

REPRODUCTIVE ABNORMALITIES IN RABBITS DUE TO VITAMIN A TOXICITY

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Abstract

Reproductive abnormalities in New Zealand White rabbits at a large commercial rabbitry were linked to an excess of dietary retinyl acetate. Fetal resorptions, abortions, and stillbirths were common in pregnant does. Examination of aborted and stillborn fetuses revealed hydrocephalus, microencephaly, and cleft palate. Analysis of the commercially prepared feed revealed a total vitamin A content of 102,278 IU/kg, of which 97,618 IU was retinyl acetate (recommended total vitamin A concentrations are 6,000-12,000 IU/kg). Levels of vitamin A in the plasma of does with reproductive disorders were 517-1,667 ng/ml (normal level is 300 ng/ml), and liver levels were 2,070-12,854 ug/g (normal range is 50-300 ug/g).

Introduction

Reproductive disorders in rabbits, such as fetal resorptions, abortions, stillbirths, and congenital anomalies may result from teratogenic agents (Hartman, 1974). Both vitamin A deficiency and toxicity induce a syndrome of such disorders with fetuses and neonates that exhibit hydrocephalus (Bondi and Sklan, 1984; Cheeke, 1987). Harrington and Newberne (1970) proposed that the progeny of does, vitamin A deficient from conception, could be used as an experimental model of hydrocephalus. The syndrome was demonstrated in incipient vitamin A deficient does by Lamming *et al.* (1954). Cheeke *et al.* (1984) showed the same syndrome (resorptions, abortions, low birth weights, increased perinatal and neonatal mortality, and hydrocephalus) in New Zealand White (NZW) does supplemented with high levels of vitamin A prior to breeding. Several questions emerge from this phenomenon. By what metabolic mechanism does an excess or deficiency of maternal vitamin A bring about a similar pathologic condition of development in the young? Are some forms of vitamin A toxic, while others are not? What is the optimum amount of vitamin A in the diet of rabbits? Are dietary or health factors involved in availability and/or utilization of vitamin A by rabbits?

We investigated an incident of hypervitaminosis A in NZW rabbits resulting from excessive levels of retinyl acetate inadvertently added to the feed (Deeb *et al.*, 1990; DiGiacomo *et al.*, 1992). The reproductive syndrome which resulted was typical of either hyper- or hypovitaminosis in rabbits. The data gathered in this investigation may be useful in directing future experimental studies to elucidate mechanisms and dosages necessary for vitamin A toxicity in rabbits.

Materials and Methods

Rabbitry

The incident occurred in a commercial rabbitry in Washington during the summer of 1989. The rabbitry raised primarily NZW rabbits, but also had NZ Black and NZW X Florida White rabbits. There were about 4,000 rabbits including about 530 breeding does. Litters were weaned at 5 to 8 weeks of age. Breeding rabbits were maintained in 1 building, and weaned rabbits were caged in 2 other buildings, by litters, until 10 weeks of age and then housed individually. Rabbits were maintained in single-tiered wire mesh cages. Approximately 150g of pelleted feed containing 18% protein and 16% fiber were provided for each rabbit daily. Water was supplied daily in metal drinking containers. Continuous artificial lighting was provided in the building with breeding rabbits.

Feed

The diet was produced locally by vendor A and 7 to 8 tons were delivered every 2 weeks to the rabbitry. The implicated shipment of feed was received June 10, 1989 and placed in a grain silo which still contained several days' supply of feed from the last shipment. The rabbitry owner noted that on June 14, 1989, many rabbits were reluctant to eat the feed and scratched it out of their troughs until June 21, 1989, when it was removed and replaced with fresh feed from the same vendor. However, the vendor subsequently stated that all feed delivered between March 29, 1989 and July 6, 1989 was defective, in that it contained a twentyfold increase in the amount of vitamin A.

Data analyses

The rabbitry maintained records on the number of does bred, the number kindled, the number of rabbits born, and the number weaned. All records were keyed to the date rabbits were bred, which occurred 3 times a week (Monday, Wednesday, and Friday). On each of these days, 9 to 28 (mean=20) does were bred. Since gestation averaged 31 days, all data were plotted by the expected date of parturition. The following parameters were calculated: kindling rate (number of does kindled/number of does bred), perinatal mortality (number dead at birth/number born), neonatal mortality (number died before weaning/number born alive), mean litter size (at birth) for all births (number of kits born/number of does kindled), mean litter size (at birth) for live births (number of kits born alive/number of does kindled) and mean litter size weaned (number of rabbits weaned/number of does kindled). In addition, fetal mortality (does aborted/does kindled and does aborted) and maternal mortality (does died during parturition/does kindled) rates were calculated. Data were plotted as a 3-day moving average. Differences in frequencies were compared using Fisher's exact test and p values < 0.05 were considered significant.

Postmortem evaluation

Does with reproductive disorders were euthanatized with an overdose of barbiturate and necropsied. Rabbits were examined grossly and representative tissue specimens were obtained. Tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 to 8 microns, and stained with hematoxylin and eosin for histopathological evaluation.

Vitamin A analyses

Plasma levels of retinol were determined by high pressure liquid chromatography (Bieri *et al.*, 1979) and vitamin A in livers and feed samples were detected fluorometrically at excitation and emission wavelengths of 330 and 470 nm, respectively (Dennison and Kirk, 1977).

Results

Reproductive rates

The various rates of reproduction were severely affected. The kindling rate decreased to about 25% of its former level (Figure 1). Perinatal and neonatal mortality rates increased severalfold (Figure 2). Correspondingly, the mean litter size also decreased significantly (Figure 3). During this time period, fetal and maternal mortality rates were significantly increased compared to 2-month time periods before and after (Table 1).

Table 1. Fetal and maternal mortality rates in rabbits that consumed a commercial diet containing excessive vitamin A

Rate	Mortality in relation to impaired reproductive rates ^a		
	Before	During	After
Fetal	4/459 (0.9) ^b	16/117 (13.7) ^c	5/454 (1.1)
Maternal	8/455 (1.8)	7/101 (6.9) ^c	3/449 (0.7)

^a Before: 5/4 to 7/3, During: 7/6 to 8/7, After: 8/10 to 10/9

^b Number affected/total (%)

^c Rate during significantly ($p < 0.01$) greater than either rate before or after

Pathologic findings

Six breeding does in various stages of gestation were necropsied. Four does aborted fetuses at 22 to 26 days gestation and were necropsied 4 to 6 days later. Two does had mild subacute endometritis indicative of uterine involution. The other 2 had moderate and severe suppurative metritis. The doe with moderate metritis had subacute suppurative inflammatory foci in multiple organs and *Pasteurella multocida* was recovered from the uterus of the doe with severe metritis. Two does that were 4 days beyond the time of normal parturition (31 days) were necropsied and found to have moderate to marked suppurative metritis. *P. multocida* was recovered from the uterus of the former. The endometritis associated with *P. multocida* infection was felt to be secondary to abortion or retained fetuses.

A total of 32 aborted or stillborn fetuses and neonates were examined (Table 2). Three aborted fetuses had hydrocephalus and 7 stillborn fetuses and neonates had hydrocephalus and cleft palate (Figures 4 and 5). Microscopic examination of the brain from affected rabbits revealed cortical atrophy, compatible with hydrocephalus. Degeneration, necrosis or inflammation was not observed in skeletal muscles.

Table 2. Congenital anomalies in fetuses and neonates from rabbits that consumed a commercial diet containing excessive vitamin A

Fetuses/ neonates	Number examined	Number with congenital anomalies		
		Hydrocephalus	Microencephaly	Cleft palate
Abortuses ^a	20	3	3	0
Stillbirths/ neonates ^b	12	7	7	3
Total	32	10	10	3

^a Less than 31 days old

^b Equal to or greater than 31 days old

Vitamin A levels in rabbits

Plasma vitamin A levels were elevated in the 10 does tested (Table 3). There was no apparent trend in the plasma levels over time. Liver vitamin A levels were greatly elevated in all 7 does examined (Table 3). In general, liver levels decreased with time. Analysis of livers from 7 aborted or stillborn fetuses revealed a mean vitamin A level of 37 ug/g (range 8-70).

Table 3. Plasma and liver vitamin A levels in does with reproductive abnormalities

Doe number	Vitamin A levels by date of sample				
	8/9-16/89 Plasma ^a	8/18-23/89 Plasma	Liver ^b	9/4/89 Plasma	9/26/89 Liver
1893B	1,140	727		681	
837D	934	781		1,123	
113A	562	517		590	
3155D	1,252			741	
2833D		663	6,110		
833D		626	10,938		
6723C		566	12,854		
4979D				899	4,533
6890D				728	2,070
3799D				1,667	7,320
9922C			9,457		

^a Normal level is 300 ng/ml (Bondi and Sklan, 1984)

^b Normal level is 50-300 ug/g dry weight (Bondi and Sklan, 1984)

Vitamin A levels in feed

The shipment of feed which rabbits were reluctant to consume had a concentration of vitamin A about 10 times greater than the recommended level (Table 4). This was due to an excessive amount of added retinyl acetate. Shipments of feed after July 16, 1989 from the same vendor (A) had vitamin A concentrations in the recommended range, as did a sample from another vendor (B) that supplied feed to the University of Washington.

Table 4. Vitamin A concentrations in commercial rabbit feeds

Source	Period fed	Vitamin A concentrations (IU/kg) ^a		
		Carotene	Retinyl acetate	Total
Vendor A	6/14-21/89	4,660	97,618	102,278
Vendor A	7/28-8/10/89	4,030	6,353	10,383
Vendor A	8/11-25/89	3,230	4,500	7,730
Vendor B		5,000	2,647	7,647

^a Recommended level = 6,000 - 12,000 (Lebas, 1980; Cheeke, 1982)

Discussion

This investigation revealed that excessive levels of dietary retinyl acetate resulted in reproductive abnormalities in rabbits. The dosage, duration, and form(s) of vitamin A which induces abnormalities in rabbits remain a subject for scientific investigation. In the natural state, for herbivores such as rabbits, the nutritional requirement for vitamin A is satisfied by the conversion of β -carotene in green plants to retinol. The conversion occurs primarily in the intestinal mucosa and decreases with increasing intake. In formulated rations, vitamin A in the form of retinyl esters, retinyl acetate or retinyl palmitate, are added to the diet. After retinol is taken up by mucosal cells, it is re-esterified, incorporated into chylomicrons and transported via lymphatics to the liver and stored primarily as retinyl palmitate (Bondi and Sklan, 1984). Retinol mobilization and delivery are regulated by rates of retinol binding protein (RBP) synthesis and secretion by the liver. Delivery of retinol to target tissues may involve specific cell surface receptors that recognize RBP (Goodman, 1984).

Studies in rodents (Mallia *et al.*, 1975) have shown that vitamin A toxicity occurs when excessive amounts of vitamin A are presented to cell membranes in association with plasma lipoproteins, rather than specifically bound to RPB. Thus, RBP may regulate not only the supply of retinol to tissues but also protect tissues from surface-active effects of the vitamin. Normally, vitamin A transport from dam to fetus is regulated within narrow limits during the critical period of rapid fetal differentiation (Goodman, 1984). Excessive intake of vitamin A in rats (Cohlan, 1953), or intraamniotic administration to rat fetuses during the critical period (Nanda and Romeo, 1977), have resulted in reduced litters carried to term and congenital deformities with the primary defects of exencephaly (Cohlan, 1953), cleft palate, micrognathia, and limb defects (Nanda and Romeo, 1977). Similar, but not identical, teratogenic effects of hypervitaminosis A were induced in hamsters and guinea pigs (Robens, 1970). The nature of the anomalies and percent of fetal resorptions depended on time of administration and dose. Some retinoids have been found to be more active teratogens than others. In rodents, retinol was less active than retinoic acid (Kochhar *et al.*, 1988) or retinyl acetate (Kochhar, 1967); however, the defects were similar (Kalter, 1989). In mice, vitamin

A treatment after the 11th day of gestation produced primarily limb defects, whereas treatment on the 9th and 10th day caused craniofacial malformations (Kalter, 1960).

In the rabbit, following treatment with retinyl acetate, maternal, but not fetal, liver levels increased and the ratio of vitamin A in fetal liver to maternal serum was lower than control values (Lorente and Miller, 1977). Kormann *et al.* (1989) reported that the addition of 40 ppm β -carotene to diets with adequate vitamin A levels resulted in improvements in kit survival and rates; and while there was a linear relationship between dietary vitamin A levels and liver stores of vitamin A, the conversion rate for β -carotene to vitamin A was decreased with increasing dietary levels of β -carotene. Thus, the rabbit may have protective mechanisms against excess β -carotene but not vitamin A, or there may be a specific function for β -carotene, as distinct from retinol. Perhaps high liver levels of vitamin A inhibit synthesis of RBP, allowing excessive amounts of toxic forms of vitamin A to reach fetal tissues during the critical period of differentiation in the rabbit.

For rabbits, the National Research Council (1977) advised that 580 IU of total vitamin A/kg of feed was required for growth, and 1,160 IU/kg for gestation. Lebas (1980) recommended dietary levels of 6,000 IU/kg for growth, 10,000 IU/kg for gestation and 12,000 for lactation. Cheeke *et al.* (1984) reported that 191,180 IU/kg of vitamin A, mainly in the form of retinyl palmitate, in the diet of nulliparous does prior to breeding caused reproductive disturbances. Moghaddam *et al.* (1987) showed that a diet with 90,000 IU/kg of vitamin A as retinyl palmitate, beginning 2 weeks prior to breeding and continuing for 3 parities, resulted in abortions and hydrocephalus. The mean liver level in does with affected litters was 3,301 (SD=2,051) IU/kg. He confirmed that liver levels more clearly reflected a toxic state than plasma levels. Yamini and Stowe (1988) reported fetal resorptions, abortions, stillbirths, and congenital malformations (cleft palate, anencephaly, exophthalmia, hydrocephalus, and other anomalies) in 2 NZW rabbitries using feed which contained 24,000 to 28,000 IU/kg of vitamin A. In our study, the implicated feed contained 97,618 IU/kg of retinyl acetate. The vendor indicated a twentyfold increase above the appropriately formulated amount of retinyl acetate supplementation and that defective feed had been delivered for more than 3 months. The rabbits objected to the diet for about a week toward the end of that period. The dramatic effect on reproduction began in the latter part of June and lasted through early August, that is, it began about 2 weeks after the rabbitry owner noted that some rabbits were reluctant to eat the feed and continued for about 6 weeks after that time. Thus, dietary levels of vitamin A as retinyl esters higher than 90,000 IU/kg, and perhaps lower, have toxic effects on reproduction in the rabbit. Whether toxicity occurs from short-term exposure or requires accumulated exposure to excessive retinyl acetate is not clear.

Conclusion

A rabbit feed supplemented with excess retinyl acetate resulted in hypervitaminosis A in does, fetal resorptions, abortions, stillbirths, and congenital anomalies, primarily hydrocephalus. To our knowledge, this is the first report of a spontaneous occurrence of vitamin A toxicity in rabbits. Until the mechanisms by which retinoids influence reproduction and the developing embryo are clarified, and toxic doses for rabbits are known,

care should be taken in using dietary supplements. Vitamin A requirements for rabbits may be better met by supplementation with β -carotene, which the rabbit regulates efficiently, rather than addition of synthetic retinyl compounds which might result in toxicity.

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FIGURE 1. Kindling rate in rabbits after consumption of commercial diet containing excessive vitamin A (time is expressed as month/day).

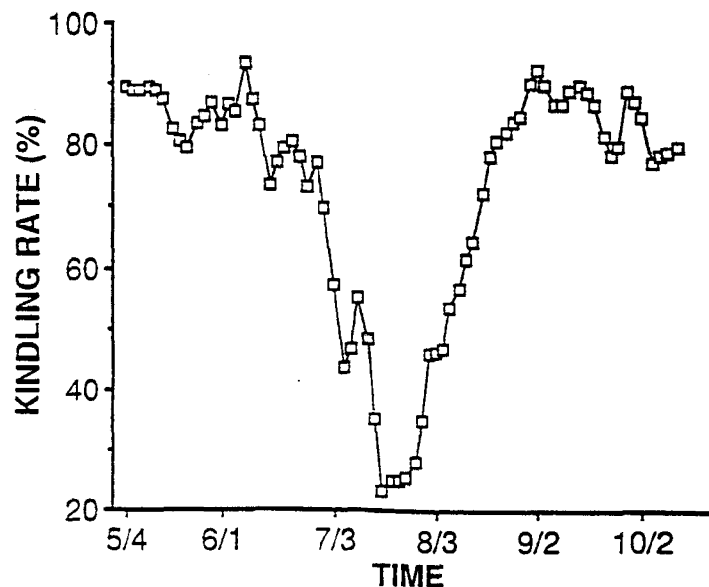


FIGURE 2. Perinatal (at birth) and neonatal (prior to weaning) mortality rates in rabbits after consumption of commercial diet containing excessive vitamin A (time is expressed as month/day).

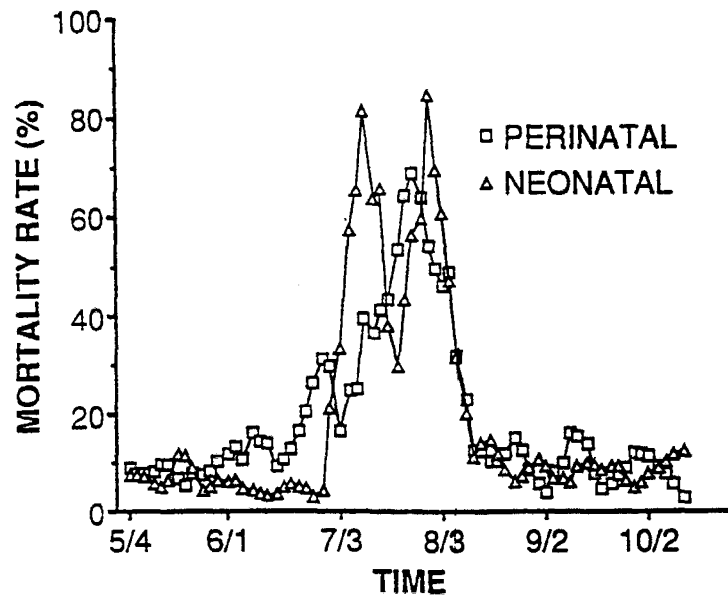


FIGURE 3. Mean litter size in rabbits after consumption of commercial diet containing excessive vitamin A (time is expressed as month/day).

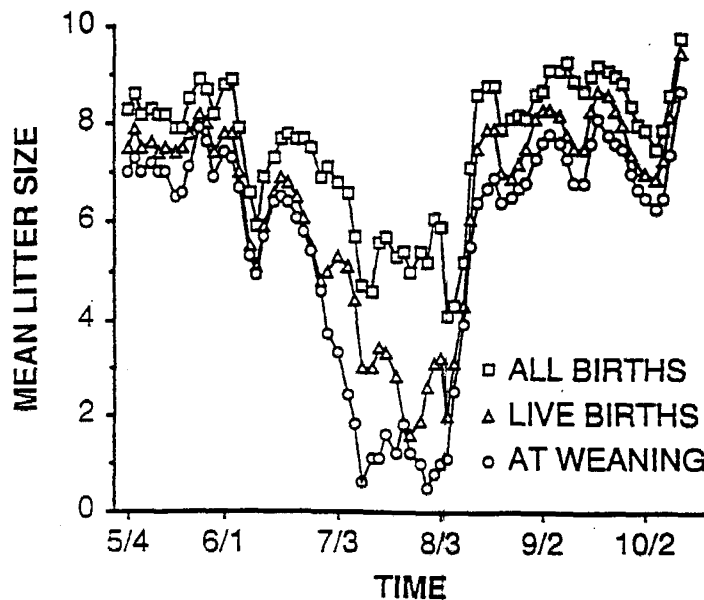


FIGURE 4. Stillborn rabbit with hydrocephalus (scale in mm).



FIGURE 5. Stillborn rabbit with cleft palate (scale in mm).

