

Effects of gossypol from cottonseed meal and dietary vitamin E on the reproductive characteristics of superovulated beef heifers^{1,2}

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ABSTRACT: Superovulated Hereford-Angus crossbred heifers (average 397 kg BW) were used to test the effect of feeding cottonseed meal (gossypol) and vitamin E on embryo quality and ovarian characteristics. Twenty-four heifers were assigned randomly to four treatments with six heifers per treatment. Treatments were the following dietary supplements: 1) SBM (soybean meal + 30 IU vitamin E/kg of diet DM); 2) SBM+E (soybean meal + 4,000 IU vitamin E · animal⁻¹ · d⁻¹); 3) CSM (cottonseed meal + 30 IU vitamin E/kg of diet DM); and 4) CSM+E (cottonseed meal + 4,000 IU vitamin E · animal⁻¹ · d⁻¹). Supplements based on cottonseed meal provided 43.5 g of total gossypol/d (37% negative isomer (-) and 63% positive isomer (+)). Blood samples were collected at the start of the experiment and every 3 wk thereafter up to 12 wk. Plasma α -tocopherol (α -T) concentration was affected by treatments ($P < 0.05$). Heifers supplemented with cottonseed meal had greater ($P < 0.05$) α -T concentration in plasma than

heifers supplemented with soybean meal at each concentration of vitamin E. Supplementation at 4,000 IU vitamin E · animal⁻¹ · d⁻¹ increased ($P < 0.05$) the concentration of α -T in plasma. Weight gain, hemoglobin and hematocrit were not affected by treatment. Erythrocyte osmotic fragility (EOF) increased ($P < 0.05$) in cottonseed meal-fed animals; however, EOF was lowered ($P < 0.05$) with vitamin E supplementation. Heifers fed CSM and CSM+E supplements had greater ($P < 0.01$) concentrations of (-), (+), and total-gossypol in plasma, corpora lutea (CL), liver, and endometrium than heifers fed SBM and SBM+E supplements. Tissue α -T concentration increased with increased dietary supplemental vitamin E, particularly in great amounts in the CL. Because there was no adverse effect of gossypol on superovulation response or embryo development despite concentrations of gossypol in endometrium that are toxic to embryos, it is likely that systems exist in the reproductive tract to limit gossypol toxicity.

Key Words: Cattle, Cottonseed Oilmeal, Gossypol, Vitamin E

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Introduction

Effects of gossypol on male bovine reproduction have been well characterized (Chenoweth et al., 1994; Vel-

asquez-Pereira et al., 1998a). In dairy cows, feeding diets containing gossypol (42.7 g free gossypol · animal⁻¹ · d⁻¹) resulted in lower hemoglobin, greater erythrocyte osmotic fragility, and death (Lindsey et al., 1980). There is also some evidence that gossypol causes infertility. Gossypol inhibited nuclear maturation of cultured bovine oocytes (Lin et al., 1994) and reduced development of bovine embryos (Zirkle et al., 1988). In a more recent study, Brocas et al. (1997) reported that development was reduced when embryos were cultured with 5 or 10 μ g/mL gossypol. Wyse et al. (1991) found that feeding 5.0 g of free gossypol · animal⁻¹ · d⁻¹ from cottonseed meal increased the number of degenerative embryos collected from superovulated Brangus heifers.

The deleterious effect of gossypol is thought to involve the generation of free radicals (de Peyster et al.,

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Table 1. Composition of dietary supplements fed to Hereford-Angus crossbred heifers for 90 d

Item	Supplement			
	SBM	SBM+E	CSM	CSM+E
Offered, kg/d ^a	4.40	4.40	4.40	4.40
DM, %	88.00	88.00	88.00	88.00
CSM, % ^a	0.00	0.00	61.60	61.60
SBM, % ^a	47.52	47.52	0.00	0.00
Ground Corn, % ^a	36.96	36.96	22.88	22.88
Limestone, % ^a	1.76	1.76	1.76	1.76
Trace minerals, % ^a	1.76	1.76	1.76	1.76
Vitamin E, IU/kg ^b	28.16	682.88	38.72	628.32
Total gossypol (AOCS), %	0.00	0.00	0.76	0.78
Free gossypol (AOCS), %	0.00	0.00	0.12	0.12
(+)-gossypol, %	0.00	0.00	55.62	55.44
(-)-gossypol, %	0.00	0.00	32.38	32.56

^aDry matter basis.

^bDry matter basis. Analysis of a composite sample from all mixing dates.

1984; Fornes et al., 1993); therefore, vitamin E as free radical reducing agent may counteract the effect of gossypol. The objectives of this experiment were to evaluate the effect of cottonseed meal at a rate to provide 14 mg free gossypol (FG)/kg BW per day on embryo quality and ovarian characteristics of superovulated beef heifers and to test whether feeding supplemental vitamin E counteracts these possible negative effects that gossypol may have on embryo quality and ovarian characteristics.

Materials and Methods

Animals, Diets, and Management. Twenty-four Hereford-Angus crossbred heifers averaging 397 kg BW and 20.7 mo of age were used in a 90-d experiment. Animals were assigned randomly to one of four dietary supplements. Diets were as follows: 1) **SBM** (soybean meal + 30 IU vitamin E/kg of diet DM), 2) **SBM+E** (soybean meal + 4,000 IU vitamin E · animal⁻¹ · d⁻¹), 3) **CSM** (cottonseed meal + 30 IU vitamin E/kg of diet DM); and 4) **CSM+E** (cottonseed meal + 4,000 IU vitamin E · animal⁻¹ · d⁻¹). Supplements based on cottonseed meal provided 43.5 g of total gossypol/d (37% negative isomer (-) and 63% positive isomer (+)) (Table 1 and 2). Vitamin E was provided as all *rac*- α -tocopheryl acetate. Diets were analyzed for α -tocopherol (Table 1) and were formulated to provide equal amounts of CP and TDN and to meet NRC (1996) nutritional requirements. Heifers were fed the supplement (Table 1) daily via Calan gates and had free access to water and poor-quality mature bermudagrass (*Cynodon dactylon*) hay with an α -tocopherol (α -T) concentration of 9.1 IU/kg DM. The protocol for all heifer procedures had been approved by the University Animal Use Committee.

Blood and Tissue Sampling and Analyses. Blood was collected every 3 wk via jugular venipuncture with an 18-gauge needle into heparinized vacutainer blood collection tubes for a total of five collections. Blood

was analyzed for erythrocyte osmotic fragility (**EOF**), hemoglobin, and hematocrit. Erythrocyte osmotic fragility, measured as percentage hemolysis, was evaluated in a 0.65% buffered saline solution as described by Risco et al. (1993). Hemoglobin was determined using a colorimetric procedure (Sigma Chemical, St. Louis, MO). Hematocrit was determined using a microhematocrit centrifuge (IEC MB Centrifuge, Needham Heights, MA).

Blood was centrifuged for 25 min at 700 × g, and plasma was removed and stored at -20°C until analyzed for α -T (all collections) and gossypol isomers (last collection). At the end of the experiment, animals were slaughtered, and portions of liver, corpus luteum (CL), endometrium, and muscle (psoas major) were collected and frozen at -20°C until analyzed for α -T, (+)- and (-)- gossypol isomers, and total gossypol. α -Tocopherol was determined following the procedure described by Njeru et al. (1992) for plasma and by Njeru et al. (1995) for tissue and feed samples.

Samples of plasma, tissues, and supplements were shipped on dry ice to Texas A & M University Agricultural Center at San Angelo, TX, for gossypol analyses. High performance liquid chromatography procedures were used to determine concentrations of (+)- and (-)-gossypol isomers in plasma, tissues, and feed supplements (Velasquez-Pereira et al., 1998 a,b,c). Free and total gossypol (TG) concentrations in the supplements were also determined by AOCS (1985 a,b).

Superovulation and Embryo Collection and Quality. After 70 d of receiving diets, superovulatory treatment was performed following the procedure described by Garcia-Bojalil et al. (1994). Heifers were slaughtered on d 6 to 7 after AI. At slaughter, whole reproductive tracts were recovered and brought to the laboratory, where embryos were flushed with a known amount of Dulbecco's PBS (Gibco, Grand Island, NY). The recovered flush was filtered and embryos recovered for quality assessment.

Table 2. Intake of gossypol (total, free, and enantiomers) by beef heifers consuming supplements containing cottonseed meal and vitamin E

Item	Supplement ^{a,b}			
	SBM	SBM+E	CSM	CSM+E
Total gossypol (AOCS)				
g/d	0.0	0.0	43.0	44.0
mg/(kg BW × d) ^c	0.0	0.0	101.4	103.8
Free gossypol (AOCS)				
g/d	0.0	0.0	7.0	7.0
mg/(kg BW × d) ^c	0.0	0.0	16.5	16.5
Gossypol (HPLC)				
(+)-gossypol				
g/d	0.0	0.0	27.2	27.7
mg/(kg BW × d) ^c	0.0	0.0	64.2	65.4
(-)-gossypol				
g/d	0.0	0.0	15.8	16.3
mg/(kg BW × d) ^c	0.0	0.0	37.3	38.4

^aSBM = soybean meal + 30 IU vitamin E/kg; SBM+E = soybean meal + 4,000 IU vitamin E · animal⁻¹ · d⁻¹; CSM = cottonseed meal + 30 IU vitamin E/kg; CSM+E = cottonseed meal + 4,000 IU vitamin E · animal⁻¹ · d⁻¹.

^bThere were no main effects or interactions ($P > 0.05$).

^cCalculated using an average 424 kg BW heifer over the entire trial.

Statistical Analyses. Plasma and weight data were analyzed by repeated measures analysis of variance in a completely randomized design using the GLM procedure of SAS (SAS Inst., Inc., Cary, NC). The Greenhouse-Geiser Epsilon was used to determine significant levels for the F -test. Analyses of variables containing single or calculated observations were performed by ANOVA (SAS Inst., Inc.) on a 2 × 2 factorial arrangement of treatments (two levels of both vitamin E and gossypol).

Results and Discussion

Animal Performance and Blood Parameters. The intake of total and free gossypol is shown in Table 2. Gossypol intake in excess of 14 mg FG kg⁻¹ BW · d⁻¹ affects metabolic and reproductive parameters of bulls (Velasquez-Pereira et al., 1998a). To achieve an intake in excess of 14 mg FG kg⁻¹ BW · d⁻¹, supplements had a high CP concentration (32% CP) that was not representative of typical diets. This high concentration of dietary CP could have affected the absorption and metabolism of gossypol by supplying a greater number of free ϵ -amino groups for gossypol to combine with in the digestive tract and then be excreted and(or) by facilitating the catabolism and detoxification of the absorbed gossypol (Abou-Donia, 1989).

Initial weight, final weight, and ADG did not differ among heifers fed the four supplemental diets (Table 3). Velasquez-Pereira et al. (1998b) also did not find any effect of gossypol on growth rate of heifers. Likewise, ADG of beef heifers supplemented with 0, 0.5, 2.5, 5, 10, and 20 g FG · animal⁻¹ · d⁻¹ did not differ in a study by Gray et al. (1993).

Plasma α -tocopherol (Figure 1) was affected by treatments. Heifers supplemented with cottonseed

meal had greater ($P < 0.05$) plasma α -T concentrations than heifers not supplemented with cottonseed meal at both concentrations of vitamin E. Vitamin E supplementation increased ($P < 0.05$) plasma α -T concentrations with both protein supplements. These observations confirm earlier results (Brocas et al., 1997; Velasquez-Pereira et al., 1998b) from which FG did not decrease plasma α -T concentration. Moreover, the observation that cottonseed meal increased plasma α -T concentration is in agreement with results of Velasquez-Pereira et al. (1998b,c). Although vitamin E is transported in blood mainly in plasma lipoproteins, erythrocyte vitamin E concentration is about 20 to 25% of total vitamin E found in plasma. The erythrocyte vitamin E pool exchanges rapidly between plasma lipoproteins and erythrocytes (Combs, 1992). The gossypol effect is time- and dose-dependent; perhaps gossypol is slowly replacing vitamin E in the erythrocytes and thereby increases plasma α -T concentration. Such a phenomenon could explain the increased α -T concentration in plasma seen in this experiment and others (Velasquez-Pereira et al., 1998c) and the effect that vitamin E has on reducing the EOF in animals fed gossypol-containing diets.

An alternative explanation to the higher plasma tocopherol concentrations on the CSM treatments has to do with the fact that vitamin E absorption from the gastrointestinal tract is aided by the presence of dietary fats; therefore, higher fat content of CSM compared to SBM (150 vs 103 g) based supplements (NRC, 1996) could have increased vitamin E absorption. In the study of Brocas et al. (1997), cottonseed meal increased plasma concentration of β -carotene, and although not significant, concentrations of α -T were numerically higher in cows fed cottonseed meal. Contrary to our results, Lane and Stuart (1990) reported that α -T and

Table 3. Effect of vitamin E and protein supplements on weight gain and ADG of beef heifers^a

Item	Supplement ^b				SE ^c
	SBM	SBM+E	CSM	CSM+E	
Initial weight, kg	402	399	399	389	13
Final weight, kg	460	450	455	439	12
90 d ADG, kg	0.64	0.57	0.63	0.54	0.06

^aLeast squares means.

^bSBM = soybean meal + 30 IU vitamin E/kg; SBM+E = soybean meal + 4,000 IU vitamin E · animal⁻¹ · d⁻¹; CSM = cottonseed meal + 30 IU vitamin E/kg; CSM+E = cottonseed meal + 4,000 IU vitamin E · animal⁻¹ · d⁻¹.

^cStandard error of the least squares means. There were no main effects or interactions ($P > 0.05$).

β -carotene concentrations in serum were decreased in dairy cows fed high concentrations of gossypol (approximately 40 g/d). In that study, however, no statistical evaluation of data were shown, and because of the difficulties in determining gossypol in a total mixed ration, the gossypol analysis reported may not represent the real values (M. Calhoun, personal communication).

Erythrocyte fragility is a very sensitive indicator of systemic gossypol status because it increases with gossypol consumption (Velasquez-Pereira et al., 1998b). Erythrocyte fragility showed a time × treatment effect ($P < 0.05$). Cottonseed meal supplementation increased ($P < 0.05$) EOF (Figure 2). However, for CSM+E supplemented heifers EOF did not differ ($P > 0.1$) from SBM and SBM+E animals during the first 9 wk of the experiment. At experiment termination (wk

12), SBM and SBM+E animals had lower ($P < 0.05$) EOF than other treatments, and vitamin E supplementation no longer had any effect on EOF. From these results, it appeared that vitamin E delayed the increase in EOF caused by gossypol consumption. The EOF increase in animals consuming a gossypol-containing feedstuff is an indicator of gossypol intake, but its use as a diagnostic tool for gossypol toxicity is questionable. α -Tocopherol as well as gossypol are located in the lipid membranes of the cell. Therefore, it may be possible that vitamin E decreased the effect of gossypol on EOF by occupying the same domain in the membrane and reducing the intercalation of gossypol into the lipid membrane of the erythrocyte. However, at the end of the experiment, this effect was not seen because gossypol effects are both time- and dose-dependent, with multiple oral doses gradually in-

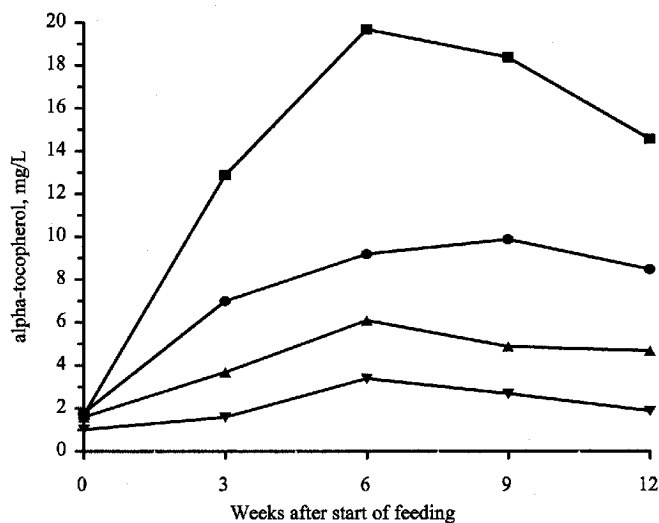


Figure 1. Plasma α -tocopherol concentration of beef heifers supplemented with: soybean meal + 30 IU vitamin E/kg (SBM ▼); soybean meal + 4,000 IU vitamin E · animal⁻¹ · d⁻¹ (SBM+E ●); cottonseed meal + 30 IU vitamin E/kg (CSM ▲); cottonseed meal + 4,000 IU vitamin E · animal⁻¹ · d⁻¹ (CSM+E ■). Standard errors were: 0.2, 0.9, 1.4, 1.1, and 0.6 at 0, 3, 6, 9, and 12 wk, respectively.

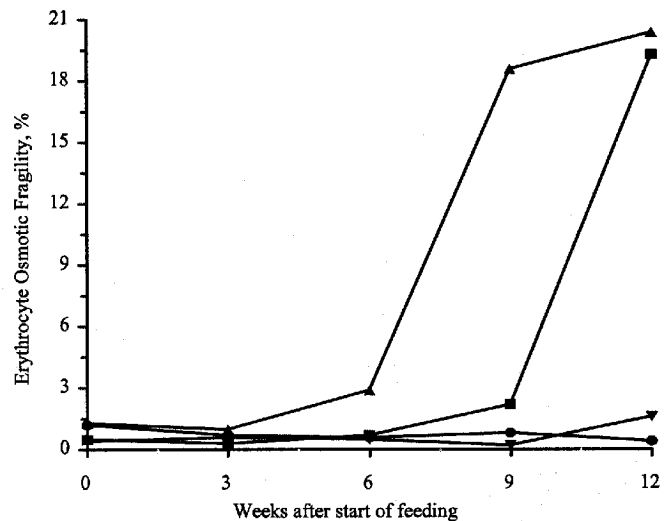


Figure 2. Erythrocyte osmotic fragility of beef heifers supplemented with: soybean meal + 30 IU vitamin E/kg (SBM ▼); soybean meal + 4,000 IU vitamin E · animal⁻¹ · d⁻¹ (SBM+E ●); cottonseed meal + 30 IU vitamin E/kg (CSM ▲); cottonseed meal + 4,000 IU vitamin E · animal⁻¹ · d⁻¹ (CSM+E ■). Standard errors were: 0.3, 0.2, 0.3, 2.4, and 2.4 at 0, 3, 6, 9, and 12 wk, respectively.

Table 4. Effect of gossypol and vitamin E supplementation on blood hemoglobin and hematocrit of beef heifers^a

Item	Collection	Supplement ^b				SE ^c
		SBM	SBM+E	CSM	CSM+E	
Hemoglobin, g/dL	0	14.6	14.6	14.2	15.2	0.5
	3	15.1	14.8	15.4	15.5	0.6
	6	15.6	15.7	15.5	15.9	0.6
	9	15.4	15.5	15.0	16.0	0.5
	12	16.9	18.0	14.8	17.6	0.7
Hematocrit, %	0	41.0	40.6	40.2	42.6	1.2
	3	42.9	41.7	43.4	43.6	1.2
	6	43.0	44.2	42.9	44.4	1.0
	9	44.1	45.2	43.6	45.8	1.1
	12	42.3	45.4	39.0	43.5	1.5

^aLeast squares means.

^bSBM = soybean meal + 30 IU vitamin E/kg; SBM+E = soybean meal + 4,000 IU vitamin E · animal⁻¹ · d⁻¹; CSM = cottonseed meal + 30 IU vitamin E/kg; CSM+E = cottonseed meal + 4,000 IU vitamin E · animal⁻¹ · d⁻¹.

^cStandard error of the least squares means. There were no main effects or interactions, except the main effect on hemoglobin at 12 wk. SBM treatments were higher ($P < 0.05$) than CSM.

creasing the amount of the toxicant retained in the body.

Blood hemoglobin and hematocrit (Table 4) were not affected ($P < 0.05$) by cottonseed meal and/or vitamin E supplementation until wk 12 when animals in the CSM treatment had lower ($P < 0.05$) concentrations. Lindsey et al. (1980) reported lower blood hemoglobin ($P < 0.07$) concentrations in dairy cows fed 3.5 and 24.2 g FG · animal⁻¹ · d⁻¹. Similar results were reported in dairy calves (Risco et al., 1992). However, Risco et al. (1993) found no clinical or hematological evidence of decreased survival of red blood cells.

Total gossypol and its isomers were greater ($P < 0.05$) for animals consuming CSM and CSM+E supplements. Total gossypol concentrations in plasma (Table 5) were higher than the safer upper limit of 5 µg/mL proposed by Calhoun et al. (1995b). Lower concentra-

tions of total gossypol in plasma were reported by Lindsey et al. (1980) in cows fed 3.5 or 24.2 g FG · animal⁻¹ · d⁻¹; however, the procedures used to measure plasma gossypol were different in the two studies. Calhoun et al. (1995a) reported that dairy cows consuming from 27.2 to 33.8 g TG · animal⁻¹ · d⁻¹ from whole cottonseed had average plasma gossypol concentrations of 3.0 µg/mL with a range of 1.2 to 5.8 µg/mL. Velasquez-Pereira et al. (1998b) reported that heifers consuming 4.5 g FG · animal⁻¹ · d⁻¹ had plasma gossypol concentrations of 4 µg/mL. In the present experiment, the gossypol intake was higher than those used by Calhoun et al. (1995a) and Velasquez-Pereira et al. (1998b); however, no signs of gossypol toxicity were seen for these animals. Gossypol enantiomers have different toxicological and pharmacokinetic effects, the (-) isomer being the most potent. Calhoun et al. (1995b) suggested that

Table 5. Concentration of plasma, corpus luteum, and endometrium gossypol (total and enantiomers) as affected by supplement in beef heifers^a

Tissue	Gossypol	Supplement ^b				SE ^c
		SBM	SBM+E	CSM	CSM+E	
Plasma, mg/L	Total	0.0	0.0	5.6	7.5	0.5
	(+)	0.0	0.0	2.9	3.7	0.2
	(-)	0.0	0.0	2.8	3.8	0.3
Corpus Luteum, (mg/kg, wet basis)	Total	6.6	10.6	47.1	58.2	3.4
	(+)	1.6	3.7	21.1	28.5	1.6
	(-)	5.1	6.5	26.0	29.6	2.6
Endometrium, (mg/kg, wet basis)	Total	32.0	27.7	78.2	87.2	12.2
	(+)	17.4	14.9	36.9	43.9	6.8
	(-)	14.6	12.8	41.2	43.3	5.5

^aLeast squares means.

^bSBM = soybean meal + 30 IU vitamin E/kg; SBM+E = soybean meal + 4,000 IU vitamin E · animal⁻¹ · d⁻¹; CSM = cottonseed meal + 30 IU vitamin E/kg; CSM+E = cottonseed meal + 4,000 IU vitamin E · animal⁻¹ · d⁻¹.

^cStandard error of the least squares means. Main treatment effects were CSM higher ($P < 0.05$) than SBM, with no interactions for all measurements.

Table 6. Effect of gossypol and vitamin E on α -tocopherol concentration in tissue of beef heifers (mg/kg of wet tissue)^a

Tissue	Supplement ^b				SE ^c
	SBM	SBM+E	CSM	CSM+E	
Corpus Luteum	23.1	108.4	39.5	100.1	6.1
Endometrium	2.7	7.2	2.6	9.3	0.6
Muscle (psoas major)	2.0	6.1	1.5	5.6	0.4
Liver	5.5	35.7	7.7	33.8	1.6

^aLeast squares means.

^bSBM = soybean meal + 30 IU vitamin E/kg; SBM+E = soybean meal + 4,000 IU vitamin E · animal⁻¹ · d⁻¹; CSM = cottonseed meal + 30 IU vitamin E/kg; CSM+E = cottonseed meal + 4,000 IU vitamin E · animal⁻¹ · d⁻¹.

^cStandard error of the least squares means. Main treatment effects were vitamin E higher ($P < 0.05$) than nonvitamin E, with no interactions for all measurements.

plasma gossypol reflects the availability of gossypol in the diet and the proportion of isomers in the gossypol source being fed. Daily dosage of gossypol was eliminated five times slower than a single oral dose in rats and mice, which indicates that multiple dosage increased retention of gossypol (Abou-Donia, 1989). This agrees with our results in which gossypol concentration in plasma increased with time at the same dosage.

Tissue Measurements. Vitamin E supplementation increased ($P < 0.05$) α -T concentration in liver, muscle, endometrium, and CL (Table 6). Gossypol supplementation did not affect ($P < 0.05$) vitamin E status. The CL was the tissue that accumulated more α -T than the other tissues examined. Steroidogenesis is accompanied by formation of oxygen radicals. Rapoport et al. (1998) reported that α -T in CL increased threefold between d 6 and 9 of the bovine estrous cycle. These researchers concluded that antioxidative mechanisms are activated to cope with steroidogenesis dependent oxyradical formation in the bovine CL (Rapoport et al., 1998). The accumulation of α -T in high steroidogenic

tissues protect against oxygen free radicals generated during the synthesis of reproductive hormones.

Vitamin E supplementation did not affect gossypol accumulation in endometrium and plasma but increased ($P < 0.05$) accumulation of gossypol in the CL (Table 5). Gossypol accumulated in endometrium and CL in very high amounts. There were also high concentrations of gossypol for the SBM and SBM+E treatment heifers. This gossypol was likely to have accumulated in these tissues when heifers were on earlier diets from previous management. The accumulation of gossypol in the endometrium and CL may indicate a potential reproductive impairment. In vitro studies have revealed an inhibitory effect of gossypol on embryo development even at low (2.5 μ g/mL) concentrations of gossypol (Brocas et al., 1997). High gossypol concentration in endometrium (approximately 16 μ g/mL, if considering 80% water in the tissue) may indicate a possible embryo development impairment because in vitro effects have been seen at concentrations lower than this amount.

Table 7. Superovulatory responses of beef heifers as affected by supplement^a

Item	Supplement ^b				SE ^c
	SBM	SBM+E	CSM	CSM+E	
Number of heifers	6	6	6	6	—
Heifers with embryos	6	6	6	5	—
Embryos per heifer	14.0	21.1	15.0	7.3	4.6
Left ovary weight, g	43.6	36.6	33.4	20.7	10.6
Right ovary weight, g	55.0	42.4	29.1	20.9	10.9
Embryo stage of development ^d	4.0	3.9	3.7	3.6	0.2
Percent blastocyst ^e	25.0	22.5	21.2	15.4	9.1

^aLeast squares means.

^bSBM = soybean meal + 30 IU vitamin E/kg; SBM+E = soybean meal + 4,000 IU vitamin E · animal⁻¹ · d⁻¹; CSM = cottonseed meal + 30 IU vitamin E/kg; CSM+E = cottonseed meal + 4,000 IU vitamin E · animal⁻¹ · d⁻¹.

^cStandard error of the least squares means. There were no main effects or interactions ($P > 0.05$).

^dDevelopment was assessed using the following scale: 0 = unfertilized oocyte; 1 = < 8 cells; 2 = 9 to 16 cells; 3 = early morula; 4 = compact morula; 5 = early blastocyst.

^eThe percent of recovered embryos that had reached the blastocyst stage of development.

Superovulation and Embryo Collection and Quality. Neither vitamin E nor gossypol concentrations had an effect on ovary weight, embryo stage of development, and embryo quality (Table 7). Gambill and Humphrey (1993) found that feeding heifers with 6.1 to 13.7 g FG · animal⁻¹ · d⁻¹ did not affect the weights or size of ovary or CL or number or size of follicles. Gray et al. (1993) reported that feeding diets containing gossypol from 0.5 to 20 g FG · animal⁻¹ · d⁻¹ did not have reproductive effects on female bovines. Also, Willard et al. (1995) did not find any effect of feeding 2 or 4 g FG · animal⁻¹ · d⁻¹ from CSM on reproductive parameters of Brahman cows and heifers. Most of the studies reporting in vivo effects of gossypol in reproduction of female bovines have not found a negative effect. In contrast, Wyse et al. (1991) found no differences on quality and development of embryos from superovulated Brangus heifers fed 5 g FG · animal⁻¹ · d⁻¹ from cottonseed meal or 15 g FG · animal⁻¹ · d⁻¹ from whole cottonseed. However, animals fed 5 g FG · animal⁻¹ · d⁻¹ from cottonseed meal had a higher percentage of degenerative embryos than animals fed 0 g FG · animal⁻¹ · d⁻¹ and the 15 g FG · animal⁻¹ · d⁻¹ from whole cottonseed (Wyse et al., 1991). Also, in vitro development of cleaved embryos was reduced by culture with 5 or 10 µm/mL gossypol (Brocas et al., 1997).

Gossypol has been reported to inhibit early embryonic development in vitro across a wide range of gossypol concentrations ranging from 2.5 (Brocas et al., 1997) to more than 12.5 µg/mL of plasma (Zirkle et al., 1988). The failure of gossypol feeding to disrupt development of embryos despite concentrations of gossypol in endometrium sufficient to reduce development suggests that mechanisms exist in the reproductive tract to counteract the deleterious effects of gossypol. In this regard, the oviduct secretes the antioxidant amino acid taurine and hypotaurine (Guerin and Menez, 1995), and it is possible that these molecules or others, such as cytoprotective molecules, limit gossypol toxicity in vivo.

Implications

Feeding of gossypol at 16.5 mg · kg⁻¹ BW · d⁻¹ does not reduce vitamin E concentration in plasma and tissue of heifers, nor did it cause any reproductive impairment. The data of this experiment may suggest that there are mechanisms in vivo that protect the embryos from the cytotoxic effect of gossypol.

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