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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) (Evgeni et al., 2014; Barratt, 2007). 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

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