

CLINICAL EXPERIENCE WITH GOSSYPOL IN NON-CHINESE MEN:

A FOLLOW-UP

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Abstract

Gossypol monoacetic acid was administered to 12 Brazilian volunteers. The initial dose was 20 mg daily for 4 months. The dose was then reduced to 60 mg weekly (20 mg three times weekly). A significant reduction in sperm motility was detected in all subjects. An increase in the number of immature cells in the ejaculate was also detected in all subjects. Severe oligospermia or azoospermia developed in all subjects at the end of the loading phase. Two years following discontinuation, 3 men were still azoospermic. Only 1 man who was azoospermic 2 years after discontinuation had a late (3 years) recovery. Two of the 3 men who were subjected to high spermatic vein ligation because of varicocele remained azoospermic 2 years after the operation. The third patient, who did not have the operation, also remained azoospermic. Of the 9 patients who recovered, 3 have fathered children during the last 2 years.

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## Introduction

Several attempts have been made to develop a male contraceptive based on steroidal formulations which inhibit gonadotropin secretion (1-4,7,8). Some of these steroid and steroid combinations induced azoospermia in most, but not in all, men treated. Some men developing oligospermia during treatment remain fertile as indicated by the occurrence of well-documented conceptions (5). In a review of 25 steroid regimens tested over a period of 7 years, it was concluded that none of the treatments appeared suitable for further development as a male contraceptive because of their incomplete effectiveness. Among the various compounds tested were megestrol acetate norethindrone, d-norgestrel, norgestrienone, gestrinone (R-2323) and medroxyprogesterone acetate (6). Other compounds which failed to induce azoospermia were cyproterone acetate (7), danazol (8) and testosterone enanthate (9).

While these studies were carried out in the West, in the East, Chinese scientists, following up on the finding that the yellow pigment of the cottonseed oil provokes sterility, were developing their own male contraceptive. The first clinical trials of gossypol in China were carried out in 1972 (10). The Chinese investigators reported that severe oligospermia with necrospermia or azoospermia developed in men taking gossypol. Following the pilot clinical trial, 14 provincial and municipal districts in various parts of China joined in a concerted effort to evaluate the contraceptive effectiveness of the drug. These expanded trials, which included 8,806 men, confirmed the major findings of the first study but brought into question the issues of reversibility and safety of the treatment. Approximately 10% of the subjects remained azospermic 6 months to 4.5 years following discontinuation of treatment. Fatigue, a disturbing side effect, occurred and appeared to be associated with hypokalemia; it affected about 10% of the Chinese patients (11). According to a more recent review of clinical data, which included the follow-up of 2,067 men who took gossypol for 6 months to several years, the incidence of hypokalemia was lower than that presented in the earlier report. Reversibility in these cases was only 73%.

In a group of 148 men who took gossypol in Nanjing from 1972 to 1977 as a contraceptive, 7 cases of hypokalemia transient paralysis, preceded by a period of fatigue, occurred (11). Administration of potassium salts at the fatigue stage, in order to cope with this undesirable side effect, appeared effective. No solution, however, has been suggested to restore spermatogenesis in those patients who remain azospermic after the treatment is discontinued.

Except for the uncertainty of reversibility and the potential to provoke hypokalemia, gossypol treatment appears to be free of side effects common to most steroidal treatments, such as weight gain, liver toxicity and loss of libido.

We have tried to repeat the Chinese experience on a small scale in a group of Brazilian volunteers in order to assess the effect of gossypol on a population, ethnically and culturally, different from the Chinese.

#### Patients and Methods

The study included 12 men selected out of a group of volunteers seeking sterilization in the Family Planning unit of our University Hospital here in Bahia. Selection was based on recently proven fertility, age between 25 and 40 years, freedom from urogenital disease and endocrinopathies. Patients having clinically detectable varicocele were not accepted. Blood chemistry determinations, which included complete blood cell count, cholesterol, glucose, triglycerides, acid phosphatase, urea, transaminase, sodium and potassium, were carried out before treatment and monthly during treatment. Sperm counts were performed twice before treatment (at approximately 1-month intervals) and every month during gossypol administration. Following discontinuation, sperm counts were performed monthly until reversal or lack of reversal was documented (16).

Luteinizing hormone releasing hormone (LHRH) response was investigated in all subjects before and during treatment. Two basal blood samples were collected at 30-minute intervals and then 100 mcg of LHRH was injected subcutaneously. Blood samples were collected every hour for an additional 3 hours in order to quantify the LH response. Leydig function was evaluated by measuring basal blood testosterone before treatment and monthly during treatment. Stimulation tests with hCG were carried out before treatment and 3 to 6 months after initiation of treatment. The test procedure was as follows: Basal blood testosterone was determined by radioimmunoassay on day 0 before the intramuscular injection of 2,000 I.U. of human chorionic gonadotropin (hCG). Testosterone levels were measured at 24-hour intervals for 4 days following the hCG injection.

Subclinical varicocele in patients who remained azoospermic 1 year after discontinuation of gossypol treatment was investigated by retrograde phlebography of the internal spermatic vein. The examination was performed on a tilting x-ray table under a fluoroscopy apparatus with television-image-intensifier photography. Catheterization of the right femoral vein was achieved under local anesthesia without additional premedication. The catheter

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was introduced over a guide wire with a flexible tip under television-image-intensifier fluoroscopy. Contrast material was injected soon after the catheter tip had reached the orifice of the left spermatic vein on the renal vein. The catheter was then withdrawn step-by-step while the patient carried out a forceful Valsalva maneuver. Visualization of the right spermatic vein was attempted by bringing the catheter tip to the orifice of that vein in the vena cava.

Gossypol monoacetic acid, generously supplied by our Chinese colleagues, was manufactured at the Institute of Materia Medica, Nanjing, China. One 20 mg tablet was given daily for 4 months. The dose was then reduced to 60 mg weekly (20 mg three times a week). This dose was maintained for 6 to 8 months, extending the total treatment duration to 10 to 12 months.

### Results

A significant ( $<0.005$ ) reduction in sperm motility was detected in all the subjects (Fig. 1). An increase in the number of immature cell forms in the ejaculate was also observed in all cases. By the end of the second month, sperm count was significantly reduced in most, but not in all, subjects. Severe oligospermia with few immotile spermatozoa or azoospermia developed by 4 months of daily gossypol administration (the so-called loading phase). Reduction to the 60 mg weekly regimen following the loading phase maintained inhibition of spermatogenesis, as illustrated in Figure 2. By the fourth month of treatment, all men were azoospermic or had only few immotile spermatozoa in the ejaculate. No significant change in semen volume was detected throughout the study period.

There was a rebound in the sperm count in 2 subjects for whom the dose was further reduced to 20 mg gossypol weekly. At the end of 2 months of weekly treatment with 20 mg gossypol, the rebound of sperm count amounted to 50% of the pretreatment level. By returning to the initial daily schedule, azoospermia was re-established in both men (Fig. 3).

Following discontinuation of gossypol treatment, sperm count returned to pretreatment levels in 6 men within 1 year. In 2 other men, sperm count rose to 50% of pretreatment levels and remained at this reduced level for 2 years after discontinuation. Motility was fully restored by the end of the second year. In 4 men, as long as 1 year following discontinuation of gossypol treatment, the sperm count remained zero (Table I). Retrograde phlebography was carried out on those 4 men. Bilateral varicocele was detected in 1 man, varicocele of the left spermatic vein in another and varicocele of the right spermatic vein in the

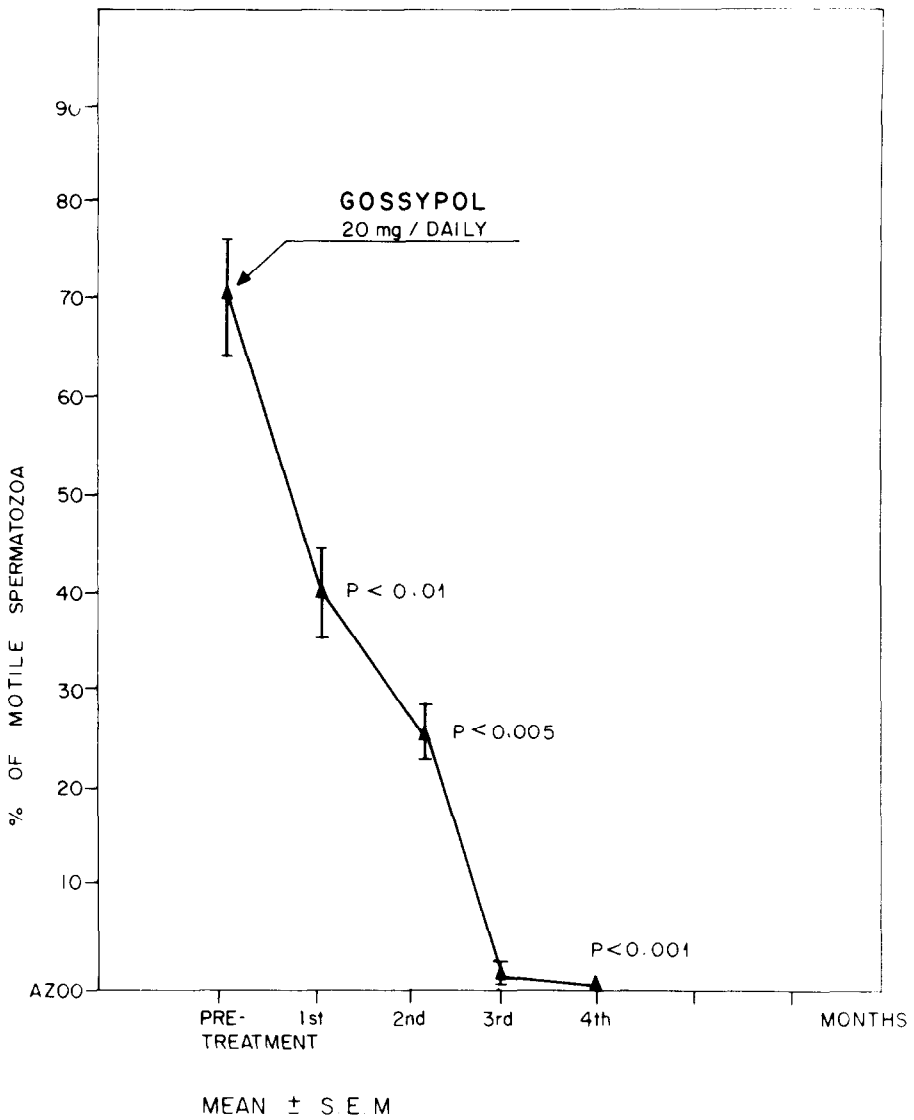


Fig. 1: Effect of gossypol on sperm motility. Note significant decrease in motility at the end of the first month of treatment. Values are mean  $\pm$  standard error (S.E.M.), n=12.

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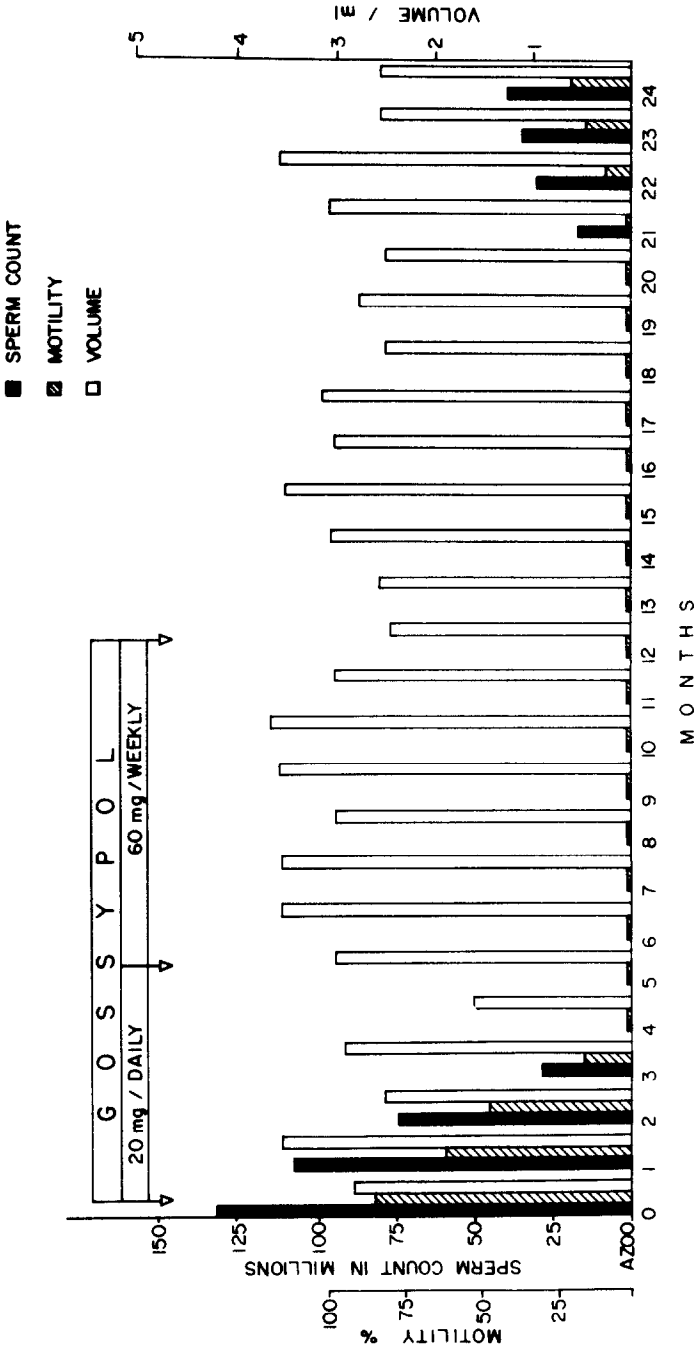


Fig. 2: Effect of gossypol on sperm count. Significant reduction is noted at 2 months. Azospermia occurs by the fourth month of treatment. Values are mean  $\pm$  S.E.M., n=10.

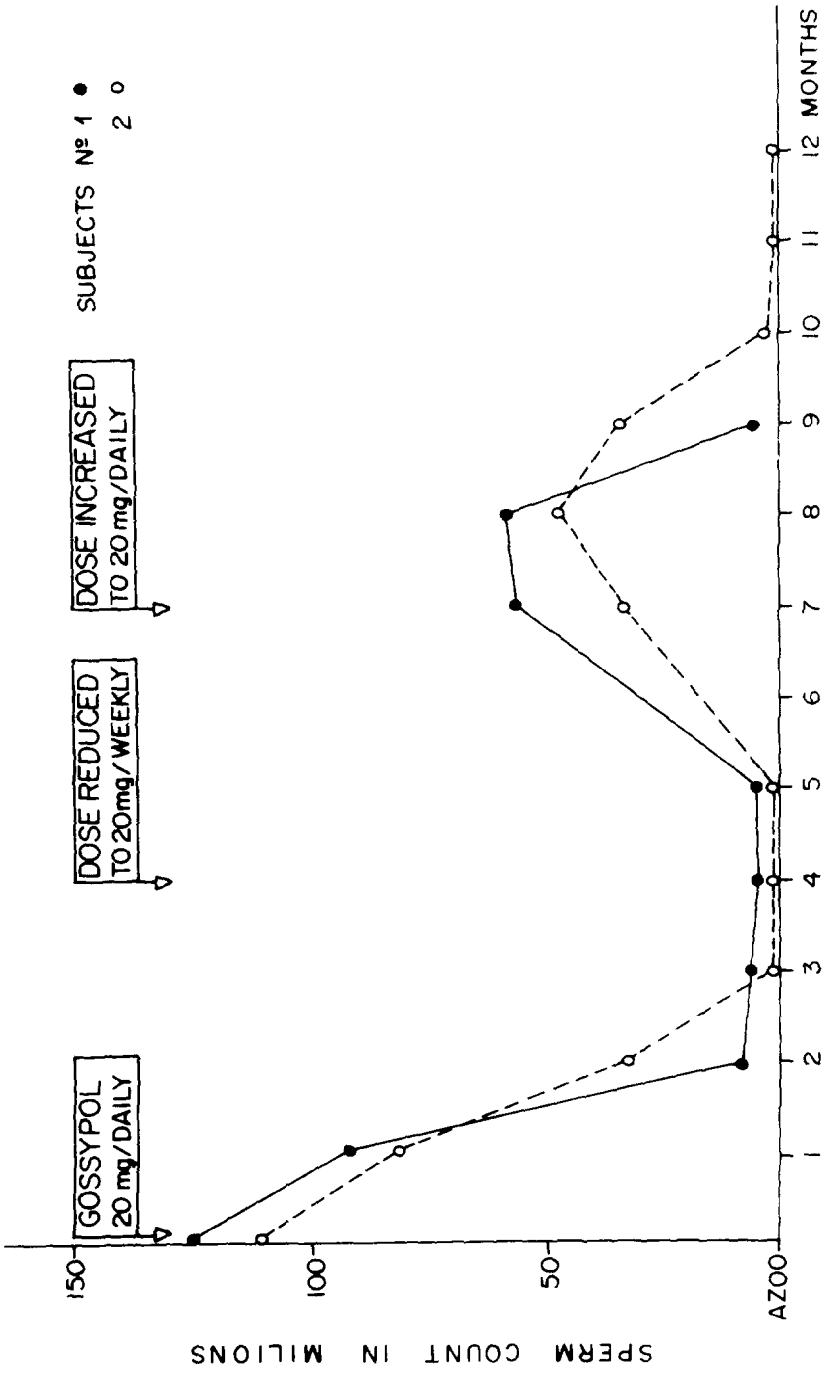


Fig. 3: Effect of gossypol on sperm count in 2 subjects. Note rebound on both sperm count and sperm motility soon after the dose is reduced to 20 mg weekly.

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Table I: Sperm concentrations in 12 subjects before, during and after gossypol therapy

SUBJECT NO.	SPERM COUNT IN MILLIONS/ML				
	BEFORE GOSSYPOL	THIRD MONTH	FOURTH MONTH	ONE YEAR AFTER DISCONTINUATION	
1	129	27	Azoo	42	
2	108	11*	Azoo	Azoo	
3	115	0	Azoo	106	
4	118	25	Azoo	122	
5	86	24	Azoo	Azoo	
6	88	10	0.2*	48	
7	123	28	Azoo	111	
8	95	26	0.5*	104	
9	91	22	Azoo	98	
10	108	0.2*	Azoo	Azoo	
11	87	0.8*	Azoo	77	
12	96	6.0	Azoo	Azoo	

\* Necrospemia



third one. In the fourth man, it was not technically possible to catheterize the spermatic veins. Figure 4 shows a typical venogram in one of these patients in whom catheterization was successful.

During gossypol treatment, no significant changes in basal testosterone levels occurred (Table II). Despite some individual variation, no statistically significant change was observed in the response to LHRH during gossypol treatment. A prompt rise in LH could be detected in all men following LHRH injection although the peak value tended to be below that of the controls (Fig. 5). The response to an hCG stimulation test was likewise unchanged during treatment. Here too, there was a tendency toward a lower response, but without statistical significance.

Blood levels of glucose, cholesterol, acid phosphatase, transaminases, triglycerides, urea, sodium and potassium remained within the normal range throughout the treatment period (Table II).

None of the 12 subjects reported changes in libido or in the frequency of intercourse. There were no significant changes in body weight or in blood pressure. Transient fatigue was reported by 1 patient.

Now, 2 years after the publication of our first paper, we have reviewed the patients who were azoospermic at the end of the first year follow-up review and found the following: Three of the 4 remain azoospermic. Only 1 patient revealed a progressive return to his original sperm count, a full 3 years after discontinuation of gossypol. Of the other 3, 2 were subjected to high spermatic vein ligation but, 2 years after the operation, they failed to recover their fertility, remaining azoospermic. The third patient declined the operation and remains azoospermic to the present.

#### Discussion

Our studies not only confirmed the Chinese experience but extended it, exposing the possible contribution of varicocele to the risk of irreversibility. In order to induce azoospermia in this selected group of volunteers, a daily dose of 20 mg of gossypol had to be maintained for 4 months. Although this time interval is slightly longer than that reported by the pioneering investigators in China, it should be noted that in the Chinese clinical trials, subjects were unselected and included older men in whom the response to gossypol could be more immediate (12).

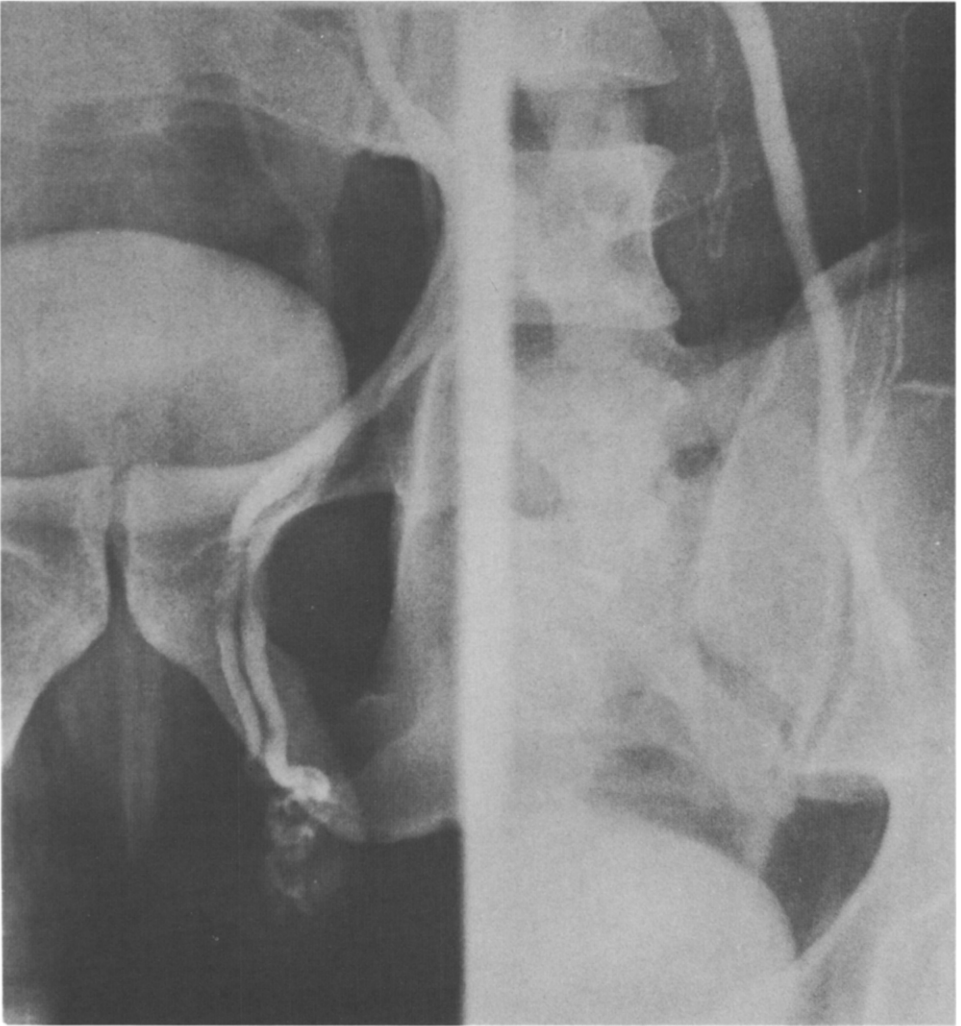


Fig. 4: Bilateral varicocele in 1 patient. Note reflux in spermatic vein.

Table II: Serum electrolytes, lipids, blood count and testosterone levels, prior to and during gossypol therapy

	Pre-Rx	1st Month	2nd Month	3rd Month	4th Month	5th Month	6th Month	12th Month
UREA mg/100 ml	16+1	22+2	22+2	32+2	30+2	29+4	31+3	19+5
GLUCOSE mg/100 ml	93+2	102+2	94+4	88+7	94+5	88+3	80+4	85+7
CHOLESTEROL mg/100 ml	196+15	184+18	194+27	193+26	187+10	206+13	230+15	220+20
TGO URF/ml	12+1	10+1	13+1	16+1	13+1	16+3	24+2	14+5
TGP URF/ml	11+1	11+1	10+1	17+3	14+1	13+2	17+2	12+3
ALKALINE PHOSPHATASE UBL	1.9+0.3	1.8+0.2	1.9+0.3	2.4+0.3	2.4+0.3	2+0.2	2.3+0.2	1.9+0.2
TRIGLYCERIDES mg/100 ml	114+16	93+8	122+25	143+23	107+16	118+12	107+25	110+10
SODIUM MEG/l	136+1	138+2	140+1	139+1	138+2	133+2	134+2	135+1
POTASSIUM MEG/l	4.2+0.1	4.2+0.1	4.3+0.1	4.3+0.3	4.2+0.3	4.6+0.1	4.3+0.2	4.3+0.2
RBC x 10 <sup>6</sup>	4.8+0.1	5+0.1	4.9+0.1	4.9+0.1	4.8+0.1	4.9+0.1	4.7+0.1	4.9+0.2
WBC x 10 <sup>3</sup>	7.6+0.6	7.4+0.5	7.1+0.6	7.3+0.3	7.7+0.3	7.3+0.3	7.7+0.4	7.5+0.7
TESTOSTERONE ng/ml	5.3+2	6.0+2.5	5.8+1.8	5.6+1.6	6.1+2.3	5.9+1.8	5.6+1.6	6.5+2.8

Mean + S.E.M.

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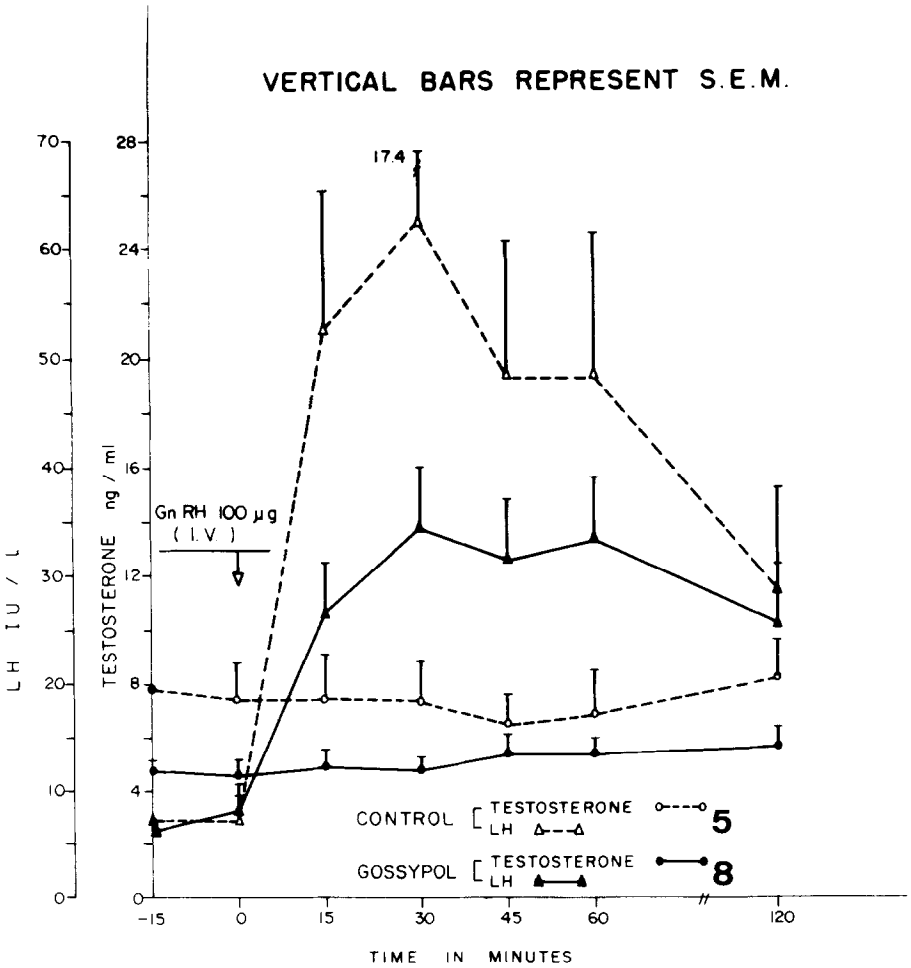


Fig. 5: LH levels following injection of LHRH (100 mcg) at time 0 in gossypol-treated and control men. No significant differences were noted between the 2 groups.

The finding of subclinical, radiologically diagnosed varicocele in 3 out of 4 men who did not have a restoration of sperm counts to pretreatment levels 1 year following discontinuation of gossypol use suggests a correlation between these two phenomena. Although it is difficult to explain how gossypol treatment could have contributed to cause valvular insufficiency of the spermatic veins and the resulting dilation of the pampiniform plexus, this possibility cannot be totally discarded. It seems more likely, however, that the vasculopathy at a subclinical level existed before the treatment started. It is sometimes not possible to diagnose varicocele and venous reflux by routine examinations as was carried out on all subjects prior to gossypol treatment. Moreover, in early stages, an obvious detrimental effect of varicocele on semen quality is observed in only 50% of patients with the condition (13).

That patients with varicocele failed to have a restoration of spermatogenesis after gossypol treatment is not completely unexpected. The altered blood circulation in the pampiniform plexus associated with varicocele has detrimental effects on testicular and epididymal functions. Sperm analyses of patients with varicocele reveal abnormalities of the tail and mid-piece development which suggest that maturation of spermatozoa in the epididymal duct does not take place normally. Later on, the spermatogenic epithelium degenerates, creating a pattern of testicular damage which is typical of varicocele. Degenerative and regressive lesions of the seminiferous tubules, interstitial tissue edema and abnormalities of the venules surrounding the tubules are usual findings. In advanced stages of varicocele-induced testicular damage, tubules may lose spermatogonia and retain only Sertoli cells (13). In some patients, even Leydig cell function may be affected with a corresponding reduction in testosterone secretion (14). A significantly higher incidence of varicocele than in the normal population has been reported for patients complaining of impotence (15).

In view of these anatomical and functional degenerative effects associated with varicocele and the changes this condition imparts to testicular circulation, it is possible that the germinal epithelium of patients with pre-existing varicocele may be particularly susceptible to long-term or even permanent damage following gossypol treatment. This possibility suggests the need for careful screening for varicocele before men desiring reversible spermatogenic arrest are treated with gossypol.

No significant alteration in Leydig cell function occurred during the prolonged gossypol treatment. The basal plasma testosterone level recorded throughout the treatment period was unchanged from normal. Testicular response to gonadotropin stimulation was not significantly below normal

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in gossypol-treated men. Pituitary responsiveness to hypothalamic-releasing factor appeared likewise to be maintained during gossypol treatment, as indicated by a response to LHRH stimulation not significantly below normal. Although not statistically significant, there was a tendency toward reduced responsiveness in both of these tests.

No significant change in plasma potassium values during the gossypol treatment period was detected in our patients. This is of particular note since it has been the occasional occurrence of hypokalemia in Chinese men treated with gossypol that has been the main reason for caution in the consideration of further clinical work with this interesting compound. The finding of normal potassium levels in Brazilian men throughout the 1 year of gossypol treatment suggests that more observations on non-Chinese men should be made.

Studies now in progress indicate that infertility may develop in gossypol-treated men well before the sperm count drops because of significant loss of sperm motility. The loss of motility may be achieved with lower doses and with no risk of permanent infertility since spermatogenesis is preserved.

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