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EFFECT OF GOSSYPOL, APOGOSSYPOL AND GOSSYPOLONE ON FERTILITY OF MALE RATS AND *IN VITRO* SPERM MOTILITY

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تاثيرات الجوسيبول و ابوجوسيبول و جوسيبولون علي خصوبة ذكور الفئران وحركة الحيوانات المنوية خارج الجسم

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تمت دراسة التاثيرات – داخل الجسم الحي – التي يحدثها الجوسيبول – وهي صبغة عديدة الفينولات من النواتج الطبيعية – ومشتقاتها ابوجوسيبول وجوسيبولون على الخصوبة في ذكور الفئران . كما درس أيضاً تأثير المركبات الثلاثة على حركة الحيوانات المنوية خارج الجسم الحي .

تأخر النمو الطبيعي للفئران المعالجة بواسطة جوسيبول وجوسيبولون بفروق احصائية واضحة مقارنة بالفئران المعالجة بواسطة ابوجوسيبول أو بحيوانات المجموعة الضابطة .

وقد أدى حقن الفئران بالجوسيبولون – ٥ ملليجرام / كيلو جرام من وزن الجسم – إلي حدوث نقص احصائي واضح لكل من عدد و حركة الحيوانات المنوية . في حين لم يظهر حقن ابوجوسيبول أي تاثير على الخصوبة ، إلا أنه أظهر تاثيراً على حركة الحيوانات المنوية خارج الجسم الحي فقط عند تركيز ٤٠ ملليجرام / ملليلتر مذيب . ويمكن اعتبار ابوجوسيبول مانع للخصوبة فقط في التركيزات المرتفعة ، وقد أظهر الجوسيبول تأثيراً متوسطاً بين الجوسيبولون – المركب عالي السمية – و ابوجوسيبول – الأقل في درجة السمية .

Key Words: Gossypol, gossypolone, and apogossypol, male contraceptive, spermicidal effect.

ABSTRACT

The effect of the naturally occurring polyphenolic pigment, gossypol, and some of its metabolites, apogossypol and gossypolone, on the fertility of male rats has been studied *in vivo*. The normal growth of the rats was significantly retarded in gossypol and gossypolone treated groups compared to either the controls or to apogossypol group. The *in vivo* effect of these compounds on sperm mobility was also examined. Gossypol acetic acid and gossypolone injection (5 mg/Kg body weight) decreased significantly the sperm count and sperm motility. Whereas apogossypol (5 mg/Kg body weight) showed insignificant effects on the sperm count and sperm motility. Gossypol and gossypolone decrease significantly the sperm mobility *in vitro* at different concentrations studied (5 up to 40 mg/ml saline). Apogossypol, only at high concentration (40 mg/ml) indicate inhibitory effect on sperm motility. Gossypol showed intermediate effect between the highly toxic compound, gossypolone, and the less toxic one, apogossypol.

INTRODUCTION

Gossypol [1,1',6,6', 7.7'-hexahydroxy -5,5' -diisopropyl-3,3, -dimethyl -(2,2'-binaphthalene)- 8,8'-dicarboxyaldehyde] is a polyphenolic compound (Fig. 1) isolated from cotton plant. It has been considered early to be a promising male contraceptive (National Coordinating Group 1978; Wang and Lei, 1979; Chang *et al.*, 1980). Gossypol is highly effective in reducing sperm count at a daily dosage of 20 mg. Gossypol probably acts directly at the level of the seminiferous tubules

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to induce oligospermia (Xue *et al.*, 1980). Gossypol does not affect the follicle stimulating hormone (FSH), luteinizing hormone (LH) or testosterone level (National Coordinating Group, 1978; Xue *et al.*, 1980). It inhibits the reproductive system and steroidogenesis in both sexes. It has been recently reported that gossypol inhibited human chorionic gonadotropins (hCG)-induced cyclic-AMP formation and progesterone secretion in cultured bovine luteal cell probably by suppressing steroidogenic enzyme activity (Gu *et al.*, 1990).

Although gossypol has been considered a very promising agent to induce infertility in animal species and in human male (National Coordinating Group, 1978; Xu *et al.*, 1990; Qian, 1985).

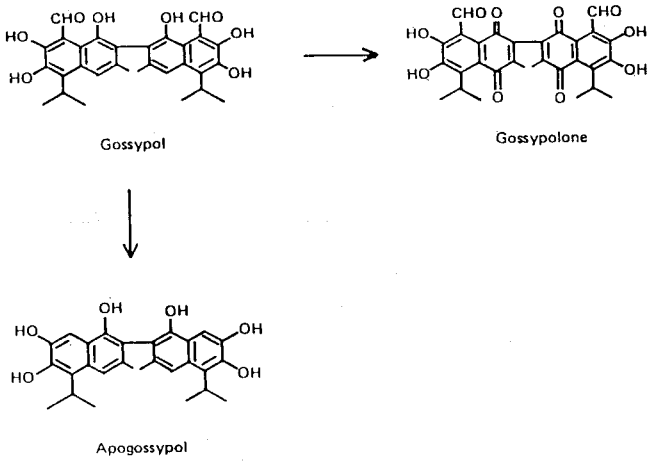

Fig. 1: Suggested gossypol metabolic pathways in the pig liver (Abou-Donia and Dieckert 1975).

The local effect of gossypol on germinal epithelium of the testis tissues was studied by several investigators (Hoffer, 1981; Shandilya *et al.*, 1982; Balwant *et al.*, 1986). They showed that the anti-spermatogenic effect of gossypol acetate is mediated within the germinal epithelium. *In vitro* studies showed that gossypol caused complete immobility of sperms at 40 mg/ml saline solution (Waller *et al.*, 1980).

Till now, little informations about the site(s) of action of gossypol or its metabolites. On the other hand, it was suggested that gossypol may be transformed biologically to apogossypol and gossypolone (Abou-Donia, 1976; Abou-Donia and Dieckert, 1975). In the present study, the effect of gossypol, gossypolone and apogossypol on body weight and fertility of male rats was studied. The drugs effect on motility of spermatozoa was also investigated *in vivo* and *in vitro*.

MATERIALS AND METHODS

Preparation of Gossypol and its Derivatives:

Gossypol was extracted from cotton seeds and crystallized as gossypol acetic acid. Recrystallized Gossypol acetic acid (GA) was obtained in the form of brilliant yellow crystals (m.p. 180-184° C, yield 0.25% of cotton seeds weight). Apogossypol was prepared from GA by the method of Lyman *et al.*, (1969). The melting point of the recrystallized AG was 235-240° C and the yield was 60% of GA. Also, gossypolone was prepared from GA according to the method described by Hass and Shirley (1965). Gossypolone m.p. was 254-258° C and the yield was 45% of GA. The chemical purity was assessed through depression of melting point, IR-and NMR-spectra. The data obtained agree with that reported in literature (O'Conner *et al.*, 1954; Nakanishi, 1962). The infrared absorption spectrum of apogossypol showed the absence of the aldehydic group as the intensity of the band at 6.2μ of that of gossypol was reduced. Also, the infrared spectrum of gossypolone showed a broad band of strong intensity at 5.95μ due to the presence of ketones (O'Conner *et al.*, 1954; Nakanishi, 1962). The spectra of the three drug preparations were identical with that of the authentic samples (Sigma Chemical Co., USA).

Animal and Treatment:

Adult male Sprague-Dawley rats were randomly housed eight rats per cage. The animals were maintained on 12 hours light/dark cycle at room temperature (25±2° C). Food and tap water were provided *ad libitum*. Rats were randomly assigned to one of four groups. control animals were injected with dimethyl sulphoxide (1 ml DMSO/Kg body weight). The animals were injected with gossypol acetic acid intraperitoneally (5 mg/ml DMSO/Kg body weight) and assigned as the group (GA). Group (AG) of rats were injected with apogossypol. Group (GN) of animals was treated in the same way with gossypolone at the same dose rate. The treatment was applied 5 days a week and up to seven weeks. Number of rats were removed each week, sacrificed 48 hours after the last injection. Animal samples were obtained to the seventh week from beginning of treatment. The coda and vas deference from each rat were obtained, minced in 1 ml Peterson and Freund (1971) medium, filtered through double 200 μm Nylon mesh to remove large membrance fragments. Sperm count and sperm motility were determined under light microscope (Cheesbrough, 1984).

In vitro study of spermicidal effect of gossypol, apogossypol and gossypolone:

Gossypol-PVP, apogossypol-PVP, and gossypolone-PVP co-precipitates were prepared by dissolving known weight of each drug in a minimum volume of diethyl ether, diluted with methanol to concentration of 10 mg/ml. On the other hand, Polyvinylpyrrolidone (PVP, M.Wt. 700000, BDH, U.K.) was dissolved in minimal amount of methanol. PVP solution was mixed with drug solution at a ratio of 4:1, (PVP-drug, w/w). Ether and methanol were removed using rotary evaporator until the volume was minimal and the co-precipitate having a fluffy consistency (Waller *et al.*, 1980). Working solutions of PVP, gossypol-PVP, apogossypol-PVP and gossypolone-PVP were obtained by diluting the respective co-precipitate with saline to one of the following concentrations; 5, 10, 20 or 40 mg/ml.

The activity was determined *in vitro* according to the method of Waller *et al.*, (1980). Human semen was obtained from volunteer donor freshly just before testing. Spermicidal activity was conducted by mixing 0.1 ml semen with 0.5 ml of the various test solutions. A drop of the mixture was placed on microscope slide and at least five fields were examined under light microscope for spermatozoa. Each test was carried out, as described, within one minute period. A second drop of the semen mixture was placed on a slide and observed for sperm mobility at five minutes time (from mixing time). The effect of each drug (at four different concentrations) on sperm mobility was measured at 1 and 5 minutes after drug addition. The control result was that for sperm mobility of 100% in PVP under the same conditions.

Statistics:

Results were expressed as mean ± S.D. for each group. The correlation coefficient between treatment period and body weight was determined. The variations between sperm motility (or sperm count) with drug type and treatment periods have been assessed using two-ways analysis (F-Test) compared with controls. Student, t-test, was used for computing the significance of *in vitro* spermicidal effect of drug compared with control experiments.

RESULTS

The effect of the drugs on male rat body weights:

Gossypol, gossypolone and apogossypol were i.p. administered into male rats at a dose rate of 5 mg/ml DMSO/Kg body weight. The animals were treated 5 days a week for 5 weeks. The body weight of the male rats was presented in Table 1. There were a significant positive correlations between the body weight and treatment period in controls and apogossypol group (+0.98 and +0.93, respectively). On the other hand, there was no significant correlation between time and body weight of rats treated with gossypol acetic acid or gossypolone.

Table 1
The effect of gossypol acetic acid (GA), apogossypol (AG) and gossypolone (GN) on the body weight of male rats.

Duration of experiment (number of rats)	Body weight (gm)*			
	DMSO	GA	AG	GN
1st week (4)	160.0±6.2	152.0±15.0	158.0±1.3	154.0±8.9
2nd week (4)	167.5±5.1	166.5±9.7	173.0±5.8	133.5±6.1
3rd week (4)	167.5±4.8	162.0±5.8	180.0±1.4	130.0±9.4
4th Week (4)	171.5±5.0	164.0±4.9	184.0±5.6	135.0±4.6
5th week (4)	174.5±5.8	166..0±7.4	184.0±7.5	140.0±4.6
6th week (4)	180.0±7.9	155.0±1.1	185.5±8.3	147.0±4.9
7th week (4)	180.0±4.1	156.0±3.3	198.0±14.7	150.0±1.6
Correlation coefficient (r)	+0.98**	-0.10	+0.93**	+0.2

* The values are expressed as Mean ± S. D.
** These values are statistically highly significant (P < 0.01).

The *in vivo* effect of drugs on the fertility of male rats:

Sperm count and motility of male rats were studied for animals treated with gossypol acetic acid, gossypolone and apogossypol.

The results in (Table 2 and 3) showed a significiant negative correlation between spermatozoa motility and treatment period, in GA group. The F-test showed a highly significant decrease ($p<0.01$) of both sperm count and sperm motility in GA and GN treated groups with respect to AG and control groups (Table 4).

The *in vitro* spermicidal activity of gossypol, apogossypol and gossypolone:

The *in vitro* effect of gossypol, apogossypol and gossypolone on sperm motility was presented in Table 5. There was a highly significant decreases in sperm mobility in gossypol and gossypolone groups (at the concentrations 5, 10, 20 and 40 mg/ml.). Apogossypol showed highly significnat decrease only at 40 mg/ml. Gossypol caused complete immobility at 40 mg/ml after five minutes. Gossypolone showed complete immobility of sperms at 20 and 40 mg/ml after 5 minutes.

Table 2
The *in vivo* effect of gossypol acetic acid (GA) apogossypol (AG) and gossypolone (GN) on the body weight of male rat sperm count.

Duration of experiment (number of rats)	Sperm count (million)*			
	DMSO	GA	AG	GN
1st week (4)	34.0±3.7	18.0±1.4	30.0±3.7	10.0±1.4
2nd week (4)	32.0±3.7	8.0±2.4	21.0±4.3	2.1±1.2
3rd week (4)	25.0±4.4	7.0±1.7	20.0±1.4	1.0±0.7
4th week (4)	25.0±1.4	10.0±1.0	22.0±2.4	--
5th week (4)	33.0±3.9	9.0±3.1	19.0±4.6	9.0±2.5
6th week (4)	38.0±1.6	10.0±1.6	25.0±4.1	5.0±1.0
7th week (4)	38.0±4.9	11.0±2.0	20.0±1.6	18.0±4.9
Correlation coefficient	+0.46	-0.32	+0.46	+0.46

* The values are expressed as Mean ± S. D.

Table 3
The *in vivo* effect of gossypol acetic acid (GA) apogossypol (AG) and gossypolone (GN) on male rat sperm count motility

Duration of experiment (number of rats)	Sperm Motility (%)*			
	DMSO	GA	AG	GN
1st week (4)	80.0±3.7	57.1±4.3	75.1±1.9	60.2±4.9
2nd week (4)	80.0±2.4	50.0±6.5	75.0±3.2	30.0±5.1
3rd week (4)	75.0±5.6	50.0±4.2	66.0±5.1	10.0±3.7
4th week (4)	72.0±1.4	30.0±4.2	60.0±2.8	00
5th week (4)	80.0±2.6	30.0±4.2	70.0±1.4	20.0±2.8
6th week (4)	85.0±0.8	40.0±1.6	75.0±4.1	50.0±8.2
7th week (4)	80.0±1.6	30.0±8.2	20.0±2.4	60.0±9.8
Correlation coefficient	+0.28	-0.82**	+0.05	+0.16

* The values are expressed as Mean ± S. D.
** The value is statistically significant (P < 0.05)

Table 4
The two way ANOVA's analysis for sperm count and sperm motility under the effect of gossypol acetic acid (GA) apogossypol (AG) and gossypolone (GN)

	F-value	Mean Value			
		DMSO	GA	AG	GN
Sperm count (million)	67.51**	32.0	10.4**	22.4	06.04**
Sperm motility (%)	25.08**	78.8	41.0**	70.86	32.86**

* These values are statistically highly significant compared to DMSO group (P < 0.01).

Table 5
The *in vitro* effect of difference concentrations of gossypol, apogossypol and gossypolone on human sperm motility (%).

Type of added compund[b]	Concentration of the added compund[a]							
	5 mg		10 mg		20 mg		40 mg	
	After 1 min	After 5 min	After 1 min	After 5 min	After 1 min	After 5 min	After 1 min	After 5 min
Polyvinyl-pyrolidone (PVP)	66.6±4.2	57.5±5.0	62.2±8.2	45.0±4.2	60.4±8.6	42.8±6.4	59.6±6.5	36.2±6.6
Gossypol-PVP Co-precipitate	37.7±5.27**	23.6±5.5**	26.8±9.6**	15.8±5.8**	8.8±4.0**	6.0±0.89**	5.8±2.5**	0.0**
Apogossypol-PVP Co-precipitate	59.8±6.94	45.0±7.7*	53.2±11.1	30.4±9.4*	41.2±10.5*	9.0±1.6*	15.0±7.1**	6.0±1.4**
Gossypolone-PVP Co-precipitate	40.0±7.1**	25.0±8.6**	25.0±8.9**	12.5±6.4**	13.2±3.4**	0.0**	7.6±1.4**	0.0**

a Values expressed as Mean ± S.D.
b These compund (5 mg, 10 mg, and 40 mg) are dissolved in PVP and 0.5 ml of each concentration was added to 0.1 ml. semen.
* These values are statistically significant ($P < 0.05$) compared to PVP-group.
** This values are statistically highly significant ($P < 0.01$ compared to PVP-group.

DISCUSSION

Much interest has been aroused dealing with the use of gossypol as male anti-fertility agent. Clinical trials were performed in China on over 800 men supported by animal studies. These studies confirmed the efficiency of the compound as a potential male antifertility drug, but also, showed up some side effects in minority of cases (National Coordinating Groups, 1978). Moreover, little is known about *in vivo* metabolism of gossypol, as well as, the role of these metabolites on its biological function. Abou-Donia and Dieckert (1975) suggested that gossypol biotransformation in animal precludes in two different pathways. In the first one, gossypol is decarbonylated to apogossypol, and in the second one, gossypol is oxidized to gossypolone (Fig. 1). However, apogossypol and gossypolone could not be detected in Cotton seeds. Trails to isolate apogossypol and gossypolone from the cotton seeds failed completely.

The effect of gossypol acetic acid on the body weight of male rats was controversially interpreted. Several workers (Hahn *et al.*, 1981, Balwant *et al.*, 1986; and El-Habet *et al.*, 1986) found that the body weight decreases under the effect of gossypol acetic acid while others demonstrated that the body weight was not affected (Wang *et al.*, 1984; Gafvels *et al.*, 1984). El-Habet *et al.*, 1986 attributed the effect of gossypol on body weight to its catabolic effect on the liver.

In the present study, the normal growth of the animals was suppressed under the effect of gossypol acetic acid and gossypolone compared to the controls (DMSO). At the same time, the body weight of the animals of apogossypol group was increased in a highly significant way ($p < 0.01$) relative to the control rats. Gossypol acetic acid and gossypolone were found to be highly significant antifertility agents ($P < 0.001$) compared to DMSO and apogossypol. The results of gossypol acetic acid agree with that of the others (National coordinating Group on Male Fertility Agents, 1978; Nadakavukaren *et al.*, 1979; Zhao and Zhang, 1988). The antifertility of gossypolone emphasized the suggestion of Abou-Donia and Dieckert (1975) that gossypol can be transformed to gossypolone in the animal.

The antifertility effect of gossypol acetic acid and gossypolone may be mediated within the germinal cells and the environmental factors (ATP, ATpase, Lactate dehydrogenase). The local effect of gossypol acetic was demonstrated by many investigators (Nadakavukaren *et al.*, 1979; Chang *et al.*, 1980; Hoffer, 1981; Shandilya *et al.*, 1982 and Balwant *et al.*, 1986). They showed that the anti-spermatogenic effect of gossypol acetic acid is mediated withing the germinal epithelium. On the other hand, other investigators have reported that gossypol acetic acid may has an effect on sperm maturation within the epididyms (Bozek *et al.*, 1981; Hadley *et al.*, 1981). Environmental factors for sperms may alter the rate of ATP generation or utilization (Mitchell *et al.*, 1976). ATP is the main energy source available for spermatozoal motility. Mitchell *et al.*, 1976 found that Na^+/K^+-activated, Mg^{++}-dependent transport ATPase is apparently situated on the sperm cell membrane.

The sperm motility was studied *in vitro* under the effect of gossypol (Waller *et al.*, 1980). It was found that gossypol-PVP co-precipitate caused complete immobility of sperms at 40 mg/ml saline solution. In the present work, gossypol-PVP, apogossypol-PVP and gossypolone-PVP co-precipitate effect on sperm motility was studied *in vitro* at different concentrations. Gossypol and gossypolone showed highly significant decrease ($P<0.01$) of the sperm mobility at 5, 10, 20 and 40 mg/ml saline solution, while apogossypol showed highly significant decrease of the sperm mobility ($P<0.01$) only at 40 mg/ml saline. On the other hand, apogossypol showed no effect on the motility of sperms of male rats at low concentration (*in vivo* and *in vitro*). Yet, it has a significant effect on the sperm mobility at a high concentraiton (*in vitro*). Accordingly, the significant effect of carbonyl group in gossypol and the carbonyl group with ketone in gossypolone may be linked with the environmental enzymes. On the other hand, gossypolone effect steroidogenesis probably by reduction of itnracellular c-AMP (Gu and Lin, 1991). Gossypolone inhibits not only 3β-hydroxysteroid dehydrogenase, but also side-chain clivage enzyme complex activity (Gu *et al.*, 1991). Apogossypol which has tertiary alcohol groups may increase the alkalinity of the environmental medium for sperms causing significant decrease of sperm mobility at high concentration.

Further studies should be performed to established the decrease of the hepatotoxic effect of gossypol. The fate of the studied derivatives in the spermatogenesis process needs more attentiaon as probable anti-fertility agents.

REFERENCES

Abou-Donia, M.B., 1976. Physiological effect and metabolism of gossypol. Residue Reviews, 61: 125-160.

Abou-Donia, M.B., and **J. W. Dieckert, 1975.** Metabolic fate of gossypol: The metabolism of ^{14}C-gossypol in swine. Toxicol. Applied Pharmacol., 31: 32-39.

Balwant, A., H. Franck and **A. Winston, 1986.** Morphologic and endocrine changes in the reproductive organs in rats implanted with gossypol acetate pellet in the testis. J. Andrology, 7: 254.

Bozek, S.A., D. R. Jensen and **J. N. Jone, 1981.** Scanning electron microscope study of spermatozoa from gossypol treated rats. Cell Tissue Res., 219-659.

Chang, M.C., G. U. Zhiping and **S. K. Saksena, 1980.** Effects of gossypol on the fertility of male rats, hamsters and rabbits. Contraception, 21: 461-469.

Cheesbroug, M., 1984. Medical laboratory Mannual for tropical countries. volume II: Microbiology, Tropical Health Technology/Butterworths.

El-Habet, A.E., A. S. El-Sharaky and **S. M. El-Sewedy, 1986.** Effect of gossypol on liver metabolic enzymes in male rats. J. Med. Res. Inst., 7: 163-176.

Gafvels, M., J. Wang, A. Bergh, J. E. Damber and **G. Selstam, 1984.** Toxic effects of the antifertility agent gossypol in male rats. Toxicology, 32: 325-333.

Gu, Y., P. K. Li, Y. Rikihisa and **R. W. Brueggemeier, 1991.** Gossypolone supresses progesterone synthesis in bovine luteal cells. J. Steroid. Biochem. Mol. Biol., 38: 709-715.

Gu, Y. and **Y. C. Lin, 1991.** Suppression of adrenocorticotropic hormone (ACTH)-induced corticosterone secretion in cultured rat adrenocortical cells by gossypol and gossypolone. Res. Commun. chem. Pathol. Pathol. Pharmacol., 72: 27-38.

Gu, Y., Y. C. Lin and **Y. Rikihisa, 1990.** Inhibitory effect of gossypol on steroidogenic pathways in cultures bovine luteal cells. Biochem. Biophys. Res. Commun., 169, 455-461.

Hass, R.H. and **D. A. Shirley, 1965.** The oxidation of gossypol. II. Formation of gossypolone with ferric chloride. J. Organ. chem., 30: 4111-4113.

Hadley, M.A., Y. C. Lin and **M. Dym, 1981.** Effects of gossypol on the reproductive system of male rats. J. Androl., 2, 190.

Hahn, D.W., C. Rusticus; A. Probst, R. Homm and **A. N. Johnson, 1981.** Antifertility and endocrine activities of gossypol in rodents. Contraception, 24: 97-105.

Hoffer, A.P. 1981. Light and electron microscopic studies on the effects of gossypol in the male rat. In: Zatuchini, G. and Osborn, C (eds): Gossypol a possible male antifertility agent. Report of workshop Research. Frontiers in Fertility Regulation, Paper number, 4: 1.

Lyman, C.M., J. T. Cronin, M. M. Trant and **G. V. Odell, 1969.** Metabolism of gossypol in the chick. J. Amer. Oil Chem. Soc., 46: 100.

Mitchell, J. A., L. Nelson and **E. S. E. Hafez, 1976.** Motility of spermatozoa. In: Hafez, E.S.E. (ed): Human semen and fertility regulation in men. p. 23, The C.V. Mosby company/Saint Louis.

Nadakavukoren, M.J., R. H. Sorensen and **J. N. Tone, 1979.** Effect of gossypol on the ultrastructure of rat spermatozoa. Cell Tissue Res., 204: 293-296.

Nakanishi, K., 1962. Infrared Absorption Spectroscopy. Holden Day, Inc., San Francisco.

National Coordinating Group On Male Antifertility Agents, 1978. Gossypol-a new antifertility agent for males. Chinese Med. J., 6: 417-428.

O'Connor, R.T., P. Von Der Haar, E. F. DuPre, L. E. Brown and **C. H. Pominski, 1954.** The infrared spectra of gossypol. J. Amer. Chem. Soc., 76: 2368.

Peterson, R.N. and **M. Freund, 1971.** Factors affecting fructose utilization and lactic acid formation by human semen. The role of glucose and pyruvic acid. Fertil. Steril., 22: 639-652.

Qian, S.A., 1985. Gossypol-Hypokalimia Interrelationship. Advance in Fertility Regulation in the Male. (Shao-Zhen Qian and M.H. Waites Geoffrey, Editors). The People's Medical Publishing House, Beijing, 1985, p. 9.

Shandilya, L., T. B. Clarkson; M. R. Adams and **J. C. Lewis, 1982.** Effects of gossypol on reproductive and endocrine functions of male cynomolgus monkeys. Biol. Reprod., 27: 241-252.

Waller, D.P., L. J. D. Zaneveld and **H. H. S. Fong, 1980.** *In vitro* spermicidal activity of gossypol. Contraception, 22: 183-187.

Wang, J.M., C. H. Gu; Z. M. Qian and **G. N. Jing, 1984.** Effect of gossypol on testicular blood flow and testosterone production in rats. J. Reprod. Fertil., 71: 127-133.

Wang, N.G. and **H. P. Lei, 1979.** Antifertility effect of gossypol acetic acid on male rats. Chin. Med. J., 59: 402.

Xu, D., W. J. Cai, B. H. Zhu, C. J. Dong, Z. C. Zheng and **Z. Q. Gao, 1988.** Clinical safety and long-term administration of gossypol in 32 cases. Contraception, 37: 129-136.

Xue, S.P., D. C. Liang, P. R. Fei, X. M. Chen, S. J. Ye, Y. Liu, Y. W. Wu, M. M. You and **X. Y. Guo, 1983.** Subcellular site of antispermatogenic effect of gossypol and its possible molecular mechanism of action. Sci. Sin (B)., 26: 614-633.

Zhao, X. and **Y. Zhang, 1988.** Effect of gossypol acetate on serum testosterone. Shengzhi Yu Biyun, 8: 66-69.