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Original Research Article

Impact of hormonal changes on the semen quality and assisted reproductive outcomes in infertile men

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ABSTRACT

This study investigated the effect of hormonal changes on semen quality, chromatin status, and assisted reproductive outcomes (intracytoplasmic sperm injection), among infertile men. In this research, 219 infertile men undergoing assisted reproductive treatment were evaluated with reproductive hormone levels (including follicle-stimulating hormone, luteinizing hormone and testosterone), semen parameters, and sperm chromatin integrity and condensation, between 2012 and 2014. Finally, the assisted reproductive outcomes in these infertile men were studied. The low rate of total sperm count, motility and morphology, fertilization and the high percentage of DNA damage, the poor zygote (Z4 grade) and embryo quality (grade D), and spontaneous miscarriage was recorded in men with high levels of follicle-stimulating hormone and luteinizing hormone. In conclusion, the changes in the follicle-stimulating hormone, luteinizing hormone, and testosterone by changes in the sperm quality, and DNA damage may have the effects on assisted reproductive outcomes (e.g., low fertilization, poor zygote and embryo quality, and high miscarriage).

Introduction

The inability to conceive within 12 months of unprotected intercourse, has been implicated in almost 10–15% of couples and this problem is defined as infertility (Evgeni et al., 2014). Approximately 50% of the cases contained the male factor infertility (Evgeni et al., 2014; Nallella et al., 2006). In most cases, the male factor infertility is traditionally characterized by semen analysis (e.g., concentration, motility, and morphology) (Barratt, 2007; Evgeni et al., 2014). Although useful information may be gathered from traditional semen analysis about fertility potential, it does not provide all the required information. Therefore, failing to achieve a pregnancy with assisted reproductive technique suggests the need for extra analysis of male fertility parameters (Lazaros et al., 2011). Therefore, the additional semen parameters should be evaluated. One of the parameters detected on reproductive outcomes, which is usually considered in semen quality, is the damaged chromatin of sperm (Colacurci et al., 2012; Seli and Sakkas, 2005) and this can affect fertility potential (López et al., 2013). As a result, damage to sperm chromatin and poor embryo quality negatively influences the fertility potential. It also causes low pregnancy rate (Lakpour et al., 2012). Some reports have indicated that when higher than 30% of the spermatozoa are identified with DNA damage; the potential for natural fertility becomes very low (Evenson et al., 2002). It has also been suggested that the sperm DNA integrity may be a more objective marker of sperm function than the standard semen analysis (Evenson et al., 2002). Several techniques were used to detect the sperm abnormalities. Toluidine blue (TB) and Aniline blue (AB) are used to observe incomplete DNA structure and chromatin condensation, respectively. These methods are sensitive, inexpensive, and simple tests (Kim et al., 2013). It has been reported that the presence of damaged DNA resulted in a lower percentage of fertilization and pregnancy (Lazaros et al., 2011).

On the other hand, several hormones (e.g., follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone), play an important role in germ cell development and spermatogenesis (Appasamy et al., 2007). Hormonal profiles may be described as a predisposing factor to spermatogenesis. In other words, the hormonal profiles (e.g. FSH, LH, testosterone, estradiol, prolactin)

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affect sperm DNA, chromatin status, and semen parameters which consequently have effects on fertilization, embryo quality, and pregnancy rates (Wei et al., 2013). Sperm DNA assay plays an important role in cases of embryo quality, implantation failure, and miscarriages (López et al., 2013). Therefore, the routine use of hormonal and sperm DNA assays can be helpful in promoting the assisted reproductive outcomes. Therefore, semen quality, male reproductive hormones, and chromatin integrity may effect on the male fertility potential and assisted reproductive outcomes.

In spite of the above data, there is insufficient evidence about reproductive hormone changes on clinical practice such as fertilization rate, zygote and embryo quality, implantation rate, and live birth rate. Therefore, this study was designed to evaluate the outcomes of assisted reproductive techniques (ART) from infertile men with different reproductive hormone levels, different semen qualities, and different chromatin integrity and condensation statuses.

Materials and methods

The study population consisted of 390 men who were considered from Alzahra Educational and Remedial Center (IVF center) and invited to participate in a study for assessing the effect of male reproductive hormone changes on the reproductive health, between May 2012 to May 2014 and informed consent was obtained from these couples. In total, 309 of the men agreed to participate in this study. Of the 309 couples who participated, 22 couples were excluded because the men were taking hormone-containing medications and had diabetes, and/or thyroid diseases, and/or past disease (e.g., cryptorchidism, testicular torsion, or chistic, etc.) which may affect the semen quality or level of reproductive hormone. Also, the couples who had faced with more than two previous failed IVF/ICSI cycles or unexplained infertility were excluded. Sixty-eight (68) subjects were excluded due to female factor infertility (e.g., ovulatory dysfunction, endometriosis, tubal diseases, and under pharmacological treatment). 219 remaining men provided both semen and blood samples for the measurement of hormone levels, and the final study population were comprised of these remaining couples. In other words, infertile couples with male factor infertility (e.g. oligozoospermia, asthenozoospermia, teratozoospermia, and oligoasthenotratozoospermia) were only included.

The men of this study had a mean age and body mass index (BMI) between 18 and 50 years and 19 to 38.5, respectively. Also, the women in this study had a mean age between 20 and 35 years and there was no statistical difference in their age and BMI. Weight and height of patients (both of men and women) were precisely measured by laboratory experts on the day of oocyte retrieval. The weight and height were measured in kilograms and centimeters, respectively. Then, the BMI was calculated as weight in kilograms divided by the squared height in meters (kg/m^2).

It should be noted that the study was approved by the Guilan University of Medical Sciences committee.

Collection of semen and blood samples

Semen samples were collected by masturbation in a sterile container on different days of sexual abstinence. The samples were liquefied for 15 min after ejaculation on the morning of oocyte retrieval, at room temperature (RT), although it may rarely take up to 60 min. Semen parameters (e.g. concentration, morphology, and motility) were analyzed according to the World Health Organization (WHO, 2010) criteria.

Blood samples were also drawn between 1 and 3 h, after the collection of semen samples on the morning of the same day. These samples were centrifuged and serum was stored at −80 °C until reproductive hormone analysis.

Determination of reproductive hormones

The LH and FSH concentrations of serum were determined with immunofluorometric techniques. The total assay variation coefficients were 2.9% and 2.6%, respectively. Testosterone level was measured directly using the Coat-A-Count RIA kit (CA), whose inter-assay and intra-assay coefficients of variation (CV) were 12% and 10%, respectively, with a sensitivity of 4 ng/dl (0.139 nmol/l). The normal range of FSH was 0.9–8.9 mIU/ml, and this range was considered 1.7 to 8.6 mIU/ml and 3–12 for LH and testosterone, respectively.

Sperm chromatin assays

Toluidine blue stain

To assay chromatin status, thin smears were prepared on the silane-coated slides. The fixation of air-dried smears was performed in 96% ethanol-acetone medium (1:1) at 4 °C for 1 h. For hydrolysis, the slides were put in 0.1 N HCl at 4 °C for 5 min, then rinsed 3 times with distilled water for 2 min and stained with 0.05% toluidine blue (TB, in 50% McIlvaine's citrate phosphate buffer, pH 3.5, Merck) for 5 min at RT. The TB stain was used to assay chromatin integrity, so that sperm head with intact chromatin and those of fragmented and abnormal chromatin indicated light blue and deep violet (purple), respectively. A total of 300 spermatozoa were seen in each slide and evaluated using a light microscope.

Aniline blue stain

Aniline blue (AB) stain was used to assay the chromatin condensation of sperm samples. In this process, smears were fixed in 4% formalin (Junsei Chemical, Tokyo, Japan), rinsed in water, and stained in 5% AB (Sigma-Aldrich Co., St. Louis, MO, USA) in a solution of 4% acetic acid (pH 3.5). Each fixation and staining were performed for 5 min at RT. The slides were rinsed with water, dried, and evaluated under a light microscope. At least 300 spermatozoa were counted. Stained dark sperms were considered as immature sperm with excessive histone and abnormal sperm chromatin.

IVF laboratory procedures

By considering sperm parameters (Oligozoospermia, Asthenozoospermia, Oligoasthenozoospermia, and Teratozoospermia), recovered oocytes were injected according to ICSI procedures, using an inverted microscope (Olympus IX70, Tokyo, Japan). The mature egg is held with a specialized holding pipette. A very delicate and sharp injection needle is used to immobilize and pick up a single sperm. This needle is then carefully inserted through the zona pellucida and into the center (cytoplasm) of the egg. The sperm is injected into the cytoplasm and the needle is carefully removed. Injected oocytes were transferred into G1PLUS (Vitrolife Co., Sweden) and incubated in a humidified atmosphere with 5% CO_2 and 37 °C.

Evaluation of fertilization was performed at 16–18 h after micro-injection with the observation of two pronuclei stage. The two pronuclei zygote assessment was performed based on the Scott et al. (2000) scoring system. On days 2 and 3, the embryos were evaluated and graded based on the Ebner et al. (2001) scoring system. Briefly, embryo classification was considered as follows: regular cells and without fragmentation (grade A); cells with lower than 25% fragmentation (grade B); cells with fragmentation

between 25 and 50% (grade C), and cells with fragmentation higher than 50% (grade D).

Suitable embryo transfer (ET) was performed as intrauterine on day 2–3 after ICSI. The chemical pregnancy was determined with increasing serum b-hCG (beta-human chorionic gonadotropin) concentration at 14 days after ET. The observation of intrauterine sac was considered as a clinical pregnancy with a heart function at 3 weeks after transfer. Abortion was also defined as pregnancy loss spontaneously after observation of pregnancy by ultrasound.

Statistical analysis

Statistical analysis was performed using SPSS version 20 (IBM, Armonk, NY, USA). The influence of hormonal changes on semen parameters (e.g. total sperm count, sperm concentration, volume, normal morphology, head defects, midpiece defects, and tail defects) and ART outcomes (e.g. zygote degree and embryo quality) have been done using multinomial logistic regression. The effect of hormonal changes on semen parameters (e.g. motility and spillage), chromatin status (e.g. abnormal condensation and damaged chromatin), and ART outcomes (e.g. fertilization, pregnancy, and abortion rates) have been done using binary logistic regression. $P \leq 0.05$ was considered to be statistically significant.

Results

In this study, three tables are presented. Tables 1 and 2 indicate the effect of hormonal changes on semen quality, DNA integrity, and condensation. Also, Table 3 evaluates the effect of these hormonal changes on ART outcomes. The results of Table 3 are the main novelty of this study. It shows the effect of hormonal changes on ART outcomes. It should be mentioned that, to the best of our knowledge, this research is the first study on the relationship between male reproductive hormone changes and assisted reproductive outcomes. The details of results from these three tables are described below.

Table 1
Association between reproductive hormone changes and seminal parameters.

		FSH			LH			Testosterone		
		TN = 76 Low N [OR] (95% CI)	TN = 82 Medium N [OR] (95% CI)	TN = 61 High N [OR] (95% CI)	TN = 74 Low N [OR] (95% CI)	TN = 86 Medium N[OR] (95% CI)	TN = 59 High N[OR] (95% CI)	TN = 71 Low N[OR] (95%CI)	TN = 88 Medium N[OR] (95%CI)	TN = 60 High N[OR] (95% CI)
Total sperm count ($\times 10^6$)	<40	40[2.05] (0.6,6.1)	9 [1.0]	12[0.34][a] (0.1,0.9)	44 [2.4] (0.7,8.2)	8 [1.0]	9 [0.2][b] (0.06,0.6)	23 [1.04] (0.3,2.9)	11 [1.0]	27[2.1] (0.7,6.03)
	41–120	13 [0.6] (0.1,2.1)	10[1.0]	8 [1.2] (0.4,3.3)	12 [0.4] (0.101.6)	13[1.0]	6 [0.6] (0.2,1.9)	9 [0.4] (0.1,1.2)	11 [1.0]	11 [3.0] (1.1,7.8)
	>120	23 [1.0]	63 [1.0]	41 [1.0]	18 [1.0]	65 [1.0]	44 [1.0]	39 [1.0]	66 [1.0]	22 [1.0]
Sperm concentration ($\times 10^6$ ml^{-1})	<20	28 [0.8] (0.3,2.2)	10 [1.0]	20 [0.6] (0.2,1.6)	28 [0.2] (0.05,0.8)	9 [1.0]	21 [0.7] (0.03,0.4)	22 [0.7] (0.2,1.9)	10 [1.0]	26 [1.2] (0.4,3.4)
	20–40	15 [0.5] (0.1,1.6)	9 [1.0]	9 [1.9] (0.7,5.4)	19 [0.1] (0.02,0.5)	11 [1.0]	3 [0.5] (0.1,1.9)	12 [0.3] (0.1,1.07)	11 [1.0]	10 [2.5] (0.9,6.7)
	>40	33[1.0]	63 [1.0]	32 [1.0]	27 [1.0]	66 [1.0]	35 [1.0]	37 [1.0]	67 [1.0]	24 [1.0]
Volume (cc)	<2	22 [0.8] (0.3,1.9)	19 [1.0]	24 [1.6] (0.6,3.7)	25 [0.7] (0.3,1.8)	16 [1.0]	24 [1.1] (0.4,2.6)	17 [0.5] (0.2,1.3)	25 [1.0]	23 [1.2] (0.5,2.9)
	3–4	20 [0.4] (0.2,1.1)	30 [1.0]	18 [1.04] (0.4,2.3)	21 [0.3] (0.1,0.8)	31 [1.0]	16 [1.06] (0.4,2.3)	23 [0.7] (0.3,1.6)	27 [1.0]	18 [1.2] (0.5,2.8)
	>4	34 [1.0]	33 [1.0]	19 [1.0]	28 [1.0]	39 [1.0]	19 [1.0]	31 [1.0]	36 [1.0]	19 [1.0]
Normal morphology (%)	<4	29 [0.6] (0.2,1.6)	19 [1.0]	40[3.4][a] (1.1,10.2)	24 [0.07] (0.01,0.3)	28 [1.0]	36 [1.16][a] (0.03,2.8)	36 [0.2] (0.06,0.6)	20 [1.0]	32 [1.08][c] (0.02,2.3)
	4–14	13 [0.2] (0.08,0.5)	27 [1.0]	12 [1.7] (0.6,4.8)	18 [0.04] (0.01,0.2)	32 [1.0]	2 [0.07] (0.01,0.3)	23 [0.1] (0.03,0.3)	25 [1.0]	4 [0.2] (0.08,0.9)
	>14	34 [1.0]	36 [1.0]	9 [1.0]	32 [1.0]	26 [1.0]	21 [1.0]	12 [1.0]	43 [1.0]	24 [1.0]
Head defects (%)	<30	32 [1.7] (0.6,4.4)	36 [1.0]	12 [0.4][a] (0.1,0.9)	26 [1.2] (0.5,2.9)	36 [1.0]	18 [0.1] (0.4,2.3)	24 [0.6] (0.3,1.5)	30 [1.0]	26 [1.07] (0.4,2.6)
	30–35	23 [1.6] (0.6,4.1)	27 [1.0]	15 [0.3][b] (0.1,0.7)	20 [1.2] (0.5,2.7)	28 [1.0]	17 [0.5] (0.2,1.2)	24 [0.9] (0.4,2.03)	23 [1.0]	18 [1.7] (0.7,4.02)
	>35	21 [1.0]	19 [1.0]	34 [1.0]	28 [1.0]	22 [1.0]	24 [1.0]	23 [1.0]	35 [1.0]	16 [1.0]
Midpiece defects (%)	<10	26 [1.1] (0.4,2.6)	37 [1.0]	17 [0.7] (0.3,1.6)	26 [1.00] (0.4,2.2)	34 [1.0]	20 [0.9] (0.3,2.07)	26 [1.7] (0.7,3.9)	38 [1.0]	16 [1.1] (0.5,2.6)
	10–20	27 [1.7] (0.7,3.9)	25 [1.0]	20 [0.6] (0.3,1.5)	26 [1.3] (0.5,2.9)	26 [1.0]	20 [1.05] (0.4,2.4)	22 [2.02] (0.9,4.5)	27 [1.0]	23 [0.9] (0.4,2.1)
	>20	23 [1.0]	20 [1.0]	24 [1.0]	22 [1.0]	26 [1.0]	19 [1.0]	23 [1.0]	23 [1.0]	21 [1.0]
Tail defects (%)	<5	30 [1.1] (0.5,2.5)	32 [1.0]	22 [0.9] (0.4,2.2)	28 [1.2] (0.5,2.9)	36 [1.0]	20 [1.1] (0.4,2.6)	28 [1.2] (0.5,2.9)	36 [1.0]	20 [0.9] (0.4,2.2)
	5–10	24 [1.2] (0.5,2.8)	23 [1.0]	20 [1.2] (0.5,2.8)	21 [1.2] (0.5,2.8)	27 [1.0]	19 [0.8] (0.3,1.8)	22 [1.4] (0.6,3.2)	25 [1.0]	20 [1.08] (0.4,2.4)
	>10	22 [1.0]	27 [1.0]	19 [1.0]	25 [1.0]	23 [1.0]	20 [1.0]	21 [1.0]	27 [1.0]	20 [1.0]
Motility (%)	<50	26 [0.1] (0.05,0.2)	27 [1.0]	49 [2.12][c] (0.05,5.2)	26 [0.2] (0.1,0.4)	34 [1.0]	42 [1.2][b] (0.1,3.5)	37 [0.7] (0.3,1.4)	29 [1.0]	36 [0.32][c] (0.1,0.6)
	>50	50 [1.0]	55 [1.0]	12 [1.0]	48 [1.0]	52 [1.0]	17 [1.0]	34 [1.0]	59 [1.0]	24 [1.0]
Spillage	No	38 [0.8] (0.4,1.6)	33 [1.0]	33 [0.5] (0.2,1.1)	33 [0.7] (0.3,1.5)	41 [1.0]	30 [0.8] (0.4,1.7)	41 [1.4] (0.7,2.9)	34 [1.0]	29 [0.6] (0.3,1.3)
	Yes	38 [1.0]	49 [1.0]	28 [1.0]	41 [1.0]	45 [1.0]	29 [1.0]	30 [1.0]	54 [1.0]	31 [1.0]

Abbreviations;: TN: Total Number; FSH: Follicle-stimulating Hormone; LH: Luteinizing Hormone. There were significant differences between hormonal changes and total sperm count, motility, normal morphology, and head defects. *P* value: [a] < 0.05, [b] < 0.01 and [c] < 0.001.The normal range (medium level) of testosterone, FSH, and LH were 3–12, 0.9–8.9, and 1.7–8.6 mIU/ml, respectively.
The reference category is expressed as [1.0].

Table 2
Hormonal changes and sperm DNA integrity and chromatin condensation.

	FSH			LH			Testosterone		
	Low TN = 76 [OR](95% CI)	Medium TN = 82 [OR](95% CI)	High TN = 61 [OR](95% CI)	Low TN = 74 [OR](95% CI)	Medium TN = 86 [OR](95% CI)	High TN = 59 [OR](95% CI)	Low TN = 71 [OR](95% CI)	Medium TN = 88 [OR](95% CI)	High TN = 60 [OR] (95% CI)
Abnormal chromatin condensation (AB)(%)	60.6 [2.1]^{c} (1.9,2.3)	34.6 [0.7]^{b} (0.5,0.8)	42.1[1.0]	48.8 [1.6]^{c} (0.9,2.6)	33.5 [0.8] (0.5,1.3)	37.1 [1.0]	69.2 [2.9]^{c} (2.6,3.3)	28.9 [0.54]^{c} (0.4,0.6)	42.8 [1.0]
Damaged chromatin (TB) (%)	39.3 [0.8]^{a} (0.8,0.9)	34.6 [0.7]^{b} (0.5,0.8)	42.2 [1.0]	47.8 [0.5]^{a} (0.3,0.8)	33.5 [0.2]^{c} (0.1,0.4)	62.8 [1.0]	69.2 [0.9] (0.7,1.1)	42.8 [0.3]^{c} (0.2,0.3)	71.02 [1.0]

Abbreviations; TN: Total Number; FSH: Follicle-stimulating Hormone; LH: Luteinizing Hormone; AB: Aniline blue; TB: Toluidine blue. There were significant differences between the hormonal changes and damaged chromatin and abnormal chromatin condensation. *P* value: $^{a} < 0.05$, $^{b} < 0.01$ and $^{c} < 0.001$. The normal range (medium level) of testosterone, FSH, and LH were 3–12, 0.9–8.9, and 1.7–8.6 mIU/ml, respectively.
The reference category is expressed as [1.0].

Table 3
Relationship between hormonal changes and IVF-ET outcomes.

		FSH			LH			Testosterone		
		Low TN = 76 [OR] (95% CI)	Medium TN = 82 [OR] (95% CI)	High TN = 61 [OR] (95% CI)	Low TN = 74 [OR] (95% CI)	Medium TN = 86 [OR] (95% CI)	High TN = 59 [OR] (95% CI)	Low TN = 71 [OR] (95% CI)	Medium TN = 88 [OR] (95% CI)	High TN = 60 [OR] (95% CI)
Fertilization rate%		69.42 [2.1]^{b} (1.2,3.8)	74.13 [2.7]^{c} (1.5,4.9)	51.51 [1.0]	70 [2.07]^{a} (1.1,3.6)	74.53 [2.5]^{b} (1.3,4.5)	53.5 [1.0]	56.3 [1.4] (0.8,2.6)	71.26 [2.1]^{b} (1.2,3.8)	53.33 [1.0]
Zygote degree%	Z1	19.11 [1.8] (0.3,8.6)	27.09 [1.5] (0.3,6.01)	26.6 [1.0]	24.19 [1.9] (0.3,9.4)	24.03 [1.4] (0.2,7.1)	30 [1.0]	25 [4.7] (0.5,44.7)	26.41 [7.7] (0.8,70.2)	5.8 [1.0]
	Z2	46.45 [1.1] (1.8,6.6)	58.82 [5.1]^{a} (0.9,27.8)	13.3 [1.0]	50.8 [4.6] (1.02,35.1)	51.9 [6.0]^{a} (0.8,27.4)	20 [1.0]	46.8 [0.8] (0.2,3.01)	50.3 [1.4] (0.4,4.7)	58.8 [1.0]
	Z3	13.23 [1.0]	22.58 [1.0]	33.3 [1.0]	16.9 [1.0]	22.11 [1.0]	40 [1.0]	26.5 [1.0]	16.9 [1.0]	29.4 [1.0]
	Z4	8.82 [0.8] (0.1,4.4)	3.8 [0.21]^{a} (0.04,1.03)	26.6 [1.0]	8.06 [1.9] (0.18,19.3)	1.9 [0.3] (0.02,4.8)	10 [1.0]	1.5 [0.2] (0.01,5.5)	6.2 [0.8] (0.2,17.8)	5.8 [1.0]
Embryo quality %	A	72 [1.4] (0.1,14.3)	71.57 [2.5] (0.2,23.2)	46.1 [1.0]	68.6 [1.7] (0.1,15.6)	71.3 [3.4] (0.3,35.5)	60 [1.0]	78.9 [3.7] (0.4,29.4)	68.59 [1.9] (0.3,9.8)	70.5 [1.0]
	B	15.27 [0.6] (0.05,7.2)	21.05 [1.4] (0.1,15.9)	23.07 [1.0]	22.28 [1.6] (0.1,20.3)	19.13 [2.7] (0.1,38)	20 [1.0]	15.7 [4.5] (0.3,54.1)	20.7 [3.5] (0.4,28.1)	11.76 [1.0]
	C	8.2 [1.0]	4.7 [1.0]	7.6 [1.0]	6.6 [1.0]	3.4 [1.0]	10 [1.0]	3.5 [1.0]	5.7 [1.0]	11.76 [1.0]
	D	4.1 [0.16] (0.01,2.3)	2.6 [0.18]^{a} (0.01,2.2)	23.07 [1.0]	2.4 [0.3] (0.01,7.2)	6.08 [0.7] (0.08,36.2)	10 [1.0]	1.7 [1.00] (0.03,29.8)	4.8 [0.6] (0.13,21.1)	5.8 [1.0]
Pregnancy rate N/ET(%)		30/73 (41.1) [1.3] (0.6,2.7)	36/82 (43.9) [1.4] (0.7,2.8)	21/61 [34.4] [1.0]	25/71 (35.2) [1.06] (0.5,2.1)	47/85 (55.2) [2.1] (1.1,4.3)	20/59 (33.8) [1.0]	28/70 (40) [0.9] (0.4,1.9)	43/85 (50.5) [1.6] (0.6,3.2)	23/60 (38.3) [1.0]
Abortion rate N/Beta^{+} (%)		10/30 (33.3%) [0.4] (0.1,1.4)	3/36 (8.3%) [0.08]^{c} (0.02,0.3)	11/21 (52.3%) [1.0]	8/25 (32%) [0.4] (0.1,1.5)	4/47 (8.5%) [0.09]^{c} (0.02,0.3)	10/20 (50%) [1.0]	7/28 (25%) [0.7] (0.2,2.6)	6/43 (13.9%) [0.3] (0.1,1.2)	7/23 (30.4%) [1.0]

Abbreviations: TN: Total Number; FSH: Follicle-stimulating Hormone; LH: luteinizing Hormone. There were significant differences between the FSH and LH hormonal changes with fertilization rate, zygote and embryo quality and abortion rate. *P* value: $^{a} < 0.05$, $^{b} < 0.01$, and $^{c} < 0.001$. The normal range (medium level) of testosterone, FSH, and LH were 3–12, 0.9–8.9, and 1.7 to 8.6 mIU/ml, respectively.
The reference category is expressed as [1.0].

Reproductive hormone changes and seminal parameters

Analysis of semen parameters (according to WHO criteria) and sperm chromatin status were performed at different hormone levels (Tables 1 and 2). These results are not surprising, as the low FSH and LH levels decrease the total sperm count to less than 40×10^{6}/ml ($P < 0.05$). The hormonal changes also affect normal sperm morphology, such that the percentage of abnormal morphology increases in men with the highest hormone levels (FSH, LH, and testosterone, $P < 0.01$).

The evaluation of sperm morphology in more detail shows that the increase in FSH and LH levels result in high head defects of sperms (>35%).

The motility of sperm cells is another parameter that was assessed and found to be influenced by testosterone. Results show that sperm motility increased in men with the highest testosterone level ($P < 0.001$). While there was a negative correlation between FSH ($P < 0.001$) and LH ($P < 0.01$) hormone levels and sperm motility (Table 1).

Hormonal changes and sperm DNA integrity, and chromatin condensation

The chromatin status of sperms was assessed using the TB and AB staining and the percentage of abnormal sperm chromatin structure and condensation was compared in men with the different hormone levels (Table 2). The Table 2 shows that there are significant differences in abnormal sperm chromatin condensation, in men with low levels of FSH (95% CI: OR of 1.908 to 2.351), LH (95% CI: OR of 0.992 to 2.642), and testosterone (95% CI: OR of 2.681

to 3.346). For sperms with damaged chromatin, there also was a positive relationship between the high levels of FSH, LH and increasing percentage of damaged sperm chromatin. Therefore, decrease and increase of reproductive hormone levels result in abnormal condensation and damage of human sperm's chromatin, respectively. In this way, the sperm chromatin changes created by hormonal changes could effect on ART outcomes.

Hormonal changes and IVF-ET outcomes

The main results of this study are visible in Table 3. The odds of having low and medium FSH is 2.1 (95% CI: OR of 1.201 to 3.809; $P < 0.01$) and 2.7 (95% CI: OR of 1.509 to 4.955; $P < 0.05$) times greater respectively, and for low and medium LH is 2.07 (95% CI: OR of 1.158 to 3.698) $P < 0.01$) and 2.5 (95% CI: OR of 1.392 to 4.575; $P < 0.05$) times greater, respectively, for men with successful fertilization, as opposed to the highest FSH, LH levels. Similar results were obtained for low (95% CI: OR of 0.818 to 2.607) and medium (95% CI: OR of 1.211 to 3.892) levels of testosterone ($P < 0.01$). In other words, this table shows that the fertilization rate decreases in men with high levels of FSH, LH, and testosterone.

The assessment of zygote degree and embryo quality shows that the odds of having medium FSH is 5.1 (95% CI: OR of 0.950 to 27.839) times greater for Z2 degree, and 0.2 (95% CI: OR of 0.044 to 1.035) times smaller for Z4 degree, as opposed to the highest FSH level and Z3 degree ($P < 0.05$). The embryo quality data also indicated that grade D of embryos were smaller in men with medium FSH level (95% CI: OR of 0.015 to 2.286; $P < 0.01$), in comparison to men with the highest FSH level and grade C. Hence, there was a negative association between zygote, embryo degree and FSH level.

While there was no significant difference in pregnancy rate among these groups ($P > 0.05$), the abortion rate was low in men with medium FSH and LH levels ($P < 0.001$) (OR = 0.08, 95% CI 0.019 to 0.356 and OR = 0.093, 95% CI 0.024 to 0.358, respectively) in comparison to the highest levels of these hormones (Table 3).

Discussion

This study analyzed the relationship between hormonal changes and semen quality, DNA integrity, and ART outcomes. Several findings obtained in this study: (i) The change of reproductive hormones affects the semen quality, (ii) The hormonal changes have effects on DNA integrity and chromatin condensation, and (iii) The hormonal changes lead to changes in the ART outcomes (e.g., decrease fertilization rate and increase miscarriage rate) in the couples with male factor infertility.

So that, the results of the current study show that the increase in testosterone level results in an increase of sperm motility and also abnormal morphology. Although, increase in FSH and LH levels leads a decrease in sperm motility, but an increase of total sperm count, abnormal sperm morphology, and head defects of sperm. Also, the increase in FSH, LH, and testosterone levels leads the increase of damaged chromatin. Therefore, the results of this study suggested that hormonal changes lead to the decrease in semen quality via effecting on motility, morphology, total sperm count, and chromatin integrity and condensation. It could also impress male fertility potential. Also, high fertilization rate was seen in the medium reproductive hormone levels (Table 3). This may reflect a better quality of sperm in comparison to other levels.

As mentioned above, the increase of FSH level results in reduced semen quality and chromatin integrity. The evaluation of embryos was done at day 3 when the embryonic genes express. Therefore, sperm quality could be reflected in the embryo quality in this day. Our results show that high FSH level decreases embryo quality. In other words, the Z4 degree of zygote and grade D of embryo increases in men with high FSH level. On the other hand, an increase of chromatin damage by hormonal changes (such as FSH) could decrease the embryo quality and increase miscarriage rate.

Our results show that increasing the level of testosterone, results in the higher motility of sperms and increase in abnormal morphology (normal morphology of <4%, Table 1). Therefore, the hormonal changes may influence semen quality via changes in sperm motility and morphology. The changes of these hormones (FSH, LH, and testosterone) cause a decrease in total sperm count, increase in sperm motility, abnormal morphology, and sperm head defects (Table 1). The results of this study suggest that a decrease in hormonal levels result in a high percentage of abnormal sperm chromatin condensation and integrity.

FSH, a gonadotropin secreted by the anterior pituitary, initiates spermatogenesis by acting on Sertoli cells (Meeker et al., 2007). FSH and LH accompanied by other hormones play roles in sperm development and subsequently affect normal sperm proportions (morphology) (Meeker et al., 2007). Therefore, hormonal changes affect spermatozoa production. To the best of our knowledge, this is the first study of the relationship between hormonal changes and semen quality, chromatin status, and assisted reproduction outcomes. The results show that serum FSH, LH, and testosterone levels are influenced male fertility potential and could reflect in the ART outcomes.

In this study, it has been shown that hormonal changes affect different semen parameters, sperm chromatin status, and reproduction outcomes. These results are consistent with several previous studies. For example, there is a significant correlation among FSH, LH, and inhibin B hormones and sperm parameters (e.g., concentration, motility, and morphology) which was reported among 1558 young men (Jensen et al., 2004). In another study, an inverse relationship of sperm concentration, motility, and morphology with FSH and LH hormone, among men from an infertility clinic have been reported (Meeker et al., 2007). The results of this study show that abnormal FSH and LH levels decline sperm parameters such as total sperm count, normal morphology (>14%), and motility. While high testosterone level, on the other hand, increases sperm motility. More analysis of sperm morphology shows that it can affect the highest FSH and LH levels. In the present study, it has been suggested that head defects (>35%) increased in these populations. On the other hand, in some IVF centers, chromatin integrity tests are not used routinely in clinical assays. Therefore, using this assay could help to promote the ART outcomes.

The structure of sperm chromatin also plays an important role in the male fertility potential. Sperm chromatin status may be influenced by serum hormones. In this study, evaluation of sperm chromatin status in different hormone levels in male infertile partners shows that the hormonal changes result in abnormal sperm chromatin condensation and damaged chromatin. Therefore, this relationship can also be a biomarker of clinical usefulness. In another study, it was reported that chromatin condensation is a valuable and independent parameter of other sperm parameters, and is significantly related to sperm morphology (Kim et al., 2013). Consistent with previous studies, our results also suggest that FSH and LH affect sperm parameters such as morphology, and subsequently sperm chromatin integrity and condensation. In another research, the association between sperm chromatin structure and quality of spermatogenesis for infertile men has been reported (Smit et al., 2010). Appasamy et al. (2007) reported that the presence of more sperm DNA damage in infertile men is negatively related to sperm motility. It indicated that sperm DNA damage can predict outcomes of assisted reproduction (Lewis et al., 2013). This biomarker correlated with decreased fertilization rate, embryo quality, and subsequently, lower pregnancy rate and higher miscarriage rate.

In this study, the fertilization rate was decreased in infertile couples with high male FSH and LH levels. It seems that low sperm motility and abnormal morphology caused by hormonal changes affect the fertilization rate. The percentage of zygote Z4 degree was also increased in the group with the highest FSH. The embryo quality was influenced by hormonal changes, such that grade D was significantly increased with the highest FSH level. The results of Table 2 indicated that the percentage of damaged chromatin increase in men with high FSH level. Therefore, it is obvious that the quality of embryo decrease in this group.

The results of this study suggest that male hormonal changes which affect semen abnormality are associated with assisted reproduction outcomes. Although there was no significant difference in the pregnancy rate of infertile couples, but spontaneous miscarriage rate was significantly increased in couples with the highest male FSH levels. These results suggest that the high FSH level and semen abnormality subsequently caused low clinical outcomes. The evaluation of reproductive outcomes was performed in men who were evaluated for hormonal changes. The results show that the increasing FSH and LH hormones have more effects on fertilization, and abortion rate. Therefore, it is assumed that these hormonal changes may impact on ART outcomes via decreasing semen and chromatin quality.

Because of male factor infertility, the treatment cycles were performed via ICSI. This can be used to help men with fertility problems, such as having a low sperm count, less motile sperm, or sperm that has difficulty in fertilizing the egg. This means that as long as some sperm can be obtained (even in very low numbers), fertilization is possible. Nevertheless, our study has limitations like other researches; for example, the samples of this study were low. We also collected only a single blood sample, and this may have been insufficient to reveal true changes because of marked fluctuations of the reproductive hormones' concentration. Also, due to the elimination of all possible causes of female infertility, unexplained infertility had not been assessed.

Conclusion

In conclusion, it has been shown that the hormonal changes affect semen parameters, fertilization rate, and IVF-ET outcomes among a cohort of men from an infertility clinic, which included only infertile men. Consistent with previous studies, the results of this study show a strong relationship between FSH, LH and testosterone changes and semen quality. It has also been shown that the abnormal sperm parameters result of hormonal alterations cause weak pregnancy outcomes in IVF-ET cycles. The miscarriage rate also increased in the highest FSH and LH levels. The clinical and diagnostic importance of the present study requires the further understanding of sperm pathology relating to other different hormones. Clearly, these findings must be confirmed by other studies.

Authors' contributions

FG conceived of the study, participated in its design and coordination, carried out the data analysis and interpretation, and drafted the manuscript. SMA is carried out data analyses and critically revised the manuscript. MA and ZZ assisted with the study design, carried out data analysis and helped to draft the manuscript. All authors read and approved the final manuscript.

Conflict of interests

The authors report no conclicts of interest and none of the authors are directly funded by (or employed by) the Government of Iran. The authors alone are responsible for the content and writitng of the paper.

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