11.2)		14.58)
Mean		6.26		5.23		5.34
SD		3.75		3.08		3.77
T(MIU/ml)	9		1		14	
Median		6.7	1	7.1		6.2
(range)		(4.2-13.2)		(5.6-13.1)		(3.4-7.3)
Mean		6.82		7.57		5.79
SD		2.87		2.01		1.29
LH(ng/ml)	9		1		14	
Median		5.3	1	5.15		5.07
(range)		(2.62-6.6)		(3.12-7.5)		(2.97-7.7)
Mean		4.88		5.13		5.04
SD		1.31		1.51		1.28
E2(pg/ml)	9		1		14	
Median		21.6	1	26.2		26.55
(range)		(14.1-		(16.9-		(14.9-63.3)
Mean		33.7)		40.7)		28.47
SD		22.73		25.63		12.13
		5.6		7.29		

TABLE 6. Hormone concentration in Alu1 infertile men genotypes.

Hormone	n	Infertile men Au1 AA	n	Infertile men Alu1 AG	n	Infertile men Alu1 GG
FSH(MIU/ml)	6		8		10	
Median		18.07		16.35		21.3
(range)		(5.45-22.7)		(4.2-47.7)		(1.9-38.4)
Mean		16.41		19.37		17.97
SD		6.54		14.16		12.01
T(MIU/ml)	6		8		10	
Median		3.42		3.7		4.6
(range)		(1.7-6.1)		(1.9-6.2)		(1.12-11.1)
Mean		3.62		3.6		4.65
SD		1.56		1.43		2.66
LH(ng/ml)	6		8		10	
Median		6.72		4.74		5.2
(range)		(1.9-8.71)		(2.9-15.3)		(1.3-8.66)
Mean		6.13		5.91		5.23
SD		2.56		3.95		2.56
E2(pg/ml)	6		8		10	
Median		20.7		23		23.5
(range)		(9.2-46)		(15.2-35)		(7.2-43)
Mean		22.79		23.66		23.94
SD		13.13		6.69		11.17

men had almost three times higher frequency of the heterozygous RsaI AG genotype than controls (Table 2 and Fig. 1). The infertile men did not differ from the controls regarding the AluI polymorphism.

3.1. Distribution of the RsaI genotypes AA ‘ AG and GG.

RsaI digestion produced one uncleaved band of 409 bp in subjects with the homozygous wild-type GG genotype, two bands of 110 and 299 bp in homozygous polymorphic AA subjects, and all three bands in heterozygous AG carriers. AluI digestion yielded one band of 405 bp in the uncleaved homozygous wild-type GG polymorphism, two bands of 163 and 242 bp in the homozygous polymorphic AA polymorphism, and all three bands in heterozygous AG subjects.

3.2. Hormone analysis

Hormone parameter of serum FSH, T, LH and E2 were compared by mean and median value in control and infertile men groups, the level of serum FSH and testosterone in infertile men were significaly difference from control group (table 3). In both controls and infertile men, the heterozygous polymorphic. RsaI AG genotype was associated with a reduction in the median concentration of LH (P = 0.057 and P = .045, respectively), compared with the homozygous wild-type RsaI GG genotype (Table 4 and Fig. 4, 5). No such differences were found for FSH, testosterone or estradiol. Regarding the AluI genotypes, No significant

differences were found for the hormones analysis. The median LH level was similar to individuals with AA, AG and GG In the infertile men and control groups. (Table 5, 6).

4. DISCUSSION

The main finding of our study was a significantly increased frequency of the ERβ RsaI AG genotype among infertile men, compared with controls. This is to our knowledge this study showing an association between ERβ genetic variants and male infertility. Recently, **Safarinejad** evaluated PvuII and XbaI polymorphisms of ERα ,and AluI and RsaI polymorphisms of ERβ versus idiopathic, oligoasthenoteratozoospermia infertile Iranian men, and showed genetic evidence that ERα and ERβ genotypes were independently associated with increase infertility risk (50). Similarly**, Lazaros** evaluated both ERα and ERβ versus sperm motility and concentration in Greek men with normal sperm count versus oligozoospermic men.The findings revealed a positive association between ERα polymorphisms and sperm motility and concentration, supporting the significance of this gene in spermatogenesis and semen quality (33). Furthermore, **Aschim** observed association between ERβ (Rsa1) polymorphism in Caucasian population and male infertility (4). However, in the Brazilian population, genetic variations in both estrogen receptors alpha (PvuII and XbaI) and beta (AluI and RsaI) were not relevant to idiopathic infertility (10). One possible explanation to reconcile the conflicting data between studies could be ethnic and geographic variation in the distribution of the polymorphisms in the estrogen receptors genes, suggesting genetic heterogeneity within the ER alpha and ER beta gene in different populations, possibly due to divergent evolutionary lineages that result in separate clusters of distinct geography (66). Consequently, the structure of linkage disequilibrium differs markedly across genomic regions and populations, and the extent of linkage disequilibrium is highly dependent on the population in which it is measured (22). Thus, the same allele may have different patterns of association with markers and haplotypes in different populations (39). In our study the finding of decreased LH levels in men with the RsaI AG genotype, despite unchanged testosterone and estradiol concentration, might indicate that this genotype implies an increased ERβ activity, leading to increased estrogen sensitivity. The finding that the RsaI AG group presented with lower LH values in controls as well as in the infertile men makes it more probable that this is a biologically relevant observation and not just a result of multiple testing. Correspondingly, decreased LH levels were reported in Chinese patients with ovulatory dysfunction presenting with combined RsaI and AluI AA genotypes (63). With regard to increased estrogen sensitivity in RsaI AG subjects, our findings are in accordance with the observation of increased risk of reduced sperm quality in men exposed to the potent estrogen diethylstilbestrol in utero (21).

The existence of periods of development during which the testis is particularly sensitive to estrogens is strongly suggested by epidemiologic study of men exposed in utero to DES. These sensitive periods may vary depending on the susceptibility of the individual, as reported for the different strains of experimental animals (60). Estrogens can induce oxidative DNA damage that can induce effects on male reproduction (67). The risk of oxidative damage and lipid peroxidation is especially high in steroid synthesizing tissues because, in addition to oxidative phosphorylation, molecular oxygen is used for steroid synthesis. Indeed, it has been shown that free radicals inhibit steroidogenesis by interfering with cholesterol transport to the mitochondria and/or the catalytic function of P450 enzymes, which leads to an increase in lipid

peroxidation and the decline of the antioxidant barrier. All these changes can alter the testicular cells, including spermatozoa, and thus sperm production, leading to an alteration of the male fertility (26). Estrogen excess during adulthood can deteriorate sperm production and maturation (6). In humans, spermatozoa generate reactive oxygen species (ROS) that are known to affect hyperactivation of spermatozoa, the acrosome reaction, and the attachment of spermatozoa to oocytes, thereby influencing fertilization of oocytes (1,16,35). Besides the beneficial effects of ROS, an excess of ROS is detrimental to spermatozoa, and leads to damage of the DNA and plasma membrane through lipid peroxidation (3). Because spermatozoa have discarded most of their cytoplasm during the final stages of spermatogenesis, the availability of cytoplasmic defensive enzymes is limited, and therefore these cells in particular are susceptible to ROS (2, 54). Increased lipid peroxidation of spermatozoa plasma membranes may lead to altered membrane fluidity, which can render sperm dysfunctional through impaired metabolism, acrosome reaction reactivity, and ability of the spermatozoa to fuse with the oocyte (2). This may result in abnormal sperm concentrations, loss of motility, and abnormal morphology of the spermatozoa, leading to loss of fertility (38). In mouse as well as in human it has been shown that estrogens are positively involved in sperm capacitation and acrosome reaction (13). The existence of ER alpha at the upper postacrosomal sperm head region and the presence of ER beta at the midpiece, at the site of mitochondria (59), is likely to be relevant for a role of estrogens in male gamete maturation and motility. It is possible that estrogens produced locally should be considered as a physiologically relevant hormone involved in the regulation of sperm motility. ER alpha polymorphisms may influence these locally acting estrogen levels, with effects on sperm motility in men with low sperm concentration. In normal-sperm-count men, ER alpha polymorphisms may influence sperm concentration, either on spermatogenesis directly or through serum estrogen levels (14). Each estrogen receptor is involved in regulating one particular function, ER beta being involved in gametogenesis and ER alpha in steroidogenesis processes. These precise mechanisms of action of estrogens in the fetal testis could help us to anticipate the effects of different estrogenic compounds depending on their affinity for each receptor. For example, genistein, which has a stronger affinity for ERb than ERa (31), would be expected to have a stronger effect on germ cells than that on steroidogenesis. Recently evidence points to a putative role for ubiquitous environmental contaminants in the occurrence of human infertility since exposure to toxic environmental pollutants can lead to epigenetic modification (biochemical modification without changes to the underlying DNA sequence such as epigenetic factor that include methylation , acetylation and phosphorylation) of critical genes, resulting in altered gene expression, Epigenetic processes are natural and essential to many organism functions, including regulation of spermatogenesis process, but if they occur improperly, there can be major adverse health and behavioral effects (57). Endocrine disruptors, including compounds in pesticides, dioxins, bisphenol A, phytoestrogens and others, have the capacity to mimic, block or modulate the endocrine system through the interaction with steroidal receptors. Numerous natural and synthetic compounds can interfere with the mammalian reproductive axis, resulting in decreased fertility (9). Furthermore, it is well known that spermatogenic arrest occurs when men are on a long-term estrogen replacement therapy before sex change surgery (5, 53), and hence, it seems plausible to assume that increased exposure to estrogens hampers spermatogenesis. This effect might be

indirect, mediated through lower gonadotropin secretion and as one of the consequences of a decreased testosterone synthesis by the Leydig cells. In our study, however, RsaI AG and RsaI GG subjects had similar circulating testosterone levels, suggesting that this was not the mechanism behind the low sperm concentration in our infertile subjects. An additional direct role of estrogen in spermatogenesis is indicated by the detection of ERβ in human testis in the Sertoli cells as well as germ cells, including round spermatids (52) and the fact that the splicing of this gene seems to be cell-dependent within the human testis (5). Although the role of ER alpha and beta in human spermatogenesis is still under investigation, a human equivalent of the αERKO mice has shown reduced number of spermatogonia, spermatocytes, and spermatids per testis as well as increased germ cell apoptosis (23). Because estrogen regulates the reabsorption of luminal fluid in the head of the epididymis, reduced sperm production is thought to be a consequence of impaired fluid resorption within the efferent ducts of the testis (17). Also, taking into account that 1) ER beta was significantly associated with impaired spermatogenesis (23, 61). 2) ER beta immunolocalization was found in sperm tails (47). In humans, biologically active aromatase and ERs (alpha and beta) were present in ejaculated spermatozoa with excess residual cytoplasm (47) and in immature germ cells (32) inaddition to Leydig cells. **Carreau** demonstrated that the amount of P450 aromatase transcripts is 30% lower in immotile than in motile spermatozoa. Moreover, the aromatase activity was 50% greater in the motile fraction compared to immotile spermatozoa. The observations of 1) decreased sperm motility in men with aromatase deficiency and 2) decrease of aromatase in immotile human spermatozoa could suggest that aromatase is involved in the acquisition of sperm motility (14). The mechanisms behind altered ERβ function in subjects with RsaI polymorphisms remain to be elucidated. The G to A change does not lead to amino acid changes in the protein. It can be speculated, however, that this polymorphism is in linkage disequilibrium with other genetic variations that could affect gene expression or function. A recent study showed that the RsaI polymorphism was in complete linkage disequilibrium with a polymorphism located at the splice acceptor site just before exon 8 in ERβ (19). This may potentially affect the splicing of this exon, leading to proteins with different properties than the wild-type ERβ (44, 46). The RsaI polymorphism could also have a direct effect through changing the nucleotide sequence and thereby the secondary structure of the ERβ mRNA, possibly leading to changes in mRNA syntheses, splicing, maturation, transport, translation, or degradation (28,56). The distribution AluI genotypes in controls was almost similar to infertile men, which may indicate no association between Alu1 polymorphism and male infertility>

In conclusion, we found an association between the RsaI genotype of the ERβ gene and male infertility, which may be related to effects on LH secretion.

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التأثيرات الكيميائية الحيوية لمعدلات الدهون البروتينية في مرضى العقم

الملخص العربي

العقم عند الرجال مرتبط بعوامل عديده ويرتبط بقوه بالعامل الجيني ففي السنوات الأخير عددا كبيرا من الدراسات ركزت على تحديد المتغيرات الجينيه التي تؤثر على الحيوانات المنوية في الإنسان. وقد تؤثر الأشكال المتعددة للجينات مستقبلات هرمون الاستروجين في العقم عند الذكور، وهذه الدراسة تهدف الي التحقق من وجود علاقه بين الاشكال المتعددة (Alu1, Rsa1) في جين مستقبل هرمون الاستروجين بيتا مع مرضي العقم مجهولين السبب . واستخدمت طريقه تفاعل البلمرة المحدد للشكل، وطريقه طول الجزء المقطوع المحدد للشكل ايضا بواسطة انزيمات القطع وذلك لتحديد الاشكال المتعددة لجين مستقبل هرمون الاستروجين بيتا في 24 رجل يعانون من العقم غير معروف السبب و34 رجل لا يعانون من العقم ويتمتعون بصحه جيده. تم اخذ عينات دم وقياس الهرمونات التناسلية وايضا تحليلات السائل المنوي في كل من المجموعتان. اثبتت الدراسة ان تردد النمط او الشكل الوراثي الغير متجانس (Rsa1 AG) في رجال العقم أكبر بثلاث مرات من الرجال الاصحاء وان هذا النمط الوراثي الغير متجانس له علاقة بانخفاض تركيز هرمون الملوتن (LH) في الدم مقارنتا بالنمط الوراثي الطبيعي (Rsa1 GG) في كلا من المجموعتان وبالتالي فان هذه النتائج تشير الي احتمال وجود علاقة بين الاشكال المتعددة لجين مستقبل هرمون الاستروجين والعقم عند الرجال.

(مجلة بنها للعلوم الطبية البيطرية: عدد 24 (1)، يونيو 2013: 343-358)