THE EFFECT OF CLOMIPHENE ON SPERMATOGENESIS AND HORMONE EXCRETION IN A PATIENT WITH KLINEFELTER’S SYNDROME

G. L. FOSS,* E. T. BELL,† F. J. W. LEWIS,‡ J. A. LORAINË† AND B. R. POLLARD§

*Bristol Subfertility Clinic; †Medical Research Council Clinical Endocrinology Research Unit, University of Edinburgh; ‡Cytodiagnostic Unit, Department of Pathology, Southmead Hospital, Bristol; and §Department of Pathology, Frenchay Hospital, Bristol

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Summary. The effect of clomiphene on the sperm count and hormone excretion is described in a patient with Klinefelter’s syndrome, having a 47 XXY karyotype, and in whom a testicular biopsy showed small areas of spermatogenesis.

Clomiphene stimulated the production of morphologically normal motile spermatozoa in the absence of any marked effect on hormone output.

Possible explanations for these findings are discussed.

INTRODUCTION

The testes in patients with Klinefelter’s syndrome are generally small and firm and show aspermatogenesis. In those cases with a 47 XXY karyotype the histological appearance of the testes are very variable. There is always Leydig cell hyperplasia, with atrophy or ghost formation of the tubules, the walls of which are usually very thickened and hyalinized. In a small proportion of patients, however, the tubules are thin-walled, containing only Sertoli cells.

In 1956 Bunge & Bradbury described three cases of chromatin positive Klinefelter’s syndrome in whom the testes showed small areas of spermatogenesis. Similar cases have been reported by Grumbach, Blanc & Engle (1957), Witschi, Nelson & Segal (1957) and Ferguson-Smith & Munro (1958); however, no chromosomal analyses were performed on these patients. Recently Steinberger, Smith & Perloff (1965) have reported data on leucocyte and fibroblast cultures in two chromatin positive cases of Klinefelter’s syndrome with a 47 XXY karyotype. In these patients the testes contained a number of tubules showing a wide range of spermatogenic cells, including a few with spermatozoa.

The aim of the present investigation is to present data on the effect of the gonadal stimulant clomiphene on sperm counts and on urinary hormone excretion in a patient with Klinefelter’s syndrome studied in the Bristol Subfertility Clinic.
OBSERVATIONS

Case report

The patient, who had a high I.Q., was referred to the clinic at the age of 28 years, having been married for 2 years. His parents were both aged 39 when he was born. He had one sister 2 years older than himself who had been married for less than 1 year and had not become pregnant. The patient's mother was aged 67, had two sisters each with children, and two brothers, one of whom was unmarried, the other being married without children. His father, who died at the age of 53, had one married brother with children and one sister unmarried.

The patient shaved daily: his libido and sexual function were normal. He was well built, with a height of 182 cm, a span of 190 cm, and he weighed 70.7 kg. Axillary and pubic hair were normal, but his body was not hirsute. There was no gynaecomastia. His penis was of average adult size, but both scrotal testes were small and firm, measuring 2.5 cm$^3$ in volume on a testicular scale which can be obtained from Messrs Thackray of Leeds (normal range 20 to 24 cm$^3$).

Of fifty oral cells examined twenty-five showed single Barr bodies. Chromosome analyses on cultures of leucocytes and skin fibroblasts were carried out, using the method of Moorhead, Nowell, Mellman, Battips & Hungerford (1960). Thirty cells from the leucocyte culture and twenty cells from the fibroblast culture were subjected to full analysis. All karyotypes were consistent with the sex complement XXY: two of them were pseudo-diploid, with a different autosomal loss in each. There was, therefore, no evidence of mosaicism from the two sources examined (Plate 1).

The mother and the patient were both Xg(a +), which gave no information of the origin of the extra X chromosome. They both had normal colour vision.

A testicular biopsy on the right side was performed before clomiphene therapy. It showed trabecular hyperplasia of the Leydig cells, and some thin-walled tubules containing spermatogenic cells as far as the spermatid stage: in one or two tubules an occasional spermatozoon was observed. In another area of the section there was a group of small, thick-walled, hyalinized tubules without the spermatogenic cells (Plate 2).

Effect of clomiphene on spermatogenesis

Seminal analyses were conducted on masturbation specimens obtained on waking. The samples were collected in plastic containers and were examined within 2 to 4 hr of production.

Several courses of clomiphene were given, the first being at a dose level of 50 mg/day and continuing for 17 weeks. Three weeks after commencement of therapy the total sperm count was 15 million, the spermatozoa being immotile. Another count performed 2 weeks later showed 12 million spermatozoa without motility; 7 weeks following the commencement of therapy there was a total count of 10 million, 10% of the spermatozoa being motile. During the remaining 10 weeks of treatment no motile spermatozoa were observed, and the dosage of clomiphene was therefore increased to 100 mg/day. Five weeks after the increase of dosage the sperm count was 8 million with 50% motility. A further increase
Fig. 1. Metaphase plate from culture of fibroblasts. × 1100.

Fig. 2. Karyotype of metaphase spread showing XXY sex complement and twenty-two pairs of autosomes.

(Facing p. 316)
Fig. 1. Testicular biopsy before treatment with clomiphene began. ×60.
Fig. 2. Testicular biopsy at higher magnification. The tubule is thin-walled and contains spermatids. ×200.

(Facing p. 317)
of dosage to 200 mg/day for two periods of 2 weeks and 6 weeks had no effect on sperm count.

No treatment was given for 30 weeks, during which time azoosperma was observed. Thereafter clomiphene was administered at a dosage of 100 mg/day for 7 days, at which time the hormone assays shown in Text-fig. 1 were performed. On the 3rd day of treatment a specimen containing 18 million spermatozoa with 60% motility was obtained, but 2 weeks later there was again complete azoosperma. Differential sperm counts showed normal morphology in 80 to 90% of the spermatozoa in the five specimens in which motility was observed.

**Effect of clomiphene on urinary hormone excretion**

The subject collected complete 24-hr urine samples continuously throughout the period of observation shown in Text-fig. 1. Assays were conducted on 24-hr or 48-hr pools of urine and the results were expressed/24-hr sample. For the estimation of 'total gonadotrophic activity' by the mouse uterus test the method used was that of Loraine & Brown (1959); results were expressed in terms of the Second International Reference Preparation for Human Menopausal Gonadotrophin (Second irp-hmg) as mg/24 hr. Assays of follicle-stimulating hormone (FSH) were conducted by the method described by Brown (1955), depending
on the augmentation of ovarian weight in mice; results were expressed in international units (i.u.)/24 hr. Pregnanetriol was measured by the method of Fotherby & Love (1960). For the estimation of 17-hydroxycorticosteroids (17-OHCS) and total 17-oxosteroids, modifications of the techniques described by Appleby, Gibson, Norymberski & Stubbs (1955) and Vestergaard (1951) were employed. Testosterone was measured by the method of Ismail & Harkness (1966).

During the control period from Days 1 to 7 of the investigation, values for 17-OHCS, 17-oxosteroids and pregnanetriol were within the normal range for a male subject (see Loraine & Bell, 1966), while testosterone output was below normal. Levels for 'total gonadotrophic activity' showed some fluctuation, but must be regarded as abnormally low for a patient with Klinefelter’s syndrome. The urinary excretion of FSH was below 5 i.u./24 hr.

Treatment with clomiphene had virtually no effect on excretion values for pregnanetriol, 17-OHCS, 17-oxosteroids and testosterone. HPG levels during treatment remained abnormally low, while FSH was undetectable. Following cessation of therapy there was a rise in levels of 'total gonadotrophic activity' and FSH, the last of three readings for 'total gonadotrophic activity' being within the range which might be anticipated in a patient with this syndrome.

**DISCUSSION**

The mechanism of action of clomiphene in women remains obscure, and it is not yet certain whether the compound acts via the hypothalamus or pituitary, or directly on the ovary (see Loraine, Bell & Harkness, 1966; Loraine, Bell, Harkness & Harrison, 1966; Bell, Loraine, Harkness & Foss, 1966; Smith, 1966). In the male even less is known about the mode and site of action of the compound. In normal men, Harkness, Bell, Loraine & Morse (1964) showed that clomiphene administration at a dosage of 100 mg/day orally caused an increase in the output of total 17-oxosteroids, dehydroepiandrosterone and oestrogens, without any consistent effect on HPG excretion as measured by the mouse uterus test. According to Brusca, Kastella & Heller (1966) clomiphene can also produce an increase in urinary testosterone excretion in normal males.

In the patient described herein clomiphene administration at a dose level of 50 mg b.i.d. for 7 days produced no marked change in urinary steroid output. These findings are similar to those reported by Lipsett, Davis, Wilson & Canfield (1965), who treated five chromatin positive cases of Klinefelter’s syndrome with human chorionic gonadotrophin, and observed little or no effect of this therapy on the output of pregnanetriol or testosterone. It is of interest to note that in the present study clomiphene appears to have depressed excretion values both for FSH and 'total gonadotrophic activity'. This effect on 'total gonadotrophic activity' is in contrast to that reported in normal males by Harkness et al. (1964). In the case of FSH the data obtained are difficult to interpret, due to lack of information on endogenous levels of this hormone both in the normal male subjects and in patients with Klinefelter’s syndrome. It should, however, be noted that throughout the whole period of study the pattern of FSH excretion is similar to that for 'total gonadotrophic activity'.

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It is generally agreed that in the testes of patients with Klinefelter's syndrome areas of spermatogenesis are rare, and it is therefore surprising that, in this case, treatment with clomiphene stimulated the production of live spermatozoa. According to Jungck, Roy, Greenblatt & Mahesh (1964), clomiphene administration at dosages ranging from 25 to 50 mg/day causes an improvement of spermatogenesis in a proportion of patients with oligozoospermia. Heller & Clermont (1964) have shown that the time elapsing between the production of a new stem cell spermatogonium and the release of its descendant spermatozoon from the testes in human subjects is approximately 74 days. However, in the patient reported herein, clomiphene administration resulted in the production of morphologically normal live spermatozoa 3 days after the beginning of therapy. The reason for this rapid response is not entirely clear, but it is possible that the compound prevented the degeneration of spermatids during stage 1 of the cycle of the seminiferous epithelium, thus allowing spermatozoa to appear in the semen. In view of the rapid effect of clomiphene on spermatogenesis in the absence of an increase in HPG output, it might be reasonable to suggest that the compound exerted a direct effect on enzyme systems in the testes which prevented spermatid degeneration.

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REFERENCES


