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REVIEW ARTICLE

The role of HOXA-9 in prediction of positive pregnancy outcome women undergoing intracytoplasmic sperm injection

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Abstract:

Background: Infertility problem is relatively common in our community and list of causes behind it is long however, they can be grouped into male factor, female factors, mixed factors and unexplained infertility. Recent experimental studies have shown that gene polymorphisms in HOX-A 9 gene can be linked to endometrial thickness, oocyte development, embryo quality and successful implantation.

Aim of the study: The aim of the current study was to assess the association of HoxA9 gene polymorphism, with implantation Success for women under intracytoplasmic sperm injection.

Patients, materials and methods: The present study included a total of 150 infertile women undergoing intracytoplasmic sperm injection. In this study participants' blood were obtained. Gene polymorphism study and DNA sequence analysis of HOXA-9 was carried out.

Result: Regarding HOXA-9 gene, there was significant difference in 211-212 and 315T>C, Tins between positive and negative pregnancy ($p < 0.05$). However, there was no significant difference in 276-277Ains Tins, 430-431Tins, 815-816Tins, 396C>G, 676C>T, 699C>TCC, 865G>T, 287T>C, 510T>C between groups ($p > 0.05$).

Conclusion: Gene polymorphism of HOXA-9 can affect on endometrial thickness leading to implantation failure.

Introduction

Infertility is defined as a disease characterized by the inability to achieve a clinical pregnancy after twelve months of regular, unprotected sex [1]. Causes of infertility Phenotypic causes of female Infertility: The main etiological factors of female infertility include ovulation disorders, tubal disorder, uterine abnormalities, endometriosis and cervical factors [2]. Genotypic causes of female infertility: X Chromosome and female infertility: As a result of an accelerated loss of primordial oocytes during female fetal development and streak gonads at birth, chromosomal abnormalities like monosomy X (Turner syndrome): cytogenetically visible deletions and duplications, and balanced and unbalanced X-autosome rearrangements are linked in humans [3]. Turner's Syndrome (TS): is defined by the absence of one X chromosome, either entirely or partially. The 45X chromosomal configuration is the most prevalent [4]. Single gene mutation: Low FSH and oestradiol, excessive LH, and sterility in females [5-6]. Congenital Adrenal Hyperplasia (CAH) Changes in the adrenal gland in women with CAH might result in hormonal imbalances may cause infertility [7]. Physiology of implantation the physiological functions of the uterine endometrium (uterine lining) are preparation for implantation, endometrial stromal fibroblasts (ESCs) terminally differentiate to secretory decidual stromal fibroblast cells (DSCs) and in the absence of conception the tissue undergoes controlled shedding, tissue repair, re-epithelialisation, regeneration and remodelling , this process is controlled by ovarian steroid hormones [8]. Implantation Embryo implantation involves the first physical and physiological interaction between the embryo and uterus, which determines the success of post-implantation conceptus development and term pregnancy outcome [9]. Homeobox genes (HOX genes) The broad family of transcription factors known as homeobox genes (HOX) controls the early development of various body components The human HOX genes are

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located at four chromosomal loci, HOXA9: A sequence-specific transcription factor of a developmental regulatory system is encoded by a gene on chromosome 7p15.2 that gives cells a distinct position on the anterior-posterior axis and is thought to be important in gene expression ,morphogenesis, and differentiation [10-11]. Homeobox Genes and infertility Disorders of reproductive function may result from any changes in the control of homeobox gene expression in the adult uterus or the developing female reproductive tracts [12].

Materials and Methods

The period of collection of patients from May 2022 until November 2022, at the infertility Teba center / Babil/ Iraq"and Al-kafeel hospital \karbala. The present study enrolled 150 infertile females undergoing ICSI cycles with an age range of 18 to 35 years and an infertility duration ranging from 2 years to 15 years. Patients' assessment Full history was obtained from infertile couples. Collection of Blood samples Five milliliter of venous blood were obtained by a sterile venipuncture under optimal condition, and divided into 2 parts: First part (2ml) was put into an ethylene diamine tetra-acetic acid (EDTA) tube [13]. DNA extraction protocol 1-Washing cells by (10%normal saline -10% probynene glycol – 80% Tris-Hcl) 2- Lysis of Cells by (0.06 g EDTA+ 0.3 g Tris-Hcl + 0.2 g of SDS 3-Proteins precipitation by sodium acetate and acetic acid 4- Precipitation and washing of DNA by 60 ml ethanol + 40 ml Tris-Hcl 5- Recovering DNA by 100 µl of DNA Elution buffer [14]. The primers of Homeobox-9

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC %	Self complementarity	Self 3' complementarity
Z19f	ACCACAAGCATAGTCAGTCAGG	Plus	22	27164978	27164999	60.03	50.00	2.00	0.00
Z19r	TACAGTAGAGCGACAATGCGAT	Minus	22	27165977	27165956	59.64	45.45	5.00	3.00
Product length	1000								

diamine tetra-acetic acid (EDTA) tube [13]. DNA extraction protocol 1-Washing cells by (10%normal saline -10% probynene glycol – 80% Tris-Hcl) 2- Lysis of Cells by (0.06 g EDTA+ 0.3 g Tris-Hcl + 0.2 g of SDS 3-Proteins precipitation by sodium acetate and acetic acid 4- Precipitation and washing of DNA by 60 ml ethanol + 40 ml Tris-Hcl 5- Recovering DNA by 100 µl of DNA Elution buffer [14]. The primers of Homeobox-9 (Hox-A9) genes which used in this study with their sequences are listed in table (1):

Molecular Weight and Integrity Estimation of DNA extraction

The molecular weight and the integrity of the DNA extracted was determined by 0.8% agarose gel-electrophoresis, the setting device at 100 volts for (30-40) minutes.

Agarose gel electrophoresis

The electrophoresis had been performed to determine the quality of the DNA extraction and to visualize the PCR product size after finishing the PCR program as shown in Figure 1. The concentration of the gels depend on the type of the product. In general, for DNA quality, the agarose gel was 1%, while it was 2% for the regular PCR products [15].

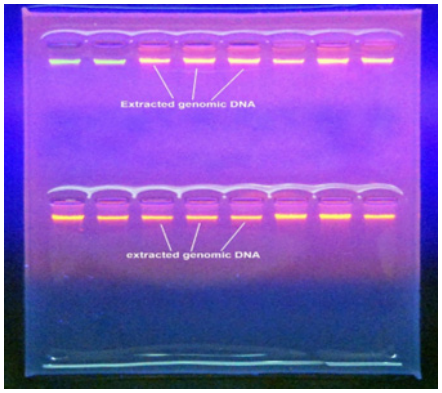

Figure (1) extracted genomic DNA electrophoresis

Conventional Polymerase chain reaction

Polymerase chain reaction (PCR) components and programs for Hox-A9 and Hox-A10 were carried out after several attempts of optimization to detect the best temperature for annealing with a total volume of 25 µl [16-17].

PCR amplification program is described in Tables (2 and 3) respectively. PCR products were assessed by 2 % agarose gel electrophoresis as described above

Table (2): Components of PCR reaction (Homeobox - 9 (Hox-A9))

Composition		Concentration	Volume
1	Master Mix	2X	12.5 µl
2	Forward primer	10 PM	1 µl
3	Revers primer	10 PM	1µl
4	DNA sample	10-20 ng\µl	2µl
5	Nucleases free water		8 µl
6	DMSO		0.5 µl
	Total volume		25 µl

Table (3): Temperatures and time of PCR Homeobox-9 (Hox-A9).

stage	steps	Temperature C°	Time	No. of Cycles
1	Initial temperature	94	5 mins	1
2	DNA denaturation	94	30 sec	35
	Primer annealing	58	30 sec	
	Extension	72	120 sec	
3	Final extention	72	5 mins	1

Results

Genetic study

There was significant difference in 211-212 Tins between positive and negative pregnancy TA 28 (77.8%),70(93.3%) and TTA 8 (22.2%) , 5(6.7%) respectively; (p = 0.038) table 4

In In addition There was no significant difference in 276-277Ains Tins between positive and negative pregnancy AC 30 (83.3%),71(94.7%) and AAC 6 (16.7%), 4(5.3%) respectively;(p =0.110) table 4

However, the 430-431Tins was not significant between positive and negative pregnancy TC 33 (91.7%),74(98.7%) and TTC 3 (8.3%) , 1(1.3%) respectively;(p =0.191) table 4.

the difference was statistically not significant between positive and negative pregnancy in 815-816 Tins GC 29 (80.6%), 49(65.3%) and GTC 7 (`19.4%),26(34.7%) respectively;(p =0.100) table4.

There was in addition insignificant difference between positive and negative pregnancy in 396C>G CC 32 (88.9%) , 65(86.7%) and CG 4 (11.1%) , 10(13.3%) respectively;(p =0.980) , C 68 (94.4%), 140(93.3%) and G 4 (5.6%) , 10(6.7%) respectively;(p =0.750) table 4.

There was no significant difference between positive and negative pregnancy in 676C>T CC 10 (27.8%),24(32.0%) and CT 26 (72.2%) , 51(68.0%) respectively;(p =0.651) , C 46 (63.9%) ,99(66.0%) and T 26 (36.1%),51(34.0%) respectively;(p =0.757).

In positive and negative pregnancy there was no significant difference in 699C>T CC 9 (25.0%), 17(22.7%) and CT 27 (75.0%) , 58(77.3%) respectively;(p =0.786) ,C 45 (62.5%),92(61.3%) and T 27 (37.5%),58(38.7%) respectively;(p =0.867).

In addition, in positive and negative pregnancy there was no significant difference in 865G>T GG 34 (94.4%), 73(97.3%) A and GT 2 (5.6%), 2(2.7%) respectively;(p =0.825), G 70 (97.2%),148(98.7%) and T 2 (2.8%) , 2(1.3%) respectively;(p =0.449).

Table 4:

Genotype	Total n = 111	Pregnancy positive n = 36	Pregnancy negative n = 75	p	OR	95% CI	
211-212Tins							
TA	98 (88.3 %)	28 (77.8%)	70(93.3%)	0.038 Y *	0.25	0.08	0.83
TTA	13 (11.7 %)	8 (22.2%)	5(6.7%)		4.00	1.20	13.28
276-277Ains							
AC	101 (91.0 %)	30 (83.3%)	71(94.7%)	0.110 Y NS	0.28	0.07	1.07
AAC	10 (9.0%)	6 (16.7%)	4(5.3%)		3.55	0.93	13.49
430-431Tins							
TC	107 (96.4%)	33 (91.7%)	74(98.7%)	0.191 Y NS	0.15	0.01	1.48
TTC	4 (3.6%)	3 (8.3%)	1(1.3%)		6.73	0.67	67.11
815-816Tins							
GC	78 (70.3%)	29 (80.6%)	49(65.3%)	0.100 C NS	2.20	0.85	5.70
GTC	33 (29.7%)	7 (`19.4%)	26(34.7%)		0.13	0.05	0.33
396C>G							
CC	97 (87.4%)	32 (88.9%)	65(86.7%)	0.980 Y NS	1.23	0.36	4.23
CG	14 (12.6%)	4 (11.1%)	10(13.3%)		0.81	0.24	2.79
C	208 (93.7%)	68 (94.4%)	140(93.3%)	0.750 C NS	1.21	0.37	4.01
G	14 (6.3%)	4 (5.6%)	10(6.7%)		0.82	0.25	2.72
676C>T							
CC	34 (30.6%)	10 (27.8%)	24(32.0%)	0.651 C NS	0.82	0.34	1.96
CT	77 (69.4%)	26 (72.2%)	51(68.0%)		1.22	0.51	2.94
C	145 (65.3%)	46 (63.9%)	99(66.0%)	0.757 C NS	0.91	0.51	1.64
T	77 (34.7%)	26 (36.1%)	51(34.0%)		1.10	0.61	1.97
699C>T							
CC	26 (23.4%)	9 (25.0%)	17(22.7%)	0.786 C NS	1.14	0.45	2.88
CT	85 (76.6%)	27 (75.0%)	58(77.3%)		0.88	0.35	2.22
C	137 (61.7%)	45 (62.5%)	92(61.3%)	0.867 C NS	0.95	0.53	1.70
T	85 (38.3%)	27 (37.5%)	58(38.7%)		1.05	0.59	1.88
865G>T							
GG	107 (96.4%)	34 (94.4%)	73(97.3%)	0.825 Y NS	0.47	0.06	3.45
GT	4 (3.6%)	2 (5.6%)	2(2.7%)		2.15	0.29	15.89
G	218 (98.2%)	70 (97.2%)	148(98.7%)	0.449 C NS	0.47	0.07	3.43
T	4 (1.8%)	2 (2.8%)	2(1.3%)		2.11	0.29	15.32

Table 5:

The difference in 287 T>C between positive and negative pregnancy was statistically not significant Codominance TT 31(86.1%),70(93.3%) , TC 5(13.9%), 4(5.3%) respectively;(p =0.128), Dominan TT 31(86.1%), 70(93.3%) ;(p =0.213), TC+TT 5(13.9%),5((%6.7%) , Recessive TT+TC 36(100.0%),74(98.7%) and CC 0(0.0%) , 1(1.3%) respectively;(p =0.486), Allele T 67(93.1%),144(96.0%) and C 5(6.9%),6(4.0%) respectively;(p =0.344).

Table 6:

Mode	287T>C	Total n = 111	Pregnancy positive n = 36	Pregnancy negative n = 75	p	OR	95% CI	
	TT	101(91.0%)	31(86.1%)	70(93.3%)	Reference	Reference	Reference	
	TC	9(8.1%)	5(13.9%)	4(5.3%)	0.128 C N S	2.82	0.71	11.23
	CC	1(0.9%)	0(0.0%)	1(1.3%)	0.507 C N S	0.75 a		
Dominant	TT	101(91.0%)	31(86.1%)	70(93.3%)	0.213 C N S	0.44	0.12	1.64
	TC+TT	10(9.0%)	5(13.9%)	5((%6.7%)	Reference			
Recessive	TT+TC	110(99.1%)	36(100.0%)	74(98.7%)	Reference			

	CC	1(0.9%)	0(0.0%)	1(1.3%)	0.486 C N S	1.47a		
Allele	T	211(95.0%)	67(93.1%)	144(96.0%)	0.344 C NS	0.56	0.16	1.89
	C	11(5.0%)	5(6.9%)	6(4.0%)		1.79	0.53	6.08

Table 7:

In addition, in positive and negative pregnancy there was no significant difference 315T>C Codominance TT 15(41.7%) 20(26.7%), TC 6(16.7%),426(34.7%) respectively;(p =0.034) and CC 15(41.7%), 29(38.7%) respectively;(p =0.425) Dominan CC 15(41.7%),29(38.7%);(p =0.762), TT+TC 21(58.3%),46(61.3%), Recessive CC+TC 21(58.3%),55(73.3%)and TT 15(41.7%) , 20(26.7%) respectively;(p =0.111), Allele T 36(50.0%),66(44.0%) and C 36(50.0%),84(56.0%) respectively;(p =0.401).

Table 8:

Mode	315T>C	Total=111	pregnancy positive	pregnancy negative	P value	OR	0.95	
codominant	TT	35(31.5%)	15(41.7%)	20(26.7%)	Reference	Reference	Reference	
	TC	32(28.8%)	6(16.7%)	26(34.7%)	0.034	0.3	0.1	0.9
	CC	44(39.6%)	15(41.7%)	29(38.7%)	0.425	0.7	0.28	1.7
dominant	CC	44(39.6%)	15(41.7%)	29(38.7%)	0.762	1.13	0.5	2.55
	TT+TC	67(60.4%)	21(58.3%)	46(61.3%)	Reference			
Recessive	CC+TC	76(68.5%)	21(58.3%)	55(73.3%)	Reference			
	TT	35(31.5%)	15(41.7%)	20(26.7%)	0.111	0.51	0.22	1.18
C	T	102(45.9%)	36(50.0%)	66(44.0%)	0.401	0.79	0.45	1.38
	C	120(54.1%)	36(50.0%)	84(56.0%)		1.27	0.72	2.24

Table 9:

In positive and negative pregnancy there was no significant difference in 510T>C codominant TT 34(94.4%),67(89.3%) and TC 2(5.6%), 6(8.0%) respectively;(p =0.616) ,CC 0(0.0%) , 2(0.316%) respectively; (P=0.316) , dominant TT 34(94.4%) 67(89.3%) respectively; (P=0.379), , TC+CC 2(5.6%) , 8(10.7%) , Recessive TT+TC 36(100.0%),73(97.3%) respectively; CC 0(0.0%),2(2.7%) respectively; (P=0.323)and Allel T 70(97.2%) , 2(2.7%) , C 2(2.8%) (P=0.23)

Table 10:

Mode	510T>C	Total=111	pregnancy positive	pregnancy negative	P value	OR	0.95	
codominant	TT	101(91.0%)	34(94.4%)	67(89.3%)	Reference	Reference	Reference	
	TC	8(7.2%)	2(5.6%)	6(8.0%)	0.616	0.7	0.13	3.4
	CC	2(1.8%)	0(0.0%)	2(0.316%)	0.316	a 2.450		
dominant	TT	101(91.0%)	34(94.4%)	67(89.3%)	0.379	2.03	0.41	10.09
	TC+CC	10(9.0%)	2(5.6%)	8(10.7%)	Reference			

Recessive	TT+TC	109(98.2%)	36(100.0%)	73(97.3%)	Reference			
	CC	2(1.8%)	0(0.0%)	2(2.7%)	0.323	a 0.403		
Allel	T	210(94.6%)	70(97.2%)	140(93.3%)	0.23	0.4	0.09	1.88
	C	12(5.4%)	2(2.8%)	10(6.7%)		2.5	0.53	11.72

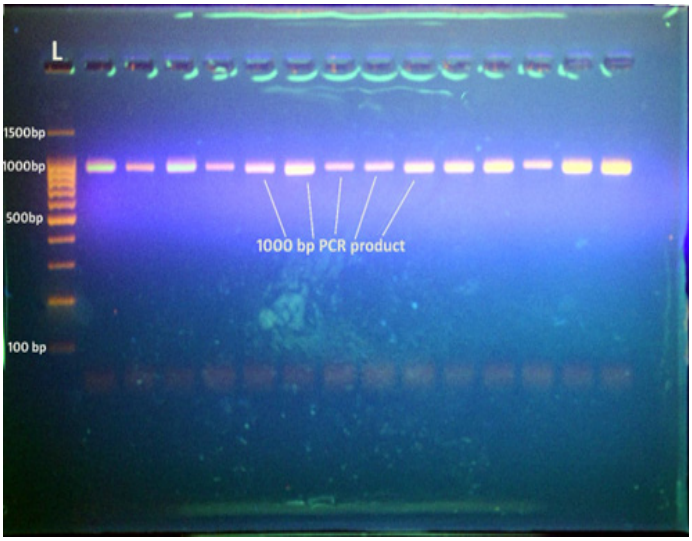

Figure (2): Electrophoresis of HOXA-9 samples, agarose concentration 2%, time 60 minutes, 50 volt.

Discussion

Gene polymorphism of HOXA-9 in association with pregnancy outcome:

In the present study we found no significant association between pregnancy outcome and the following genetic loci, 276-277Ains, 430-431Tins, 815-816Tins, 396C>G, 676C>T, 699C>T, 865G>T, and 287T>C indicating that these changes in nucleotide sequences did not affect gene expression of HOXA9 and therefore the endometrial thickness and embryo implantation is not going to be affected. However, changes in sequences in loci 211-212Tins and 315T>C SNP, TC genotype in co-dominance mode were significant. We suggest, that the change in 211-212Tins to be TTA resulted in 4 times higher chance of getting pregnancy because of rise in gene expression of HOXA-9 leading to higher tissue level and greater growth of endometrium and better endometrial receptivity. In addition, we suggest that changes in 315T>C SNP in the codominance mode resulted in reduction in the expression level of HOXA-9 because of reduced chance of pregnancy by 70 % (Odds ratio =0.03) and the reduced expression of this gene resulted in lower tissue level of HOXA-9 and therefore, the endometrial thickness and receptivity and implantation were all negatively affected.

Following thorough search in available published articles, no previous study has linked the changes in nucleotide sequences with pregnancy outcome in women undergoing assisted reproductive techniques, thus this point of our research is original and novel.

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