TESTOSTERONE IN THE TREATMENT OF INFERTILITY ASSOCIATED WITH INADEQUATE LUTEAL PHASE

by

B. N. CHAKRAVARTY,* M.O. (Cal), M.R.C.O.G. (Lond)

and

SUBHAS MUKHERJEE,** M.R.C.O.G. (Lond), Ph.D. (Edin), Ph.D. (Cal).

Introduction

Normal ovulatory mechanism does not always guarantee the subsequent presence of an adequate corpus luteum. Such inadequate luteal phase does not prevent fertilisation but the fertilised ovum is silently aborted because the endometrium is poorly developed to allow the fertilised ovum to be implanted. The condition, clinically appears, as one of ordinary sterility. The underlying cause of this hypoluteal phase leading to apparent infertility is not very clear. This is because, in the biology of human conception, the mechanism involved for the maintenance of corpus luteum from the time of ovulation till the time when HCG becomes available in adequate quantities is ill understood. For this purpose, substances like Pituitary LH and prolactin have been involved without general agreement.

Mukherjee (1972) and Mukherjee and Chakravarty (1974), while working on the probable role of testosterone and the related androgenic steroids on the female reproductive organs of rats briefly reported a potent positive luteotrophic effect of testosterone and dehydroepiandrosterone (DHEA) in this species, as well as in the human female.

The object of this communication is to corroborate this experimental observation on the basis of clinical trial with testosterone in a group of infertile women in whom the infertility was believed to be due to inadequacy of corpus luteum function.

Material and Methods

The luteotrophic effect of testosterone was initially experimented on rats. It was subsequently observed on normally menstruating women and finally the therapeutic effects of testosterone was successfully tried on 38 infertile women, in whom the infertility was believed to be due to the hypoluteal state of corpus luteum.

Animal Experiments

(a) *Animals Used*: 19-21 days old intact immature inbred Wister female rats weighing 30-35 g. (b) Preparation of the model for producing heavily luteinized ovaries: This was achieved by subcutaneous injection of 50.00 IU of PMS followed approximately 72 hours later by 20.00 IU of HCG. It was computed that from the 7th day (after HCG administration) onwards and at least for a period of 14 days, the combined weight of the corpora lutea in the ovaries thus produced weigh-

*Reader, Department of Gynaecology & Obstetrics.

**Reader, Department of Physiology.

N.R.S. Medical College, Calcutta.

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ed approximately 75%—90% of the total ovarian weight, (dry weight as well as wet weight), under all experimental conditions used. (c) Routes of injection: Subcutaneous (separate sites for different materials). (d) Effect of testosterone and dehydroepiandrosterone on PMS-HCG pretreated rat ovarian weight. Groups of rats as specified above were pretreated with PMS and HCG. One injection of testosterone phenyl propionate 20.0 mg. or dehydroepiandorsterone sulphate in ethyl oleate 5.0 mg. on alternate days (4 such) were given to PMS-HCG pretreated groups of rats, the first steroid injection being given on the day immediately after the animals had their HCG. Groups of experimental (testosterone treated or DHEA treated) and control (only PMS-HCG treated) animals were sacrificed at phased intervals and the ovaries weighed in wet state after cleaning as in Parlow's assay (Parlow, 1958) or ovarian cholesterol depletion assay for LH (Bell *et al*, 1964; Mukherjee *et al*, 1965). Results are shown in Figs. 1 and 2.

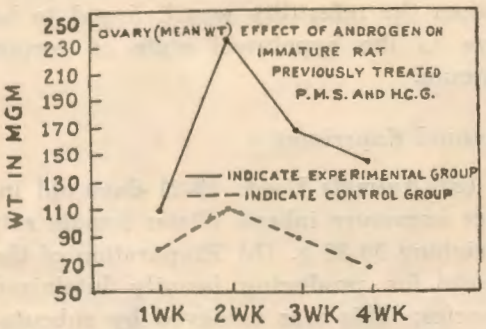

FIG.1 OVARIAN WEIGHT AT WEEKLY INTERVALS FOLLOWING PMS-HCG PRETREATMENT WITH AND WITHOUT TESTOSTERONE

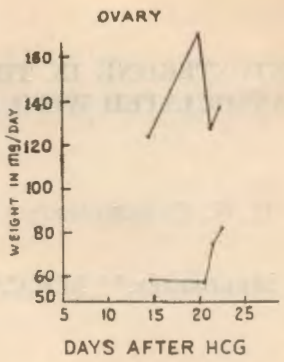

FIG. 2 RAT OVARIAN WEIGHT AT 5 DAYS INTERVAL FOLLOWING PMS-HCG PRETREATMENT WITH DEHYDROEPIANDROSTERONE TREATMENT, TOP GRAPH AGAINST CONTROL BOTTOM GRAPH

Human Experiments

(A) *In Normally Menstruating Women*:

(a) Methods of urinary excretion of total estrogens i.e. total of estrone, estriol and estradiol-17 B (Browne, 1955), with compulsory saponification procedure applied for all samples (b) Urinary pregnanediol according to the method of Klopper *et al* (1955) (c) for serial study of urinary steroid excretion during normal menstrual cycle, 24-hour serial collections were made for 2 consecutive cycles. Testosterone phenylpropionate 100 mg. was given i.m. on the day of the "thermal shift" as evidenced by basal body temperature recordings. Results are shown in Fig. 3.

(B) *In Infertile Women, in whom Infertility was believed to be due to Inadequate Corpus Luteum Phase*:

Testosterone as a luteotrophic drug was tried in 83 infertile women, out of whom, 38 have conceived so far. Clinical data of the successful cases are detailed in the following Tables. In women, who had

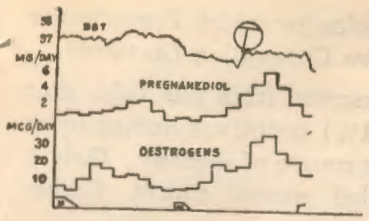

FIG. 3 SERIAL DETERMINATIONS (72 h. SAMPLES) OF URINARY ESTROGENS AND URINARY PREGNANEDIOL ALONG WITH DAILY BASAL BODY TEMPERATURE (BBT, O-O) RECORDINGS IN A CONTROL CYCLE AND FOLLOWING TESTOSTERONE ADMINISTRATION T, DURING THE EARLY LUTEAL PHASE, IN A NORMALLY MENSTRUATING YOUNG WOMAN AGED 26 YEARS. MENSTRUAL EPISODES, M.

normal menstrual cycles, testosterone in the form of perandren tablets (5 mgm, CIBA) was administered, one tablet daily at bed time for 5-7 consecutive days starting from 14th day counting from first day of the last menstrual period. In cases who had irregular rhythm of menstrual cycle, but were still ovulating, the exact date of ovulation was predetermined by Basal Body Temperature chart, and testosterone in the similar dose and schedule was administered from the day of the thermal nadir. The drug was administered for 3 consecutive cycles.

Following conception, each patient was treated with allyloestrinol (gestanin organon), for variable periods of pregnancy, because there was an apprehension that inadequate luteal phase, which was the cause of infertility could also lead to abortion, when these cases subsequently conceived.

Type of Endometrium

Endometrial biopsy formed the main basis of diagnosis of inadequate luteal phase. The usual finding in such an endometrium is inadequate glandular development commonly in association

Results

TABLE I
Type of Endometrium

Type of endometrium	No. of cases	Per cent
Early secretory "Out of phase"	34	89.48
Late secretory	3	7.89
Mixed	1	2.63

with patches of dense stroma. The biopsy material was obtained just in the premenstrual period or within 24 hours of onset of period. Prior to testosterone therapy, all except 12 cases were treated with allyoestrinol (Gestanin) or retroprogesterone (Duphaston-Interfran-Duphar) for 10 days starting from the earlier part of the luteal phase. This drug was continued in general for 3 consecutive cycles. If conception did not occur a re-biopsy was done and persistence of inadequate luteal phase indicated administration of testosterone. The result of biopsy are given in Table I.

It will be observed that in 3 cases the endometrium was in late secretory phase, and yet they did not concieve inspite of adequate progestogen therapy and therefore, testosterone was used apparently with good success.

Basal body temperature, urinary pregnandiol estimation and vaginal cytology are few amongst others which may also help in the diagnosis of inadequate luteal phase. In the present study, however, systematic record of these observation was not maintained and as such our main parameter was based on endometrial biopsy.

Type of Infertility

Majority of cases, 32 (84.22%) were primarily infertile, while 6 (15.78%) had secondary infertility. Of those who had

secondary infertility, 5 had history of previous abortion in the first trimester. Abortion and infertility may both be due to inadequacy of corpus luteum. Subsequent conception with testosterone and successful management with gestanin in early months of current pregnancy sufficiently prove the possible existence of luteal deficiency in these cases.

Age

Thirty-three cases in the series were below the age of 30, at least 5 (13.16%) were in the age group of 31 to 35 years. This suggests that testosterone is essential for luteotrophic activity throughout the reproductive career of women irrespective of age.

Duration of Infertility

This table adequately suggests the effectivity of testosterone therapy in cases who remained infertile for quite a long time. Eighteen cases (47.4%) were successfully treated with testosterone who failed to conceive for 5-10 years following their marriage (Table II).

TABLE II
Duration of Infertility

Duration in years	No. of cases	Per cent
2 to 3 years	7	18.4
3 to 5 years	13	34.2
5 to 10 years	18	47.4

Interval between First Visit and Conception

The efficacy of the therapy is further corroborated by the short interval within which these patient conceived since their initial vist to our clinic. Eighteen women (47.38%) became pregnant within one year while 15 (39.47%) concieved within two years and 5 after two years.

Number of Cycles for which Testosterone was used before Conception Occurred

It will be observed from this Table that 35 cases (92.11%) conceived during or at the end of first course of therapy. Only 3 (7.89%) needed second course (Table III).

TABLE III
Duration of Therapy

No. of cycles for which the drug was used	No. of cases	Per cent
One cycle	2	5.26
Two cycles	11	28.95
Three cycles	22	57.90
More than three cycles	3	7.89

Previous Treatment for Infertility

Besides routine investigations, 26 cases had some sort of treatment, either medical or surgical, for the cure of infertility. Being encouraged with the results of testosterone therapy, we have instituted this treatment in 12 cases directly following preliminary investigations. In these 12 cases, we have been successful but there are others where we have failed and we believe that cause of infertility in these 'failure' cases must be something else in addition to inadequate corpus luteum phase (Table IV).

TABLE IV
Previous Treatment for Infertility

Type of treatment	No. of cases	Per cent
Gestanin or Duphaston in luteal phase	23	60.55
Gilliams	3	7.89
No. treatment	12	31.56

Pregnancy Outcome

The outcome of pregnancy and labour are detailed in Table V.

TABLE V
Pregnancy Outcome

Outcome of Pregnancy	No. of cases	Per cent
Normal delivery	22	56.28
Forceps delivery	8	21.49
Casarean section	4	10.73
Abortion	1 +	2.63
Undelivered as yet	4 +	7.89

Figures marked asterisk have overlapped. The one, who had abortion, subsequently concieved with another course of testesterone therapy and is yet to deliver.

There was no untoward manifestation during pregnancy except in one case who had abortion. She was not treated with gestanin in the earlier months of pregnancy. She did not conceive for 18 months after her previous abortion, but subsequently conceived following another course of testosterone tablets. This time she conceived following the first cycle therapy. Gestanin in heavy dose was administered during the first trimester and she has reached 32 weeks at the time of this report. This case illustrates the possible existence of hypoluteal phase, by which the two episodes of infertility and the previous abortion can be reasonably explained.

None of the babies had any stigma of congenital malformation or any evidence of virilization.

Comments

In about 20 per cent of infertile women who are curetted premenstrually, the endometrium displays some evidence suggestive of inadequate progesterone influence (Balin, 1967).

The usual treatment of such corpus luteum inadequacy is substitutional therapy with progesterone or progestogens. Palmar (1959) however, believes that administration of progestogens may improve the quality of secretory endometrium but the drugs suppress progesterone production by the ovaries. The effect primarily depends upon the speed with which the embryo, aided to embed by exogenous progesterone, can muster sufficient chorionic gonadotrophins to stimulate the corpus luteum to persist.

On the other hand, our experiments (Figs 1 and 2) suggest that androgens help to maintain the structural and functional integrity of the corpus luteum. Androgens, therefore, preserve and stimulate the corpus luteum to produce more progesterone necessary to build up an adequate secretory endometrium and thus prepare the bed for the fertilised ovum to be implanted. Further it appears (Mukherjee, 1974) that androgens not only exert a luteotrophic effect in the postovulatory phase but also antagonise the possible luteolytic activity of progesterone-prostaglandin complex. In hypoluteal phase, therefore, it is the androgen and not the progesterone which appears to be responsible for maintenance of corpus luteum from the time of fertilisation till the time of placentation.

The mechanism by which testosterone exerts luteotrophic action is not very clear. It is difficult to surmise whether it is a direct effect on the ovary or whether hypothalamic-pituitary-gonadal axis is also involved for this luteotrophic activity on the corpus luteum.

However, it is known that testosterone increases the secretion of pituitary gonadotrophins (Loraine and Bell, 1971). Also it is known that testosterone reduces the responsiveness of the ovaries to pituitary gonadotrophins (Diczfalusy, 1962). At the present state of our knowledge it seems difficult to comment on whether the luteotrophic effect of testosterone is exerted directly or whether the increased secre-

tion of gonadotrophin or altered ovarian responsiveness to gonadotrophins are also involved. The last effect is a possibility since there is some evidence that LH might have some luteolytic action.

The therapeutic effect of testosterone in the treatment of infertility associated with inadequate luteal phase has been corroborated by the success achieved in 38 cases reported in this series. Existence of inadequate luteal phase in these cases was proved by at least two biopsies in two cycles supported in a few cases by either shortened elevated or a discordant post-ovulatory basal body temperature chart.

In some cases, even if the endometrium is normal secretory, exogenous stimulation is indicated, because in these cases endometrium needs more than the customary quantity of endogenous progesterone to develop properly (Balin, 1967). Three cases in the present series had late secretory endometrium and they conceived with testosterone supplementation.

Five out of six cases of secondary infertility reported in this series had previous history of abortion. Abortion in these cases could presumably be due to inadequate luteal phase, the existence of which was corroborated by the quick response achieved following testosterone therapy. One case conceived after testosterone therapy but she aborted at twelve weeks because prophylactic progestogen was not advised in the earlier months of pregnancy. She remained infertile for 18 months and then conceived with the first course of testosterone tablets. This case strongly suggests the possible existence of hypoluteal phase and efficacy of testosterone for such defect.

While other treatments failed to induce pregnancy in these "apparently" normal infertile women, success was achieved during or at the end of first course of therapy in 35 out of 38 cases (92.11%). Eighteen of them (47.4%) had infertility for a period varying between five to ten years. These observations indicate the rational use of testosterone and further confirm that the cases reviewed in the present study did not conceive simply "by chance".

Although apparently novel, the general acceptance of treatment of female infertility by so-called "male sex hormones" must await further trial by more obstetricians with larger groups of cases. However, the dosage of androgen used is very much within the safe pharmacological requirements and uptill now, in thirty-four babies already delivered, there was no evidence of any abnormality.

Summary

1. The luteotrophic activity of testosterone has been suggested based on animal experiments and supported by trial in the postovulatory phase of normally menstruating women.

2. Testosterone not only helps to maintain the structural integrity of corpus luteum but also antagonises the luteolytic activity of progesterone-prostoglandin complex.

3. Based on these observations, therapeutic trial was conducted on 83 infertile women in whom infertility was believed to be associated with inadequate luteal phase.

4. Thirty-eight (45.7%) have conceived so far. Endometrial histology was the main parameter by which diagnosis of hypoluteal phase was confirmed.

5. History, duration of infertility, nature of previous treatment and the interval between testosterone therapy and conception were some of the other factors which corroborated existence of inadequate progesterone phase in these cases.

6. The dose of testosterone used was within safe pharmacological limits and no untoward effect was noticed in thirty-four new born babies delivered so far.

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References

1. Balin, H.: Quoted from "Diagnosis and treatment of Menstrual disorders and sterility" by S. L. Israel, 5th ed. Harper & Row Pub. London, P. 470, 1967.
2. Bell, E. T., Mukherjee, S. and Loraine, J. A.: J. Endocrinol. 28: 321, 1964.
3. Browne, J. B.: Biochem. J. 60: 85, 1955.
4. Diczfalusy, E.: Rec. Prog. Horm. Res. 18: 381, 1962.
5. Klopper, A., Michie, E. A. and Browne, J. B.: J. Endocrinol. 12: 209, 1955.
6. Loraine, J. A. and Bell, E. T.: In clinical Application of Hormone assays, E. & S. Livingstone, Edinburgh, 69, 1971.
7. Mukherjee, S., Bell, E. T. and Loraine, J. A.: J. Endocrinol. 31: 197, 1965.
8. Mukherjee, S.: Proceedings of International Conference of Family Planning I.M.A. New Delhi, P F 6, 1972.
9. Mukherjee, S. and Chakravarty, S.: Proceedings of the 5th Asia and Oceania Congress of Endocrinology Vol. I, Page 142, 1974.
10. Palmar, A.: Fertil. & Steril. 8: 220, 1959.
11. Parlow, A. F.: Fed. Proc. 17: 402, 1958.