Clinicopathological Studies On Gossypol and Infertility In Friesian Cows

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ABSTRACT

Twenty-six Friesian cows were divided into 3 groups to elucidate the deleterious effect of gossypol on some blood-, biochemical parameters, besides the induced lesions in the liver, kidneys, ovaries and uterus. Moreover, their impact on fertility was considered. Gp.(1) was the control (5 cows). They were fed on a balanced ration. The remaining 21 cows were daily supplied by 12 Kg concentrated ration per cow for 3 month, then one cow was slaughtered at the end of the third month. The remaining 20 cows were equally divided into two groups (gps.2&3). Gp.(2) was IM injected with 5 ml receptal per cow with cystic-ovaries, and IM injected with folliigon (1000 IU) for each cow with smooth inactive ovaries. The animals were artificially inseminated after treatment. Gp.(3) was treated by changing the ration to a balanced one till the signs of heat appeared, and the animals were artificially inseminated. Blood samples were collected from the jugular vein of all animals, 3 month from the beginning of the experiment and after 48 hrs. post- insemination. The serum was separated for biochemical analysis. Specimens were collected from the liver, kidneys, uterus and ovaries of the slaughtered cow at the end of the third month. The specimens were fixed in 10% neutral buffered formalin. Five micron-thick paraffin sections were prepared, stained with H&E and examined microscopically.

The AST, ALT, ALP, total protein, albumin, globulin, total bilirubin, direct and indirect bilirubin were significantly increased. The renal function parameters revealed increased BUN and creatinine besides hypophosphatemia, hypokalemia and normal sodium level. Hypercalcemia with decreased estrogen and progesterone were found. All the changed parameters returned to their normal levels after providing the cows with the hormonal and nutritional treatment. Hepatic, renal and ovarian lesions were found.

It could be concluded that feeding cottonseed-meal to Friesian cows for a long period caused hepatic, renal and ovarian lesions together with hormonal disturbance. The majority of those changes disappeared after the hormonal and nutritional treatment. It is recommended to minimize or avoid the cottonseed-meal for the Friesian cattle.

INTRODUCTION

Gossypol seems to disrupt the estrous cycle and pregnancy in cattle. Moreover, it may interfer with the hormonal regulation of the ovary besides inducing a cytotoxic effect on the uterus and embryo *(1)*.

Serum biochemical changes

Elevated plasma concentration of gossypol, plasma total protein, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in mature dairy cows fed excessive amounts of cottonseed-meal for 9 weeks *(2)*. Dairy cows, given 35% and 55% whole cotton- seed for 3 weeks; revealed high levels of urea, creatinine, total bilirubin, calcium, alanine aminotransferase, alkaline phosphatase, total protein and albumin in addition to a low level of serum phosphorus *(3)*. Rabbits, on a diet supplemented with 5% cottonseed cake, revealed a high level of serum alanine aminotransferase *(4)*. High values of alanine aminotranferase, serum total protein and serum total bilirubin with a normal value of serum aspartate amino- transferase were found in lambs, given a high amount of free gossypol for 30 days *(5)*. Beef heifers, fed on diets containing gossypol (8.2 or 16.3 g/day) for 62 day, revealed a high plasma concentration of the liver enzymes in addition to a low potassium-ion concentration *(6)*. Increased levels of aspartate aminotransferase, total protein, creatinine and albumin, but the level of blood urea decreased in lactaining cows, daily fed 15% whole cottonseed-meal containing 23 gm free gossypol for 2 weeks *(7)*. The clinicopathological data, obtained from lambs given 15% and 30% cottonseed cake for 30 and 60 day respectively, revealed high values of plasma total protein, globulin, albumin and urea *(8)*.

Infertility studies

The mean serum progesterone concentration was not affected in heifers, treated with gossypol *(6)*. Gossypol-acetic acid was not responsible for the endocrine imbalance and disturbance in the ovarian hormone secretion *(6)*. The progesterone-production by the cultured bovine luteal cells was hindered in the presence of gossypol *in vitro*, and the gossypol acetic acid inhibited the conversion of cholesterol and pregnenolone to progesterone *(9)*.

Gossypol can cause a reduction in fertility and decrease the sperm number in cows and bulls *(10)*.

Pathological changes

Hepatic vascular dystrophic and necrotic lesions were observed in sheep, given cotton-husks, containing gossypol *(11)*. Anasarca and hepatic congestion were reported in gossypol-intoxicated ruminants *(1)*.

MATERIAL AND METHODS

I. Material

1. Experimental animals

Twenty-six healthy Friesian cows (four year old), belonged to a military farm,* were put under hygienic conditions.

They were given balanced rations and observed for three weeks before starting the experiment.

2. Drugs

a. Receptal (Hoechst), a synthetic hormone that releases luteinizing hormone (LH) and follicle stimulating hormone (FSH) from the anterior lobe of the pituitary. Each 10 ml bottle contains 0.0042/ml buserelin acetate, imported by the Egyptian Company for Drug Trading.

b. Folligon (intervet), pregnant mare serum gonadotrophin hormone, (PMSG), 1000 or 5000 I.U. The serum gonadotrophin per vial, was imported by the Egyptian Company for Drug Trading.

3. Ration

a. A balanced ration was given during the green season. It consisted of 30 kg clover, 6 kg concentrated ration and 2 kg wheat-straw *(12)*.

b. The balanced ration, in the dry season, consisted of 5 kg drees, 30 kg darawa, 6 kg concentrated ration and 5kg wheat-straw *(12)*.

c. The concentrated ration included 30% cottonseed-meal, 2% calcium carbonate, 1% sodium chloride, 1% mineral mixture; 26% rice-straw and 40% maize *(12)*.

II. Methods

1. Experimental design

Twenty-six Friesian cows were divided into 3 groups . Gp.(1) was the control (5 cows). It was fed on a balanced ration. Twenty-one Friesian cows were daily supplied with 12 kg concentrated ration /animal for 3 months; then one animal was slaughtered . The remaining 20 cows were equally divided into gps. (2&3). Gp.(2) was intramuscularly injected with 5 ml receptal for each cow with cystic ovaries, and intramuscularly injected with folligon (1000 I.U.) for each cow with smooth inactive ovaries. The animals were artificially inseminated after the treatment. Gp.(3) was put on a balanced ration till the appearance of the signs of the heat (6-8 week), then artificially inseminated. All cows were examined for pregnancy by rectal palpation.

2. Sampling

Five ml blood-samples were drawn from the jugular vein of each cow , 3 month after the beginning of the experiment, and 48 hr after insemination. The collected blood was left to coagulate, then centrifuged in centrifuge tubes. The serum was separated for the biochemical studies.

3. Clinical biochemistry

All the biochemical testes were performed using test-kits of bio-Merieux-France.

The serum aspartate transferase (AST) and serum alanine aminotransferase (ALT) were determined *(13)*. The serum alkaline phosphatase was measured *(14)*. The serum total protein *(15)*, and albumin *(16)* were determined. The serum globulin was

calculated as the difference between the total protein and albumin. The serum total bilirubin and direct bilirubin were estimated *(17)*. The indirect bilirubin and blood-urea-nitrogen were determined *(18)*. The serum creatinine *(19)*, calcium *(20)*, inorganic phosphorus *(21)*, sodium *(22)* and potassium *(23)* were determined. The serum estrogen and progesterone were measured by radioimmunoassay (RIA) kits, obtained from the Gamma Trade Company *(24)*.

4. Histopathology

Specimens were collected from the liver, kidneys, ovaries and uterus, from the slaughtered cow. The specimens were examined macroscopically, then fixed in 10% neutral buffered formalin. Five micron thick paraffin sections were prepared. stained by H&E and examined microscopically *(25)*.

RESULTS

A. Liver function tests

Cows, fed cottonseed-meal for 3 months, showed a highly significant increase in the AST, ALT and ALP at the ratios of 24.95% , 38.49% and 50.77%, respectively. Moreover,a significant increase in the globulin and bilirubin (total , direct and indirect) was encountered at the ratios of 10.31%, 18.75%, 19.05% and 18.18% respectively, in addition to insignificant increase in the total protein and albumin (Table 1). A highly significant increase in the ALP, total protein and globulin was observed at the ratios of 43.42%, 41.69% and 86.91% respectively (gp.2). A significant increase in the total, direct and indirect bilirubin at the ratios of 12.50%, 9.52% and 18.18% was respectively encountered in addition to an insignificant change in the other liver function tests (Table 1). Gp. (3) showed a highly significant increase in the ALP and indirect bilirubin at the ratios of 34.66% and 63.64% respectively. Moreover, a significant increase in the globulin and total bilirubin was found at the ratios of 9.19% and 18.75%, in addition to insignificant change in the other liver function tests (Table 1).

B. Renal function tests

Cows, fed gossypol for 3 months, revealed a highly significant increase in the urea, creatinine and calcium levels at the ratios of 49.40% 90.58% and 16.31% respectively, and a highly significant decrease in the phosphorus and potassium at the ratios of 30.42% and 43.11% respectively, in addition to an insignificant change in the serum sodium (Table 2).

Gp. (2) showed a highly significant increase in the creatinine at the ratio of 36.23%, and a significant increase in the potassium at the ratio of 9.19% in addition to insignificant change in the other renal function parameters (Table 2). Gp (3) showed a significant increase in the creatinine and potassium at the ratios of 17.39% and 13.13% respectively, in addition to insignificant change in the other renal function parameters (Table 2).

c. Hormonal results

Cows, fed gossypol for 3 months, showed a highly significant decrease in the estrogen and progesterone at the ratios of 48.68% and 69.42% respectively. Gps.(2&3) revealed a highly significant decrease in the progesterone at the ratios of 54.85% and 82.04% respectively after the hormonal and nutritional treatments, in addition to an insignificant change in the estrogen level (Table 2).

3. Pathological findings in the slaughtered cow

Congestion of the hepatic blood vessels and centrolobular veins were observed. Centrolobular coagulative necroses, infiltrated with lymphocytes, were seen (fig.1). The necrotic areas were irregularly replaced with blood (fig. 2). The portal tracts were infiltrated with lymphocytes and macrophages. The hepatic parenchyma was focally replaced by aggregations of lymphocytes. Periportal hydropic degeneration was seen (fig. 3).

The kidneys showed severe congestion of the medullary blood vessels and intravascular hemolysis, besides brown pigments (fig.4). Periglomerular hyalinized fibrous tissue was observed. The Bowman's capsule was thickened and the glomerular cavity was dilated with edematous eosinophilic fluid which extended among the capillaries of the

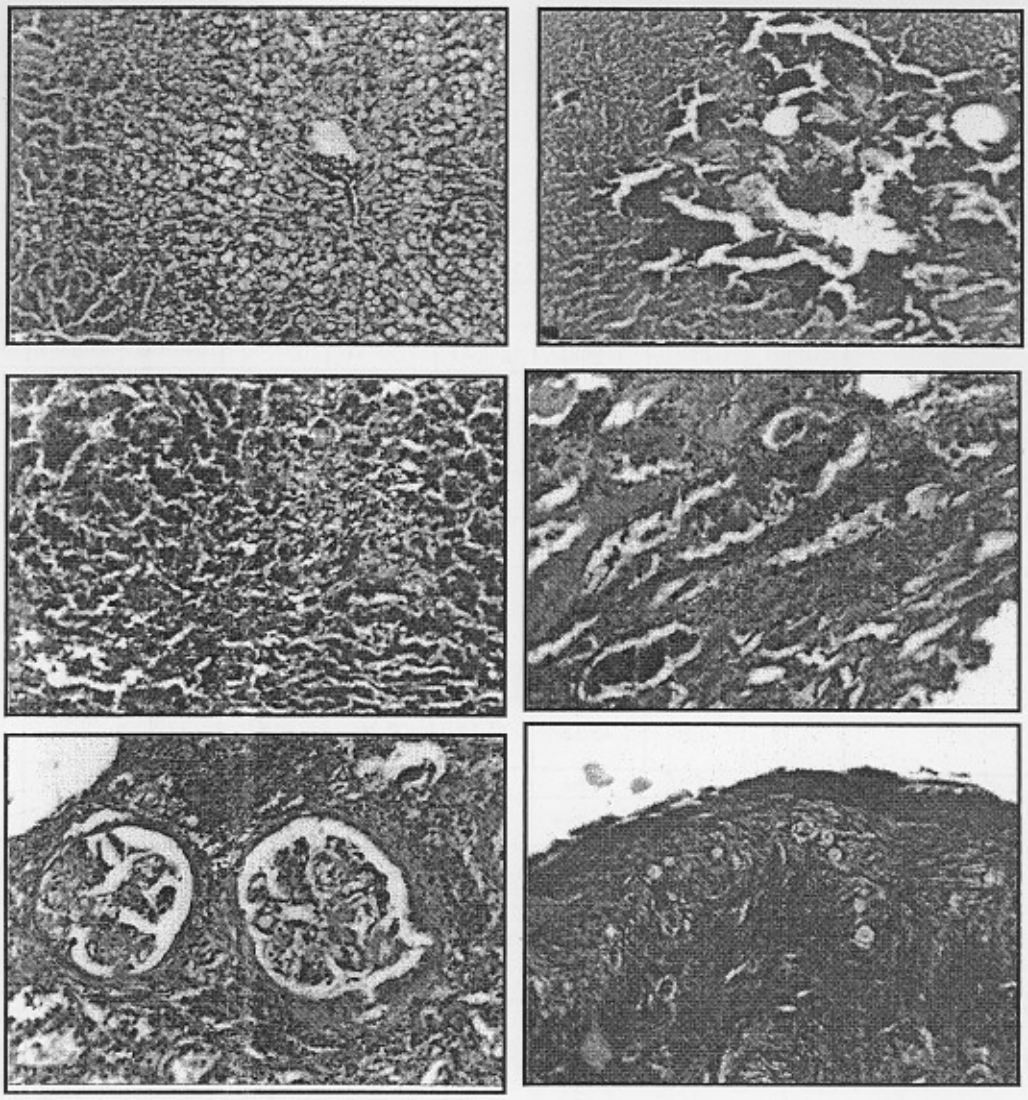
glomerular tufts (fig. 5). The ovarian capsule was thickened and the primordial follicles were atretic, where their ova were absent (smooth ovaries) (fig. 6). The stromal fibrous connective tissue was proliferated. Some ovaries showed few cystic graafian follicles besides degenerated and necrotic granulosa-cell-layer. The uterus was microscopically normal.

Table 1. Liver function tests (mean values ±S.E) in Friesian cows,given gossypol before and after treatment.

Groups	AST (U/ml)	ALT (U/ml)	ALP (U/ml)	TP (gm/dl)	Alb (gm/dl)	Glob (gm/dl)	TB (mg/dl)	DB (mg/dl)	Ind B (mg/dl)
Control (1)	60.61	15.12	12.35	7.46	3.87	3.59	0.23	0.21	0.11
	± 0.95	± 0.22	± 0.27	± 0.07	± 0.02	± 0.06	± 0.01	± 0.002	± 0.01
Tested animals (before treatment)	75.73	20.94	18.62	7.91	3.95	3.96	0.38	0.25	0.13
	± 1.14**	± 0.8**	± 0.37**	± 0.08	± 0.12	± 0.17*	± 0.01*	± 0.01*	± 0.01*
% dif	24.95	38.49	50.77	6.03	2.07	10.31	18.75	19.05	18.18
Gp. (2)	59.53	15.08	17.7	10.57	3.86	6.71	0.36	0.23	0.13
	± 1.01	± 0.45	± 0.54**	± 3.37**	± 0.02	± 3.36**	± 0.01*	± 0.01*	± 0.01*
% dif	-1.78	-0.26	43.32	41.69	0.26	86.91	12.5	9.52	18.18
Gp. (3)	57.63	14.91	16.63	7.81	3.89	3.92	0.38	0.2	0.18
	± 1.4	± 0.24	± 0.3**	± 0.13	± 0.02	± 0.13*	± 0.01*	± 0.01	± 0.01**
% dif	-4.92	-1.39	34.66	4.69	0.52	9.19	18.75	-4.76	63.64

Table 2. Renal function tests (mean values ± SE) in Friesian cows gived gossypol before and after treatment and hormonal changes

Groups	Urea (mg%)	Creat (mg%)	Ca (mg%)	P (meq/l)	Na (meq/l)	K (meg/l)	Estrogen (pg/dl)	Progesterone (mg/ml)
Control	23.34 ±0.52	1.38 ±0.04	11.10 ±0.36	5.26 ±0.05	143.91 ±0.32	4.57 ±0.10	7.21 ±0.52	2.06 ±0.32
Tested animals (before treatment)	34.87 ±0.77**	2.63 ±0.10**	12.91 ±0.17**	3.66 ±0.15**	133.53 ±1.12	2.60 ±0.12**	3.70 ±0.1	0.63 ±0.06**
% dif.	+49.40	+90.58	+16.31	-30.42	-7.21	-43.11	-48.68	-69.42
(gp.2)	23.85 ±1.12	1.88 ±0.05**	11.08 ±1.04	5.61 ±0.11	147.47 ±1.13	4.99 ±0.08*	7.21 ±0.33	0.93 ±0.3**
% dif.	+2.19	+36.23	-0.18	+6.65	+2.47	+9.19	-0.00	-54.85
(gp.3)	23.36 ±1.02	1.62 ±0.08*	10.98 ±0.20	5.73 ±0.20	146.80 ±1.58	5.17 ±0.12*	7.40 ±2.64	0.37 ±0.06**
% dif.	+0.086	+17.39	-1.08	+8.94	+2.01	+13.13	+2.64	-28.04



Figs. (1-6): Cow fed gossypol for 3 month, then slaughtered.

Fig. 1-Liver showing centrolobular coagulative necrosis infiltrated with lymphocytes. H&E., X 300.
Fig. 2- Liver showing irregular blood spaces replacing necrotic areas. H&E., X 150.
Fig. 3- Liver showing periportal hydropic degeneration. H&E.,. X 300.
Fig. 4- Kidney showing severe congestion of the medullary blood vessels with hemolysis. H&E., X 300.
Fig. 5- Kidney showing thickened Bowman' capsule which is dilated with eosino- philic material. Hyalinized periglomerular fibrous tissue. H&E.,.,.X 300.
Fig. 6- Ovary with a smooth surface, thickened wall and atretic primordial follicles. H&E.,. X 150.

DISCUSSION

Nutrition is intimately related to fertility, in farm animals. Serious breeding problems have been attributed to gossypol in the food of farm animals *(1)*.

The current study showed that cows, fed gossypol for 3 months, suffered hepatic damage, which was elucidated clinically and histopathologically. The increase in the aspartate aminotransferase (AST), alanine aminotranferase (ALT) and alkaline phosphatase (ALP) enzymes indicated that a liver damage was produced by the gossypol. Hisopathologically, the liver showed centrolobular coagulative necrosis and congestion.

The increased levels of the total protein, albumin and globulin, in the present investigation, after feeding gossypol, may be due to the high level of protein in the ration. Similar results were previously reported *(7&8)*. The increase in the bilirubin, after using gossypol may be attributed to its toxic effect on the liver and hemolytic effect on the erythrocytes *(2)*. The current findings are in accordance with the above mentioned pathogenesis as congestion of the hepatic blood vessels , degenerative changes and centrolobular hepatic necrosis were found in the present work.

Our results are in agreement with others *(5)*, who found increased levels of urea and creatinine, after using gossypol. Such deviation may be due to the effect of gossypol on the kidneys. The current investigation revealed congestion, degeneration and hyaline renal casts. Also, the increase in the urea may be due to the high protein content in the concentrated ration (in the tested animals fed on gossypol). Our results are in agreement with other studies *(7,8)*.

The hypercalcemia, seen in the present work after gossypol administration, may be due to the feeding of the concentrated ration (containing calcium carbonate) or the metabolism of calcium and phosphorus which are closely related. Hypophosphatemia is always accompanied by hypercalcemia. Similar results were previously obtained *(3)*. The hypercalcemia and hypophosphatemia, due to gossypol toxicosis,are partially in agreement with others *(26)*.

Insignificant change in blood-sodium was previously reported *(27)*. They stated that there was no significant relationship between fertility of dairy cows and blood concentration of sodium. The hypokalemia, seen in the present work, may be due to low potassium level in the ration *(6)*.

The decreased estrogen, seen in the present work after feeding gossypol, may be due to the inhibition of the ovarian estrogenesis as a result of gossypol administration. This result was confirmed histopathologically by multiple cystic graafian follicles, smooth ovaries and degenerated granulosa-cell layer. Similar results were previously obtained *(28)*. The progesterone level was decreased, after feeding gossypol, as a result of inhibited conversion of cholesterol and pregnenolone to progesterone by gossypol. Similar results were obtained by other workers *(9,29,30)*. Gossypol increased percentages of dead sperm and abnormal sperm and reductions in sperm concentration, total sperm output, percentage incidence of forward motility and total functional sperm fraction *(31)*. The disturbed ovarian hormonal levels get along with the current histopathological findings of cystic follicles, degenerated and necrotic granulosa-cells, besides smooth ovaries.

Most-blood parameters regained their control level after the hormonal and nutritional treatment. The parameters,which were still deviated, may need a longer time to restore their control level.

It could be concluded that feeding cottonseed-meal for a long time caused hepatic, renal and ovarian damage together with hormonal disturbance in the Friesian cattle. The majority of these changes regained their normal status, .after the hormonal and nutritional treatment. It is recommended to minimize or avoid the cottonseed-meal for the Friezian cattle.

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الملخص العربى

دراسات باثولوجية إكلينيكية على الجوسيبول كسبب للعقم فى الأبقار الفريزيان

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**مدير إدارة الخدمات البيطرية بالقوات المسلحة

لقد أصبح من الضرورى مع زيادة السكان إيجاد مصادر لزيادة الإنتاج الحيوانى ودراسة المشاكل التى تعترض هذا الهدف.

الغذاء يلعب دوراً رئيسيا وهاما فى هذا المجال ومن المكونات الغذائيه المعروفة كسب بذرة القطن ولقد ثبت ان مادة الجوسيبول توجد فى بذرة القطن و تؤثر تأثيرا كبيراً على خصوبة الحيوانات ولذلك أجرى هذا البحث فى محاولة لدراسة تأثيرها على بعض الوظائف الباثولوجية الإكلينيكية (الهرمونات والأنزيمات والأملاح) بالآضافه الى التغيرات المرضيه المرافقه فى الأبقار الفريزيان.

أجرى هذه الدراسة على ٢٦ من الأبقار الفريزيان بمزرعة ٦ أكتوبر بالقوات المسلحة عمرها ٦ عام وأثبتت نتائج الفحوص الإكلينيكية نشاط المبايض مع خلوها من الأمراض , قسمت هذه الحيوانات الى ٣ مجموعات

الأولى ٥ حيوانات وأعتبرت المجموعة الضابطة وتم تغذيتها بالعليقة المتوازنة طوال فترة التجربة. باقى الابقار غذيت على عليقه مركزه ٣٠% كسب بذرة القطن بواقع ١٢ كجم لكل حيوان لمدة ٣ شهر ثم ذبحت بقره وبقى ٢٠ قسمت الى مجموعتين (٢،٣).

المجموعة (٢) وعددها ١٠ حيونات وتم معالجتها هرمونياً

(folligon ,Receptal) ثم لقحت صناعياً وأخذت منها عينات دم بعد التلقيح الصناعى بـ ٤٨ ساعة .

المجموعة (٣) وعددها ١٠ حيوانات تم تغذيتها على العليقة الطبيعية ومتابعتها حتى ظهور الشبق عليها بصورة طبيعية ثم لقحت صناعياً وأخذت منها عينات دم بعد التلقيح الصناعى بـ ٤٨ ساعة٠

ثم أخذ عينات دم من جميع الحيوانات بعد ثلاثة شهور وبعد ٤٨ ساعة من التلقيح الصناعى (تم بعد المعالجة سواء هرمونياً أو بتعديل العليقة) لتعيين معدلات الاسبارتات أمينوترانسفيراز – الألانين أمينو ترانسفيراز – الألكالين فوسفاتيز – البروتين الكلى – الألبيومين – الجلوبيولين البلليروبين الكلى والمباشر وغير المباشر – اليوريا – الكرياتينن - الكالسيوم – الفوسفور – الصوديوم البوتاسيوم بالإضافة إلى تقدير مستوى هرمون الاستروجين والبروجستيرون ٠

وأسفرت هذه الدارسة على النتائج الآتية :

وجد بالفحص الأكلينيكى أن بعض الحيوانات مصابه بتحوصل فى المبيض والبعض الآخر بخمول فى المبيض. لوحظ طول الفترة بين دورات الشبق مع ضعف الشبق فى بعض الحيوانات ثم أختفاء الشبق بعد شهرين فى حالات خمول المبايض وبعض حالات تحوصل المبيض. وجد أيضا استطالة دورات الشبق مع عدم انتظامها فى بعض حالات تحوصل المبيض٠

تغيرات السيروم شملت زيادة فى الاسبارتات امينوترانسفيريز الألانين أمينو ترانسفيريز والألكالين فوسفاتيز والبلليروبين كما وجود زيادة فى البروتين الكلى الألبيومين والجلوبيولين بعد التغذية على كسب بذرة القطن وأيضا وجد زيادة فى نسبة اليوريا والكرياتينين ونقص فى الفسفور والبوتاسيوم ونقص غير معنوى فى الصوديوم بالأضافة إلى زيادة الكالسيوم بعد التغذية على كسب بذرة القطن أما التغيرات فى الهرمونات فكانت نقص فى مستوى الاستروجين والبروجستيرون٠

بعد العلاج الهرمونى وبتعديل العليقة وجد ان معظم هذه التغيرات عادت إلى المستوى الطبيعى وكان هناك تغيرات اخرى ربما تحتاج إلى وقت أطول لكى تعود للمستوى الطبيعيى٠

أما التغيرات فى الأنسجة فكانت فى الكبد : أحتقان للاوعية الدموية الكبدية والوريد المركزى بالإضافة إلى التورم الغيمى والتنكس الفجوى والمائى ببعض الخلايا الكبدية , كما لوحظ تورم غيمى وتنكس فجوى ومائى بالنبيات الكلوية كما لوحظ وجود ارتشاح للخلايا الليمفاوية حول الكبيبات الكلوية لوحظ وجود العديد من أكياس جراف المتحوصلة بالمبيض ولوحظ وجود بويضات غير ناضحة داخل أكياس جراف وفى بعض الحالات كانت البويضة غير موجودة (المبيض أملس)٠

نستخلص من هذه التجربة أن التغذية على كسب بذرة القطن لمدة طويلة يسبب تغيرات فى الكبد والكليتين و أضطراب الهرمونات فى الأبقار الفريزيان بالاضافة إلى تغيرات فى المبايض , اختفت معظم هذه التغيرات بعد العلاج الهرمونى أو بتعديل العليقة٠

ينصح بتحجيم كمية كسب بذرة القطن او بمنعها فى عليقة الابقار الفرزيان.