

Effectiveness of Combined Empirical Therapies and Double IUI Procedures in Treatment of Male Factor Infertility

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Abstract

Objective: Current information on using anti-estrogenic compounds, antioxidant vitamins and minerals in treatment of male factor infertility still remains controversial. Herein, we investigated the pregnancy outcome in male factor infertile patients using a combination of non-specific empiric modalities and Intra Uterine Insemination (IUI) procedures.

Subjects and Methods: The study involved a group of 33 infertile couples with mild male factor infertility who previously failed two IUI attempts. The patients received tamoxifen, vitamin E, zinc, and selenium for three months prior to their third IUI treatment cycle. Four important parameters were mainly noted: sperm concentration, motility, forward progression and the percentage normal forms.

Results: There was no difference between these parameters in semen samples of our study group in the first and second IUI treatment cycles ($p < 0.96$, $p < 0.23$, $p < 0.59$, $p < 0.84$ respectively). However, after completion of the empiric therapy course and in the third IUI treatment cycle, significant differences in overall values for the four semen parameters were detected in comparison to the earlier two IUI cycles (range $p < 0.005$ to $p < 0.0005$), except for semen volume and sperm normal forms, resulting in a chemical pregnancy rate of 30.3%, a clinical pregnancy rate of 21.2% and a delivery rate of 18.1%. Grouping the female patients according to the Body Mass Index (BMI) showed imperative differences in pregnancy outcome, yet there was no clear effect of age over pregnancy success rates in our study group.

Conclusion: Combined empirical therapies can improve semen parameters in infertile men with mild male factor. Double insemination procedures with improved semen samples, can contribute in increasing the chances of pregnancy and life birth more significantly in females with lower BMI.

Keywords: Semen; Tamoxifen; Vitamin E; Zinc; Selenium; BMI; ART

Introduction

The causes of male infertility are complex and more than one factor may contribute to the etiology. One of the most common features of idiopathic male infertility is testicular malfunction. Normal spermatogenesis is a result of normal testicular anatomy, histology, physiology and proper hormonal regulation through the pituitary gonadal axis [1]. Disturbances in any of these features may lead to testicular malfunction and abnormal semen parameters. Many methods have been proposed and implemented to treat these conditions taking into consideration the natural physiology of male endocrine system, the endogenous milieu for spermatogenesis and exogenous factors that may affect testicular output. Hitherto, no individual agent solely has been recommended for effective treatment of male factor infertility, and patients are mostly advised to undergo Assisted Reproduction Technologies (ART) for parenthood. Until present time, there is some controversy in the role of oral empiric therapies for treatment of male sub fertility. The mechanisms by which these drugs target the endogenous aspects of spermatogenesis and how they improve sperm integrity still need to be elucidated. Oral administration of L-carnitine for three months in patients with idiopathic oligoasthenozoospermia resulted in improvement of sperm motility and morphology in candidates need Intracytoplasmic Sperm Injection (ICSI) [2].

Hormonal treatment aims mainly at improving levels of endogenous follicle stimulating hormone and/or androgen and subsequent spermatogenesis. Treatment with gonadotrophins such as Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) may be effective in patients with hypogonadotrophic hypogonadism

[3,4]. Tamoxifen citrate is an anti-estrogenic agent, used as a single prophylactic agent in hormone responsive breast cancer. This drug is used to improve sperm quality in patients with asthenozoospermia. The role of this drug in inducing and improving spermatogenesis is still controversial. The beneficial effect of tamoxifen is thought to be limited to improvement in the number of live spermatozoa with no substantial effect on sperm motility and morphology [5].

The adverse effect of tamoxifen on spermatogenesis was shown in rats where increasing doses of tamoxifen resulted in shrinkage and atrophy of somniferous tubules, showing reduced sperm density showing abnormal morphology [6]. The significant role of estrogens as modulators of spermatogenesis, germ cell survival and apoptosis is discussed in a review article and the authors suggested a possible role for oestrogen-regulated genes involved in spermatogenesis and germ cell differentiation [7].

Prostatic secretions, and therefore semen are rich in zinc. Oral

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administration of zinc to malnourished patients can improve sperm quality [8]. Deficiency of some minerals such as zinc and selenium may result in low motility and viability of sperm [9]. Zinc is thought to be important in stabilization of sperm chromatin and sperm membrane. Interestingly, oral daily administration of 220 mg zinc sulfate for four months showed an increase in the level of seminal zinc significantly. This was associated with increased sperm count, sperm motility and sperm morphology, with positive impact on pregnancy [9]. Similarly, selenium supplementation in sub fertile men with low selenium status managed to improve sperm motility and the chances of successful conception [10].

Biochemical factors that may affect sperm quality include Reactive Oxygen Species (ROS) causing sperm DNA damage and poor outcome of assisted reproduction treatments [11]. Cytoplasmic enzymes such as Superoxide Dismutase (SOD), catalase, and Glutathione Peroxidase (GPX) are endogenous antioxidants that neutralize the free radicals in semen. None enzymatic antioxidants in semen include vitamin C, vitamin E, pyruvate, glutathione, and carnitine [12].

Samples of men with idiopathic infertility show higher levels of Reactive Oxygen Species (ROS) and lower antioxidant properties than fertile controls [13]. *In vitro* studies demonstrated a clear correlation between seminal leukocyte concentration and ROS production and presence of ROS and abnormal sperm function [14,15]. Reactive oxygen species (ROS) have detrimental effect on sperm DNA, and therefore to fertilization related events of sperm.

Infertile men with leukocytospermia show lower sperm motility and associated with lower concentration of vitamin E in their sperm membrane [16]. This is supported by the study showing fertile men possess semen total antioxidant capacity higher than infertile men [17].

One of the frequent questions of male infertility specialist is whether the oral administration of vitamin E can reach the male reproductive tract and improve sperm function. The improving effects of oral vitamin E and other antioxidants was demonstrated on sperm function and shown that plasma concentration may not reflect vitamin E concentration in sperm membrane [9]. Oral supplementation of male partners with antioxidants was found to be associated with increased pregnancy rate in their ART treatment cycles [18,19].

In view of the above background, our study was designed using a protocol implementing a combination of several types of empirical therapies in idiopathic male factor infertility followed by double intra uterine insemination procedure to examine any positive effect on pregnancy outcome in patients with history of failed IUI attempts.

Materials and Methods

The study

The study was approved by the Research and Ethics Committee of the College of Medicine and Medical Sciences, Arabian Gulf University, Kingdom of Bahrain. All persons gave their informed consent prior to their inclusion in the study. This study was conducted over a period of 15 months from October 2010- to Jan 2012.

Criteria for patient selection

A pool of 33 couples, presenting either primary or secondary infertility with 3-7 years duration, experienced failed IUI trials in two consecutive attempts. Patients were counseled and given choice to

advance to the third trial with double insemination after treatment of the male spouse with combined empirical therapy for three months.

Female patients: Age 22-46 years ($x=29.63$), were investigated prior to treatment with intrauterine insemination. All female patients had patent Fallopian tubes as per their hysterosalpingogram test and normal levels of serum FSH, LH, E2 and prolactin measured on day 2 of their cycles. No ovarian cyst or uterine abnormalities were found upon ultrasound scan.

Male patients: Age 23-51 years ($x=32.02$) presented impaired semen samples in two earlier IUI attempts. In these two occasions, semen analysis for our patients were performed according to WHO standards (strict criteria) by having the cut off limits for semen volume >1.5 ml, for sperm density $>15 \times 10^6$, for total sperm motility $>40\%$, for sperm progressive motility $>32\%$, for sperm viability $>58\%$ and for normal forms sperms $>4\%$ [19]. Also, all patients had physical examination as per WHO standards and requested to have blood fertility hormone measurement for FSH, LH, prolactin and testosterone [20].

Empirical therapy treatment of men

Prior to entering the treatment cycle, patients were counselled and asked to provide a written consent. Men were advised to start taking tamoxifen 10 mg (Nolvadex-D, Astra Zeneca pharmaceuticals Ltd, Wilmington, USA) twice a day, vitamin E 400 IU daily (α -tocopherol, nature's Bounty Inc., Bohemia, NY11716, USA), zinc 15 mg (Jamieson Laboratories Ltd., Ontario, Canada) twice a day and selenium (L-selenomethionine) 200 mg daily (Solgar vitamin and herb, New Jersey, USA). As the duration of a cycle of spermatogenesis in man is approximated to 64 days [21], this treatment was continued for three 3 months prior to the double IUI cycle.

Ovarian induction

Ovarian induction in females was performed as follows: Baseline transvaginal sonography was done on Day 3 of menstruation. Recombinant FSH (rec-FSH) preparations-follitropin Alfa (Gonal-F; Merck Serono S.p.A., Bari, Italy) administered sub-cutaneously between 50 and 150 IU/day, begun on cycle Day 3 of menstruation and continued until leading follicle was 17-18 mm. Serial estradiol (E2), LH and progesterone measurements were performed in all cycles to rule out premature luteinisation. All 33 patients showed a minimum of 2 follicles and maximum of 4 with size 16-18 mm, were permitted to continue the treatment cycle. Ovulation was induced by Ovitrele (choriogonadotropin alpha, 250 μ g; Merck Serono, Sp.A. Modugno (BA), Italy) administration when serum progesterone level was 1 ng/ml. The first insemination was performed at 36 hours post HCG and the second 8- 12 hours later. The luteal phase was supported by vaginal Crinone gel (8%; Merck Serono, Watford, UK) daily for 15 days, Folic acid 5 mg (Vifolin, the Arab Pharmaceutical Manufacturing co. Ltd., Salt- Jordan) OD, Estrofem 2 mg (NovoNordisk A/S, Bagsvaerd, Denmark) BD.

Calculation of the body mass index in females

The body mass indices of female spouses were calculated by dividing weight in kilograms by the square of the height in meters (Kg/m^2). We followed WHO classification as following: underweight <18.50 , normal weight 18.5-24.99, overweight 25-29.99 and obese >30 [22].

Sperm preparations

Semen specimens were produced by masturbation and collected for

insemination within 1 h of production. Semen samples were evaluated according to WHO strict criteria and sperm washing and selection were carried out by direct swim up technique, as this method has been proven to yield best quality sperm with high motility, less debris and best hypo osmotic score [19,23]. The harvested quality sperms were suspended in a final volume of 0.8 ml of sperm prep medium (LifeGlobal, USA), to be used for IUI. All semen samples were analysed and prepared by the same andrologist in the same lab and under the same conditions.

Inseminations

Patients were scheduled for IUI once they showed at least two follicles of 18 mm in size and adequate endometrial thickness of >10 mm [24]. Insemination with prepared sperm was done with a sterile catheter (Sure view Wallace embryo replacement catheter, Smith Medical int. Kent, UK) and a 1 ml syringe. The catheter was gently passed through the cervical canal and the sperm suspension expelled slowly into the uterine cavity close to the tubal junction in a volume of 0.8 ml. The female patients were asked to rest in supine position for 30 minutes after IUI. The inseminations in the first and the second trials were performed once in all patients immediately after ovulation as per ultra sound examination. In the third trial, one insemination was performed 36 hours after HCG injection and followed by another within 8 hours period. Patients were asked to have multiple intercourses after the procedures for the next following day, and then abstain for the rest of the cycle.

Pregnancy test

Blood pregnancy test performed on day 14 of insemination being positive when β HCG was higher than 25 IU/ml (chemical pregnancy). Patients were given Duphaston 10 mg twice a day, oral folic acid 5mg once daily (Vifolin, the Arab Pharmaceutical Manufacturing co. ltd., Salt- Jordan) and vaginal progesterone support once daily (Crinone gel, 8%; Merck Serono, Watford, UK)). Patients showing positive pregnancy tests were advised to continue same medications for 16 weeks. All pregnancies were monitored by ultra sound at two weeks intervals, first for detection of gestation sac and embryonic pole (clinical pregnancy) and then followed up by scans every two to three weeks for detection of foetal heart beats and foetal development.

Statistical analysis

The ANOVA test was used to compare levels of significance between the different groups. The Student's t-test, unpaired method, was used to measure statistical significance between two semen analysis recording

groups. In all tests $p < 0.05$ was taken as the level of significance. All tests of significance were two sided.

Results

Physical examination and fertility hormones measurement in all men did not reveal any abnormality that may contribute to their sub fertility.

Semen analysis results of our patients in the two initial single IUI cycles showed impaired sperm progressive motility and forward progression being lower than standard values suggested by WHO [20]. Other semen parameters were either within the normal range for sperm concentration or just close to the WHO cut off values for total motility and normal sperm morphology. There were no differences in semen parameters what so ever in the first and the second insemination cycles: count ($19.03 \times 10^6 \pm 8.4 \times 10^6$, $19.48 \times 10^6 \pm 8.81 \times 10^6$) ($p < 0.96$), motility ($39.5\% \pm 9.05\%$, $42.15\% \pm 7.4\%$) ($p < 0.23$), forward progression (1.81 ± 0.59 , 1.75 ± 0.43) ($p < 0.59$) and percentage of normal form spermatozoa ($5.83\% \pm 1.11\%$, $6.65\% \pm 1.73\%$) ($p < 0.84$) (Table 1).

Comparing these parameters with the corresponding ones obtained from semen samples after oral treatment of patients with empirical therapy showed significant differences in form of enhancement in overall value for the sperm concentration (23.7 ± 9.77), sperm motility (47.9 ± 13.9), sperm forward progression (2.16 ± 0.6) and level of morphologically normal sperm (6.41 ± 1.53) (Table 1).

The pregnancy success rate/couple in the third double IUI trial was calculated in our study group and found to be 30.3% for the chemical pregnancy, 21.2% for clinical pregnancy and 18.1% for life birth (Table 2). These figures are comparable with absolute negative results obtained earlier in two single insemination cycles prior to treatment of male partners with empirical therapy.

The pregnancy success rates were calculated in relation to the ages of the female spouse and to their body mass indices. Females were grouped according to the age into three groups: 20-25, 26-30, 31-35, 36-40 and >41 years. Interestingly, Women with age group of 26-30 years demonstrated the highest pregnancy success rate with uneventful full term pregnancy and life birth (9.09%), followed by women with age group 31-35 years (6.0%) and women with age group 20-25 years (3.0%) (Table 3).

Also, our results showed a very imperative difference in pregnancy outcome when females were grouped according to the body mass

| | 1 st IUI | 2 nd IUI | 3 rd IUI | P values (1 st IUI and 2 nd IUI compared to 3 rd IUI) |
|-------------------------------|---------------------|---------------------|---------------------|--|
| Volume | 2.16 ± 0.77 | 2.05 ± 0.58 | 2.09 ± 1.8 | 0.5 |
| Count x 10 ⁶ | 19.03 ± 8.48 | 19.48 ± 8.81 | 23.76 ± 9.77 | 0.00002, 0.00004 |
| Motility % | 39.57 ± 9.50 | 42.15 ± 7.46 | 47.90 ± 13.91 | 0.001, 0.004 |
| Progressive Motility % | 18.6 ± 0.46 | 20.57 ± 6.68 | 32.34 ± 10.34 | 0.0001, 0.0001 |
| Forward Progression/4 | 1.81 ± 0.59 | 1.75 ± 0.43 | 2.16 ± 0.62 | 0.002, 0.0005 |
| Normal Forms % | 5.83 ± 1.11 | 6.65 ± 1.73 | 6.41 ± 1.53 | 0.02, 0.38 |
| Swim Up Count×10 ⁶ | 10.06 ± 6.67 | 9.87 ± 5.75 | 15.60 ± 11.57 | 0.004, 0.005 |
| Swim Up Forward Progression/4 | 2.06 ± 0.70 | 2.27 ± 0.71 | 3.03 ± 0.82 | 0.0001, 0.0001 |

Table 1: Comparison of semen parameters in 3 IUI trials showing the mean ± standard deviation.

| Patients | Chemical Pregnancy | | Clinical pregnancy | | >5weeks gestation miscarriages | | Delivered | Live birth rate |
|----------|--------------------|------|--------------------|------|--------------------------------|-----|-----------|-----------------|
| | No | % | No | % | No | % | | |
| 33 | 10 | 30.3 | 7 | 21.2 | 1 | 3.0 | 6 | 18.1 |

Table 2: Overall pregnancy success rate/couple in the third double IUI trial.

| Age Range | No of patients | No of Chemical Pregnancy | No of Clinical pregnancy | >5weeks gestation miscarriages | Delivered | Live birth rate (%) |
|-----------|----------------|--------------------------|--------------------------|--------------------------------|-----------|---------------------|
| 20- 25 | 10 | 4 | 1 | 0 | 1 | 3.0 |
| 26- 30 | 9 | 3 | 3 | 0 | 3 | 9.09 |
| 31-35 | 8 | 3 | 3 | 1 | 2 | 6.0 |
| 36-40 | 5 | 0 | 0 | 0 | 0 | 0 |
| 41-46 | 1 | 0 | 0 | 0 | 0 | 0 |

Table 3: Showing the results of IUI outcome in relation to age of the female spouse. The percentages of live births are expressed in relation to the total number of couples.

| BMI Range | No of Patients | No of Chemical Pregnancy | No of Clinical pregnancy | >5weeks gestation miscarriages | Delivered | Live birth rate (%) |
|------------|----------------|--------------------------|--------------------------|--------------------------------|-----------|---------------------|
| <18.5 | 10 | 5 | 5 | 0 | 5 | 15.1 |
| 18.5-24.99 | 11 | 2 | 2 | 1 | 1 | 3.0 |
| 25-29.9 | 6 | 2 | 0 | 0 | 0 | 0 |
| 30- 35 | 6 | 1 | 0 | 0 | 0 | 0 |

Table 4: Showing the results of IUI outcome in relation to the body mass index (BMI, WHO classification) of the female spouse.

index. In 33 patients, 5 females with BMI <18.5 showed positive results for chemical pregnancy and continued until full term and delivery (live birth rate of 15.1%). Females with BMI 18.5-24.99 showed 2 chemical pregnancies with one miscarriage at 5 weeks gestation and one full term delivery (live birth rate of 3.0%). Females with BMI >25-29.9 showed 2 chemical pregnancies and did not continue beyond this stage while no pregnancies were detected in the rest of the patients having BMI more than 30 (Table 4).

Discussion

With introduction of the technique Intracytoplasmic Sperm Injection (ICSI) childless couples can be treated even in cases with severe male factor infertility. However, milder therapeutic options are preferred to this invasive and costly method in cases with suboptimal semen samples. Intra Uterine Insemination (IUI) is a useful and cost effective therapeutic modality used for treatment of idiopathic male factor infertility. Successful IUI outcome is highly dependent on semen parameters to be as close as possible to the normal ranges recommended by the world Health Organization [19]. To meet patient's favorable expectations in IUI treatment cycles, efforts should be made towards improving the motility, the normal morphology and the concentration of sperm in semen samples used for insemination. Despite many reports on non-effectiveness of individual empirical therapies in this context, some of these treatments have managed to upgrade semen samples from azoospermia to samples possessing few thousands of sperm, more than adequate for ICSI trials. Such treatment outcomes are considered a bonus for the treatment method and should not be taken as incidental or a blow of luck. Therefore, our efforts were focused on optimizing sperm quantity and quality necessary for oocyte fertilization by adopting two strategies. Patients, who had two earlier failed IUI treatment cycles were counseled to accept a tailored program designed for their third IUI trial. Our aim was to improve sperm compromised parameters such as progressive motility and sperm forward progression by a three month course of empirical therapies. Oral administration of antiestrogen agent (tamoxifen), antioxidants (vitamin E) and minerals (zinc and selenium) for three months managed to improve semen criteria needed for pregnancy. There was a noticeable increase in all parameters after treatment and the differences were significant in progressive sperm concentration.

Also, an additional approach to increase the positive outcome of IUI cycles was by employing a double insemination method in the third

attempt. The rationale behind this strategy was two fold: first to increase the number of motile spermatozoa in the Fallopian tubes during the time of fertilization, and second to accommodate the asynchronous ovulation of oocytes after the rupture of the leading follicle. Literature review showed conflicting results about the superiority of double insemination over single insemination. In a randomized study, double insemination showed significant increase in pregnancy rates in patients with male factor, but not in patients with idiopathic infertility [25]. In a systemic review of three studies from Cochrane library to evaluate the double insemination in treatment of infertile couples, no significant effect on pregnancy rate/couple was observed in comparison to single insemination [26]. A more recent meta-analysis on double versus single insemination for idiopathic infertility found no clear benefit in overall clinical pregnancy rates [27]. Disappointing results in these trials might have been related to more complex issues of infertility in both couples rather than the compromised values for seminal parameters in males.

Also, to minimize the risk of multiple pregnancies, a mild ovarian stimulation was applied. The ovaries were carefully monitored for production of a maximum of 4 follicles, at least two of which were suitable for fertilization at the time of insemination. Timing of IUI in relation to the time of follicle rupture is extremely crucial. The optimum time for fertilization has been determined to be from the instant of ovulation up to 40 hours after HCG injection [28]. In another study IUI performed 24 hours after HCG with single insemination did not differ in the pregnancy outcome to show much difference than double insemination at 12 and 36 hours post HCG [29]. Regardless of the exact time of insemination, it is agreed that oocytes are fertilizable for 12-16 hours post ovulation [30]. In our strategy, we had our first insemination at 36 hours post HCG and the second 8 hours later (44h). Therefore, the timings of our inseminations were in coordination with the fertilization window of the oocytes.

Positive pregnancy outcome in the third IUI trial gave us confidence in our strategy towards improving our results. According to semen analysis data after consumption of tamoxifen, vitamin E and minerals, we prioritize their effect in improving pregnancy outcome in our patients. Yet, to confirm these results in a future study, it is advisable to measure the seminal Zinc and Selenium in male patients before administration of these medications.

Moreover, contribution of the double insemination method cannot be ruled out in this improvement and the positive outcome could have

been due to the collective effect of all the manoeuvres used. Despite the controversies regarding the non effectiveness of any one of these modalities at a time, we think that these strategies should be employed together for treatment of idiopathic male factor infertility in future.

A correlation between the pregnancy rate and the body mass index of the female partners was observed in our study group, where 83.3% of women who conceived and continued to full term and delivery had BMI equal or less than 18.5. This observation could be purely incidental and we may require a larger study group to extrapolate a valid conclusion regarding the effect of body mass index on IUI pregnancy outcome in couples with unexplained male factor infertility. In a retrospective analysis of 3000 IUI trials, higher pregnancy success rates were achieved in women with body mass indices equal or higher than 25 [31]. The authors attributed their findings to treatments for anovulation in overweight women during the course of IUI. In our study, although IUI was successful mostly in women with age group between 35 and 30 years, the size of our study group was not adequate to investigate clearly the effect of female age over pregnancy.

Vaginal ultrasound on the day of insemination showed a normal endometrial thickness in all 33 females and found to be within the expected range suitable for conception [25]. Nevertheless, in addition to the optimum endometrial thickness, the incidence and continuum of pregnancy in our patients might have been supported by other endogenous factors needed for conception.

In conclusion, double IUI procedures can result in better pregnancy and life birth rates in couples with male factor infertility. The pre requisite for successful IUI in male factor infertility is to follow a regimen of empirical therapies, which can be effective in some men, to improve their seminal parameters. Confirmation of our findings needs a large controlled study group of infertile couples, mainly presenting unexplained or mild male factor. We highly recommend that in our future studies semen levels of vitamin E, zinc, and selenium to be measured before commencement of empirical therapy. Such modality may clarify the beneficiary role of empirical therapies in improving seminal parameters in selective candidates.

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