

## EVALUATION OF EFFECTS OF CLOMIPHENE CITRATE ON SERUM TESTOSTERONE & FSH LEVELS AND SEMINAL PARAMETERS IN IDIOPATHIC OLIGOSPERMIA

SAFEER ZAMAN\*, SIBGHA ZULFIQAR\* AND M. SHOAB KHAN\*\*

\*Department of Physiology, Shaikh Zayed Federal Postgraduate Medical Institute, Lahore

\*\*Department of Biochemistry, Bannu Medical College, Bannu - Pakistan

### ABSTRACT

*Clomiphene citrate is a synthetic nonsteroidal drug that acts as an antioestrogen and competitively binds to oestrogen receptors in the hypothalamus and pituitary. This blocks the action of the normally low levels of oestrogen on the male hormone axis and results in increased secretion of GnRH, FSH and LH. The enhanced output of these hormones increases testosterone production and sperm production. A non randomised clinical trial was conducted in a sample of 21 idiopathic oligospermic men from local population with a mean age of  $27.75 \pm 4.13$  years, to evaluate the effects of clomiphene citrate in a dose of 100mg/day for five days, on serum testosterone, serum FSH, semen volume, sperm density, and total sperm motility. After clomiphene citrate therapy, mean serum testosterone level showed highly significant increase ( $p < 0.05$ ), mean serum FSH level showed significant increase ( $p < 0.1$ ). Semen analyses were done at periodic intervals, 1<sup>st</sup> at day 1 i.e. before the 1<sup>st</sup> dose of clomiphene citrate, 2<sup>nd</sup> at 6<sup>th</sup> day i.e. 24 hours after the last dose of clomiphene citrate, and 3<sup>rd</sup> at 74<sup>th</sup> day of the trial. Clomiphene citrate administration resulted in statistically significant increase in the mean semen volume at day 6 ( $p < 0.05$ ), increase in the sperm density at day 74 ( $p < 0.05$ ), and increase in the total sperm motility at day 6 ( $p < 0.05$ ).*

### INTRODUCTION

Infertility remains a serious socio-medical problem worldwide. It has received a considerable global attention in recent years.<sup>1</sup> Infertility is defined as the inability to conceive after one year of unprotected sexual intercourse.<sup>2,7</sup> Approximately 15% of couples are unable to initiate pregnancy without some form of assistance or therapy.<sup>3,7,24,7</sup> Evaluation of infertile couples has revealed that male factor infertility contribute to the problem in up to 50% of cases.<sup>2,3,4,6,9</sup> In male dominant society generally and in our society particularly where illiteracy and poverty are more prevalent, men hardly agree to get investigated for infertility.<sup>1,5</sup> Oligospermia is defined as a sperm density (count) less than 20 million/ml.<sup>3,6,7</sup> Spermatogenesis is a chronological process spanning about 74 days.<sup>7,8</sup> One of the difficult aspects of treating infertile men is that there are no recommended medical therapies for men with idiopathic oligospermia.<sup>9</sup>

Clomiphene citrate is a synthetic nonsteroidal drug that acts as an antiestrogen and competitively binds to oestrogen receptors in the hypothalamus and pituitary. This blocks the action of the normally low levels of oestrogen on the male hormone axis and results in increased secretion of GnRH, FSH and LH. The enhanced output of these hormones increase testosterone production and spe-

rm production.<sup>10</sup> The effects of clomiphene citrate for the treatment of male factor infertility are still controversial.<sup>3,11</sup> This drug has been trialed in many countries to study its effects on male hormones and different sets of seminal parameters in male infertility. The objective of this study was to evaluate the effects of clomiphene citrate on serum testosterone, serum FSH, seminal volume, sperm density and total sperm motility in a sample of local population suffering from idiopathic oligospermia and determine its role in male infertility.

### MATERIALS AND METHODS

This study was carried out in the Department of Physiology Shaikh Zayed Federal Postgraduate Medical Institute, Lahore in collaboration with Physiology Department Bannu Medical College, Bannu, N.W.F.P. and NHRC, Shaikh Zayed Medical Complex, Lahore during the months of September to November 2008. It was a Non-randomized controlled clinical trial with a sample size of 21. Subjects having sperm density  $< 20$  million/ml between the ages of 20-35 years, with no known cause for reduced count (excluding known causes by proper history taking, general physical examination and relevant investigations with the available facilities) were included in the trial. First 21 available patients were taken into account. The male partners of couples, married for more than

one year, living together, issueless and fulfilling all the inclusion and exclusion criteria were included in the trial.

Every patient was given 100 mg of clomiphene citrate (Clomid) per day for 5 consecutive days orally. Semen samples were collected from subjects by masturbation following strict abstinence of three days from intercourse. The patients were strictly instructed not to use saliva or any lubricant, as these are spermicidal. Only liquid paraffin was allowed if needed. A clean sterile wide mouthed jar of 20 ml was provided to each patient for semen collection. First semen sample was collected before the administration of 1<sup>st</sup> dose of clomiphene citrate, 2<sup>nd</sup> sample 24 hours after the last dose of clomiphene citrate, and 3<sup>rd</sup> sample on 74<sup>th</sup> day of the trial. Two samples of 5 ml blood were taken from antecubital vein from each subject, 1<sup>st</sup> before the administration of 1<sup>st</sup> dose of clomiphene citrate and 2<sup>nd</sup> 24 hours after the last dose of the drug.

Semen analyses were performed within an hour of receiving the samples for semen volume sperm density and sperm motility according to WHO protocol. Seminal volume was measured directly on a graduated pipette, after giving 20-30 minutes for liquefaction. Sperm density was determined by using haemocytometer (Neubauer counting chamber).<sup>6,12</sup> The percentage of motile spermatozoa was taken as the proportion of prog-

ressive spermatozoa moving either slowly or rapidly relative to the hundred successive sperms under microscope.<sup>13</sup> The serum samples were analysed for testosterone and FSH levels using standard ELISA method at NHRC, Shaikh Zayed Medical Complex, Lahore.

Mean hormonal levels at day 1 and day 6 were compared by paired t-test., while means of the three seminal parameters at different periodic intervals were compared by ANOVA. A p-value of <0.05 was considered significant.

## RESULTS

Serum testosterone increased from  $4.38 \pm 0.83$  ng/ml at day 1 (i.e. pre-trial) to  $8.06 \pm 1.17$  ng/ml at day 6 (i.e. post-trial) - a highly significant increase ( $p < 0.05$ ). Serum FSH increased from  $5.73 \pm 3.09$  Miu/ml at day 1 (pre trial level) to  $7.05 \pm 3.53$  Miu/ml at day 6 (post trial level), which was considered not a significant increase at 5% level of probability, but it was a significant increase at 10% level of probability. Analysis of the three seminal parameters i.e. semen volume, sperm density, and sperm motility showed that the mean semen volume which was  $2.71 \pm 0.79$  ml at day 1 (pre trial), increased to  $4.18 \pm 0.95$  ml at day 6 (post-trial), a highly significant increase ( $p < 0.05$ ). The mean semen volume at day 74 dropped to  $3.47 \pm 1.02$  ml, but still significantly higher from day 1 ( $p < 0.05$ ). The results for the sperm density showed that

at day 1 it was  $13.24 \pm 3.03$  million/ml (pre trial), at day 6 it was  $13.52 \pm 3.00$  million/ml (post trial), no significant difference was observed from the mean value of pre trial, but the mean sperm density at day 74 was  $20.23 \pm 3.74$  million/ml,

significantly higher from both day 1 and day 6 ( $p < 0.05$ ). The study showed that the mean value of total motile sperm count before the clomiphene citrate trial in the group was  $56.43 \pm 8.54\%$ , which increased to  $66.19 \pm 8.20\%$  at day 6 (post trial) that is a significant increase ( $p < 0.05$ ). The total sperm motility at day 74 was  $58.57 \pm 9.64\%$ , statistically equal to that of day 1.

## DISCUSSION

In a literature review of the use of clomiphene citrate in the treatment of idiopathic oligospermia, differing dosa-

**Table 1:** Mean comparison of serum testosterone and serum FSH at day 1 and day 6 by paired t-test.

Parameter	At Day 1		At Day 6		t-ratio	p-value
	Mean	SD	Mean	SD		
Testosterone in serum	4.38	$\pm 0.83$	8.06	$\pm 1.17$	-13.54**	0.000
Serum FSH	5.73	$\pm 3.09$	7.05	$\pm 3.53$	-1.81*	0.085

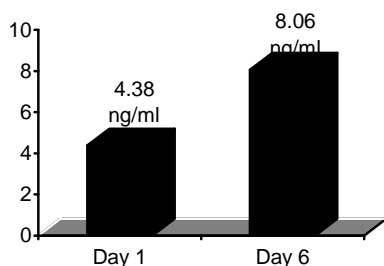
\*\* Significant at both 1% and 5% level of probability;

\* significant at 10% probability level

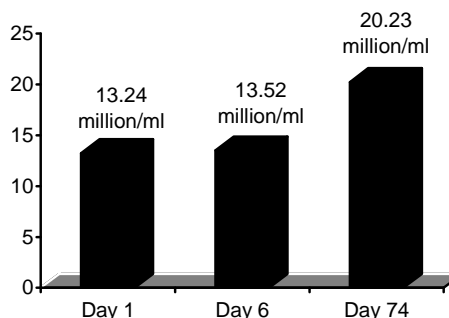
**Table 2:** The average effect of hormonal changes on seminal parameters i.e. semen volume, sperm density, and sperm motility by ANOVA.

Semen Volume (SV) In ml		Sperm density (SD) In million/ml		Sperm motility (SM) In % age	
Days	Mean	Days	Mean	Days	Mean
1	2.71 c	1	13.24 b	1	56.43 b
6	4.19 a	6	13.52 b	6	66.19 a
74	3.47 b	74	20.24 a	74	58.57 b
LSD*	0.5706	-	2.0206	-	5.44

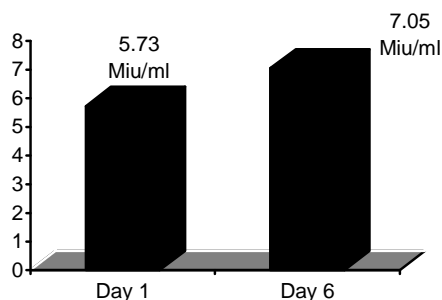
\*LSD is the least significant difference at  $p < 0.05$ ; the means in each column followed by different letters are significantly different.



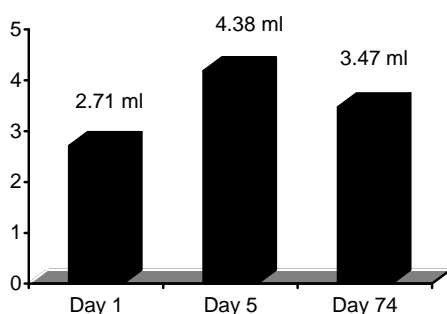
**Fig. 1:** Changes in serum testosterone levels.



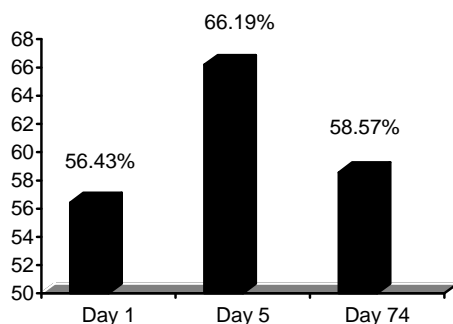
**Fig. 4:** Changes in sperm density.



**Fig. 2:** Change in serum FSH levels.



**Fig. 3:** Changes in semen volume.



**Fig. 5:** Changes in total sperm motility.

ges and protocols are reported with the most common being 50mg daily, 50 mg every other day,

25 mg every other day, or 25 mg daily for 25 days followed by a drug free interval of 5 days. In this study clomiphene citrate was given orally to each patient for five days in a dose of 100mg per day, as Gonazale had done in his study.<sup>14</sup> The rationale for use of this drug was based on its effects in increasing endogenous gonadotropin-releasing hormone secretion from the hypothalamus and gonadotropin-hormone secretion from the pituitary, thus increasing intratesticular testosterone concentration, a fundamental requirement for spermatogenesis.<sup>15</sup>

In the current study, treatment with clomiphene citrate for five days resulted in a highly significant increase in the mean serum level of testosterone ( $p < 0.05$ ), and an increase in the mean serum FSH level ( $P < 0.1$ ), comparable results to the study done by Gonzales and his colleagues.<sup>14</sup>

The study also showed a highly significant increase in the mean semen volume at day 6 ( $p < 0.05$ ). The mean semen volume at day 74 showed a drop as compared to that of day 6, but still significantly higher from day 1 ( $p < 0.05$ ). It is established by most of the authors that 2/3 of the semen volume is produced by seminal vesicles, and increased endogenous serum testosterone level is related to seminal vesicle function.<sup>16,17</sup> The highly significant increase in the semen volume at day 6 can be explained by the highly significant increase in the serum testosterone at day 6 from day 1. The increase at day 74 from day 1 might be due to variations in the abstinence interval, as studies have shown that with each day of abstinence (up to 1 week), semen volume can rise by up to 0.4 ml.<sup>18</sup>

The sperm density showed a significant increase at day 74 ( $p < 0.05$ ). It is obvious that no significant change was expected at day 6, since sperms take about 74 days to become fully mature. The significant increase at day 74 was in accordance with most of the other studies.<sup>15,19,20</sup> Clomiphene citrate administration also resulted in statistically

significant increase in the mean value of total motile sperm count at day 6 ( $p < 0.05$ ). This was in accordance to the results obtained in the studies conducted by Patankar.<sup>19</sup> The total sperm motility at day 74 was statistically equivalent to that of day 1.

One of the favourable outcomes of this clinical trial was that four female partners got pregnant out of the 21 couples whose male partners completed this trial. In comparison Hayashi and his colleague got 4 pregnancies out of the 30 idiopathic infertile men placed in a trial of clomiphene citrate.<sup>20</sup>

It is **concluded** that clomiphene citrate exerts its effects on spermatogenesis by raising the endogenous serum FSH and testosterone levels. This increase in endogenous gonadotropins manifests itself in improving sperm count, sperm motility and semen volume. Because of the relatively smaller number of our patients, we recommend that further prospective randomised studies should be done to assess the role of clomiphene citrate in the treatment of idiopathic oligospermia.

#### ACKNOWLEDGEMENT

We are very thankful to Miss Farkhanda Ghafoor (Senior Research Officer) and Miss Rabel Javed (Research Officer) of NHRC Shaikh Zayed Medical Complex for facilitating us in the hormonal assays, and to Mr. Shah Noor Khan (Laboratory Technician) for helping us in semen analysis at the Physiology Department Bannu Medical College Bannu.

#### REFERENCES

- Ranyo M. Rikhasor, Shankar Lal Rathi, Malik Hussain Jalbani, Zafar Ali Pirzado, Siraj Ahmad Abbasi; Semen Analysis of Infertile Men and Plasma Levels of LH, FSH & Testosterone in Oligospermia; Original Paper, 2001; (Vol. 7) No 2: 30-2.
- Claudia M.B.Carvalho, Luciana W.Zuccherato, Luciana Bastos-Rodrigues, Fabricio R.Santos, Sergio D.J.Pena; No Association Found Between gr/gr Deletions and Infertility in Brazillian Males; Molecular Human Reproduction, 2006; (Vol. 12) No. 4: pp. 269-73.
- Sergio G. Moreira, Jr., Larry I. Lipshultz; Management of Male Infertility; www.duj.com/Article/Moreira.html 2008.
- Spitz A, Kim ED, Lipshultz LI; Contemporary Approach to the Male Infertily Evaluation; Obstet Gynecol Clin North Am. 2000; Sep; 27 (3): 487-516.
- Malik Hussain Jalbani, Ranyo M. Rikhasor, Shanker Lal Pathai, Ghulam Akbar Solangi, Rafia Baloch; Causes of Azoospermia in Infertile Men; Original Paper, 2001; (Vol. 7) No. 2: pp. 36-38.
- Rajvi H. Mehta, Sanjay Makwana, Geetha M. Ranga, R. J. Srinivasan, S.S. Virk.; Prevalences of oligozoospermia and azoospermia in male partners of infertile couples from different parts of India; Asian J Androl; 2006; 8 (1): 89-93.
- Jonathan Rubenstein, Robert E Brannigan; Infertility, Male; eMedicine; www.emedicine.com/med/TOPICTOPIC1167.HTM 2008, pp. 1-31.
- William F. Ganong, Review of Medical Physiology, 22nd Edition, 2005; pp. 427-42.
- Jacob Rajfer; Carnitine and Male Infertility; Reviews in Urology, 2006; (Vol. 8) No. 4: pp. 235-6.
- Stuart S. Howards; Treatment of Male Infertility; The new England Journal of Medicine, 1995; 332 (5): 312-7.
- Wael M.B. Noseir; Fertility in Rams Treated With Clomiphene Citrate; http://www.priory.com/vet/rams.htm ,2008
- Federation Francaise des CECOS, Jacques Auger, Pierre Jouannet; Evidence of regional differences of semen quality among fertile French men; Human Reproduction, 1997; 12 (4): 74.
- Ashok A. Frances M. Edmund S.; Assessing Sperm Function; Urol Clin N Am. 2008; 35: 157-71.
- Gonzales GF; Basal serum testosterone as an indicator of response to clomiphene treatment in human epididymis, seminal vesicles and prostate; Andrologia, 2002; 34 (5): 308-16.
- Alayman Hussein, Yasar Ozogok, Lawrence Ross, Craig Niederberger; Clomiphene Administration for cases of Non-obstructive Azoospermia: A Multi-center Study; Journal of Andrology, 2005; 26 (6): 787-91.
- Dana A. Ohl, Sussanne A. Quallich, Jens Sonkesen, Nancy L. Brackett, Charles M. Lynne; Anejaculation and retrograde Ejaculation; Urol Clin n Am. 2008; 35: 211-20.
- Gustavo F. Gonzales; Function of Seminal Vesicles and Their Role in Male Fertility; Asian J Androl. 2001 Dec.: 251-54.
- Emil A. Tanagho, Jack W. McAninch; Smith's General Urology; 16<sup>th</sup> Edition, 2004; pp. 678-708.
- Patankar SS, Kaore SB, Sawane MV, Mishra NV, Desshkar AM; Effect of clomiphene citrate on sperm density in male partners of infertile couples; Indian J Physiol Pharmacol. 2007; 51 (2): 195-8.
- Hayashi N, Sugimura Y, Hori N, Yamamoto I, Tazima K, Tochigi H, Kawamura J; Clomiphene citrate therapy in idiopathic male infertility; Hinyokika Kyo, 1988; 34 (5): 847-50.
- Paulson Df, Wacksman J; Clomiphene Citrate in the Male Infertility, J Urol; 1976.