Role of Clomiphene Citrate and Tamoxiphene Citrate in Mice Sperm Motility and Activity

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Abstract:

Background:

Both clomiphene citrate and tamoxiphene citrate, as an anti estrogen, is the most common prescribed medication for male infertility.In humans, ant estrogens interfere with the normal negative feedback of sex steroids at the hypothalamus and pituitary, thereby increasing endogenous gonadotropin releasing hormone (GnRH) secretion from the hypothalamus and follicle stimulating hormone (FSH) and luteinizing hormone (LH) secretion directly from the pituitary.

Objective:

The objective of this study was to investigate the effect of clomiphene citrate (CC) and tamoxiphene citrate (TC) administered to male mice on quality of sperm motility and activity.

Materials and Methods:

Ninety six male mice were divided into two major groups, and every one of these groups was subdivided into three minor groups involving control and two treated groups. In the CC study, 48 mice were administrated orally 0, 0.0125 mg and 0.025 mg as control (G 1), low dose (G 2) and high dose (G 3) for 42 days. While, in the TC study, 48 mice were administrated orally 0, 0.01 mg and 0.02 mg as control (G 1), low dose (G 2) and high dose (G 3) for 42 days. Then, the male mice were sacrificed, both vas deferens were collected and assessment sperm motility and activity.

Results: The results of present study appeared that the use high dose of CC or TC significantly increment ($P<0.05$) sperm motility and activity as compared to control groups.

Conclusions:

Based on the results of this study, it can be concluded that the administration of high dose of either CC or TC increases sperm motility and activity.

Key words: Clomiphene citrate,Tamoxiphene citrate, mice sperm motility and activity.

Introduction:

Semen parameters have a limited power to predict spontaneous or assisted conception [1]. Despite the advancements in diagnostic methodology, up to 25% of patients explain abnormal semen analysis for which no etiology can be identified ,this condition is referred to as idiopathic male infertility. Semen parameters of these patients demonstrate wide range of abnormalities. Approximately 10% of patients demonstrate isolated oligozoospermia, abnormalities of all semen parameters are found in 43% of patients, defects in motility and viability alone are present in 39% of patients, where as isolated morphologic abnormalities occur in 8% .The pharmacologic therapy for these cases should be employed for a 3-6 months period so that at least one full spermatogenic cycle will be incorporated[2].The difference between tamoxiphene citrate (TC) and other anti- estrogen like clomiphene citrate is that it is selective estrogen receptor modulators with no estrogenic activity in men and it has no cumulative effect as compared to clomiphene citrate CC [3]. Clomiphene citrate (CC) was first discovered in 1956 by the Merrel Company [4].In 1967, the FDA approved clomiphene citrate for clinical usage. Since approval, both substances have been used for the treatment of breast cancer and induction of ovulation, and till date, they still are being widely used for this purpose [5]. CC is the most widely used pharmacological preparation among the ovulation-inducing drugs. Pregnancy rate with CC is 30-40% which is not as good as the ovulation rate (70-80%) [6]. Tamoxiphene citrate (TC) was first discovered in 1962 by Dr. Arthur L. Walpole [7]. Tamoxiphene citrate as another anti-estrogenic compound very similar to CC in structure has been evaluated as a fertility agent in the recent past. With limited literature on the use of TC for ovulation induction, ovulation rates have been reported as 50-90% and pregnancy rates as 30-50% [8]. Tamoxiphene citrate has shown reasonably good results in CC failure cases too tamoxiphene citrate acts in a similar manner of clomiphene citrate but lacks the estrogenic activity of clomiphene citrate, dosage of 10-20mg/day have been most commonly employed [9]. Therefore; the aim of the study is to investigate the effect of CC and TC on sperm concentration in mice as a model for human being.

Materials and Methods:

Animal:

Ninety six male mice were divided into two major groups, and every one of these groups was subdivided into three minor groups involving control and two treated groups. The males (25- 27gm) were taken from the Animal House unit at High Institute for Infertility Diagnosis and Assisted Reproductive Technologies**.** The animals were housed in plastic cages (4 mice / cage) measuring about (29x15x12) cm. Floors of cages were covered with soft crushed wood shaving; all cages were washed two times per week with 70% alcohol throughout the period of the study. They were kept in a room supplied with an air conditioner to keep temperature (~25°C) with a photoperiod (12-14) hours (Christian and Williams,1976).Tap water and pellet diet were available for the animals.

Preparation of drug:

Clomiphene Citrate preparation:

Clomiphene citrate (CC) dose was prepared by taking one tablet of CC 50 mg and dissolved in 200mL distilled water to prepare 0.025 mg / 0.1mL as high concentration of CC as stock

solution, from this stock solution 10 mL were taken and added of 10 mL of distilled water to obtain 0.0125 mg /0.1 mL as low concentration.

Tamoxiphene citrate preparation:

Tamoxiphene citrate (TC) dose was prepared by taking one tablet of TC (20) mg and dissolved in 20mL distilled water to prepare 1mg concentration of TC as stock solution, from this stock solution, 0. 1 and 0.02 mL were taken using automatic pipette add of 0.9mL and 0.8mL of distilled water to obtain the final concentrations 0.01mg/0.1mL and 0.02mg/0.1mL respectively.

Administration route and periods:

The animals were administered orally for 42 days and each one mouse was administrated 0.1mL of each drug .

Sperm collection:

At end of administration period the male mice were scarified by cervical dislocation, then both the vas differences were collected and cleaned from adipose and other tissues using SMART medium.

- Sperm concentration:

Concentration of spermatozoa (sperm/ml) was calculated from the mean number of spermatozoa in ten high powers microscopic fields under magnification of (400). This number was multiplied by a factor of one Million [10, 11].

Statistical Analysis: The data were statistically analyzed using SPSS/PC version 18 software (SPSS, Chicago). Sperm parameters were analyzed using complete randomized design (CRD) (one way ANOVA). Differences among means were compared using the Duncan multiple ranges test 0.05 was considered statistically significant difference according to Duncan, [12].

Results:

Clomiphene citrate study:

Figure (1) explains effect of different clomiphene citrate doses orally administered male mice for six weeks on sperm concentration. There was a significant decrement ($P<0.05$) in the control group as compared with both treatments (low and high dose).Also, observed significant reduction ($P<0.05$) in the sperm concentration using low dose as compared with high dose.

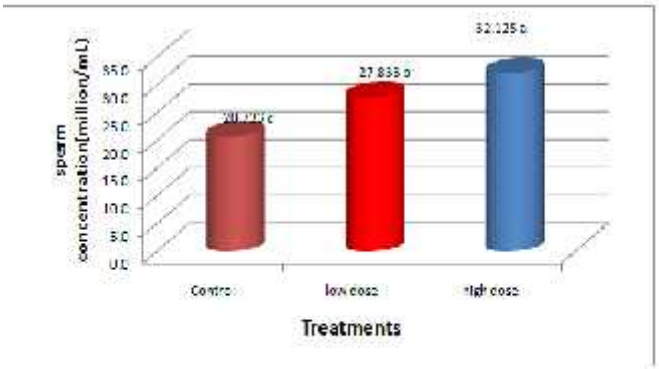

Figure (1): Effect of different clomiphene citrate doses orally administered to male mice for six weeks on sperm concentration.

- Means with different superscripts are significantly different ($P<0.05$)
- 16 mice for each group were administered orally.

Tamoxiphene citrate study:

Figure (2) explains effect of different tamoxiphene citrate doses orally administered to male mice for six weeks on sperm concentration. A significant decline ($P<0.05$) was found in the control group and low TC dose as compared to high dose. However, no significant difference ($P>0.05$) was assessed between control group and low dose.

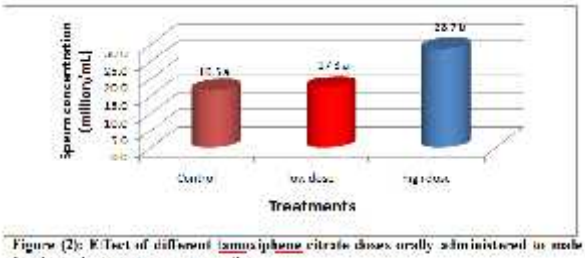

Figure (2): Effect of different tamoxiphene citrate doses orally administered to male mice for six weeks on sperm concentration.

- Means with different superscripts are significantly different ($P<0.05$).
- Means with similar superscripts are non significantly different ($P>0.05$).
- 10 mice for each group were administered orally.

Comparison between clomiphene citrate and tamoxiphene citrate:

Figure (3) explains comparison between effect of different doses of clomiphene citrate and tamoxiphene citrate orally administered to male mice for six weeks on sperm concentration. No significant difference (P>0.05) was observed between both control groups. Also, non significant differences (P>0.05) was found between both high dose groups. While, significant increment (P<0.05) was obtained between low dose of CC as compared with low dose of TC treated.

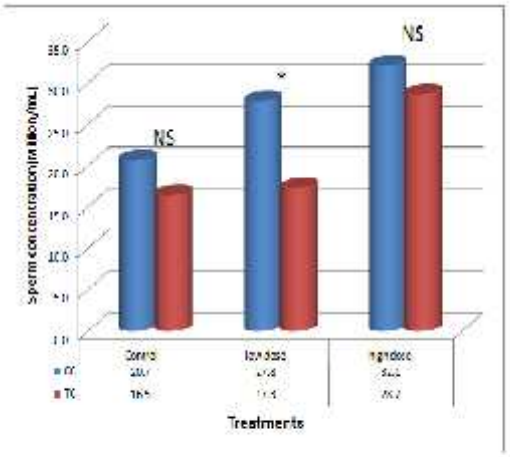

Figure (3): Comparison between effect of different doses of clomiphene citrate and tamoxiphene citrate orally administered to male mice for six weeks on sperm concentration.
*: significant different (P<0.05).
NS: Non significant.
+ 16 mice for each group were administered orally.

Discussion:

In the present study, clomiphene citrate (CC) and tamoxiphene citrate (TC) were selected as a result of widely used and consumed drugs for enhancement semen quality and sperm parameters, in addition to be as fertility stimulator drugs as a comparative study in male mice as a model of human being CC [13]. According to three groups of clomiphene citrate including low dose, high dose and controls were used. There was significant increment the sperm concentration for both treatment groups as compared to control group. Also, it was observed a significant reduction using low dose when compared to high dose. Here, in the present study, CC acts as estrogenic activity. Consequently, serum LH level increase and therefore testosterone increase else. Finally, enhancement rate of spermatogenesis and sperm production occur. With same manual showed for TC as a mode of action. The present results were agreed with those found in study done by Rajender and co-workers (2009). They observed that using clomiphene citrate was administered to patients appeared remarkable improvement in their seminal parameters over a period of 3 months and 6 months treatment. Researchers certified that this increase in endogenous gonadotropins manifests itself in improving the sperm count [14, 15].

In the line with current World Health Organization recommendations, it is well known that FSH is considered as an essential hormone for the initiation of spermatogenesis and Sertoli cell function, while LH is essential for stimulation of testosterone synthesis by the Leydig cell. This physiological increase in gonadotropin hormones which is reflected in increment in sperm concentration, this indicated an increase levels of testosterone, since the excess of testosterone mediate negative feedback effect on the anterior pituitary. Thus, clomiphene citrate exerts its effect on spermatogenesis by raising the endogenous serum FSH, LH and testosterone levels to initiate and maintain gametogenesis [16].

Furthermore, these results are agreed with the result of a study done on 90 patients complaining from idiopathic oligospermia treated with 20 mg of tamoxiphene citrate for three months in the Hospital of the University of Pennsylvania [14]. In contrast to the previous results disagree with those found in study with other studies at McMaster University. They used tamoxiphene for duration continues for 6 to 14 months, and concluded that the anti-estrogen therapy doesn't work in

infertile male [17].
This physiological increase in gonadotropin hormones which is reflected in increment in some hormone levels, this indicated an increase levels of testosterone [18]. These results were agreed Malley and Strot(2001)[16]. They concluded that the improvement in the sperm concentration reveals directly the effect of this selective antiestrogen drug on spermatogenesis which is due to the direct effect of TC on the gonadotropin releasing hormone (GnRH)-stimulating and the release of luteinizing hormone and follicle-stimulating hormone and indirectly on the release of testosterone hormone [16].

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