

EFFECTS OF VITAMINS A, C AND E ON THE REPRODUCTIVE PERFORMANCE OF HEAT-STRESSED FEMALE RABBITS IN EGYPT

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Summary

Ninety-six Bouscat does were assigned to eight experimental groups which were orally administered vitamins A, C and E as follows: no supplementation (control), 6000 IU vitamin A (group 2), 50 mg vitamin C (group 3), 25 mg vitamin E (group 4), 6000 IU vitamin A + 50 mg vitamin C (group 5), 6000 IU vitamin A + 25 mg vitamin E (group 6), 50 mg vitamin C + 25 mg vitamin E (group 7) and 6000 IU vitamin A + 50 mg vitamin C + 25 mg vitamin E (group 8)/animal/day. Treatment began 2 weeks prior to first mating. The experiment was carried out during the summer season in Egypt (May-September). Treatments continued throughout the experimental period. Four does from each group were sacrificed on day 12 of pregnancy to determine ovulation rate, implantation sites and pre- and post-implantation embryonic survival rate; the other does were left until parturition. Litter size, birth weight, and stillbirths were recorded. Postnatal mortality rate and body weight were recorded weekly during the suckling period. There were no significant differences in corpora lutea, implantation sites, survival embryos, litter size and birth weight between groups, but there were some advances in the vitamin supplemented groups compared to the control group. There was a significant increase ($P < 0.05$) in weaning weight and mortality at birth and at the 1st week in group 2 compared to the other groups. Total mortality rate in the control group was high, indicating that the does were under heat stress, and very low in group 7 (C+E), indicating the beneficial effects of vitamins C and E on postnatal mortality. Supplementation by vitamin A alone in the summer season had an unfavorable effect on reproductive performance. The effect of heat stress through the summer season on early embryonic mortality was not great compared to its effect on postnatal mortality.

Introduction

At the present time, rabbit production and interest in rabbit breeding are increasing in Egypt. Breeding is not generally done during summer due to environmental conditions unfavorable to reproductive performance (El-Fouly *et al.*, 1977; Ismail, 1988), but is confined to the period from September to April. The harmful effects of high ambient temperatures on reproductive processes (pregnancy, embryonic survival and post-natal

mortality) vary with the breed, temperature, duration of exposure, type of exposure, gestation length and stage of pregnancy.

Vitamin A is necessary for growth, cellular differentiation, fertility and maintenance of pregnancy (Cheeke, 1987; Rachman *et al.*, 1987; McDowell, 1989). Vitamin E has an important role in fertility, reproduction, prevention of peroxide damage to tissues and muscular dystrophy. Vitamin E provides disease resistance by protecting leukocytes and macrophages during phagocytosis and increasing immunity responses (Reddy *et al.*, 1987b) in addition to synthesizing ascorbic acid (Scott *et al.*, 1982). Vitamin C is sufficient in small quantities under normal situations, but larger amounts are required during adverse environmental conditions, physiological stress and disease conditions (McDowell, 1989). The effects of these vitamins on rabbit reproduction during the summer season is still not clear. This investigation was undertaken to fulfill the following objectives:

1. To study the influence of vitamin supplementation on reproductive performance,
2. To study the interrelationship between vitamins A, C and E in all possible combinations and their effects during pregnancy and the suckling period,
3. To determine which vitamin or combination of vitamins enables does to overcome the harmful effects of the summer season,
4. To elucidate any antagonistic effects existing between vitamins, and
5. To study vitamin balance, especially in cases of supplementation above recommended levels.

Materials and Methods

Ninety-six mature Bouscat does about 15 months of age were randomly placed in individual wire cages, 12 does per group. A commercial pelleted rabbit diet (Ismail, 1988) meeting NRC (1977) nutrient requirements (16% CP, 63.12 TDN and 12.62% CF, 12,000 IU vitamin A, 50 mg vitamin E and 200 mg vitamin C/kg diet) and water were available ad libitum. The experiment was carried out during the summer season in Egypt (from May until September, where temperatures ranged between 33° C during the day to 23° C during the night with an average relative humidity of 83%). Treatments consisted of orally administered vitamins A (retinol palmitate), C and E as follows: no supplementation (control), 6000 IU vitamin A (A), 50 mg vitamin C (C), 25 mg vitamin E (E), 6000 IU vitamin A + 50 mg vitamin C (A+C), 6000 IU vitamin A + 25 mg vitamin E (A+E), 50 mg vitamin C + 25 mg vitamin E (C+E) and 6000 IU vitamin A + 50 mg vitamin C + 25 mg vitamin E (A+C+E)/animal/day for treatments 1, 2, 3, 4, 5, 6, 7, and 8, respectively. The does had been treated 2 weeks prior to first mating. After being assigned to treatments, the does were bred to Bouscat bucks. Treatments continued throughout the experimental period. Does were palpated for pregnancy on day 10. Four pregnant does from each group were sacrificed on day 12 of pregnancy to determine number of corpora lutea, implantation sites, dead and survival embryos. The other does were left until parturition, after which litter

size, birth weight, and mortality at birth (stillbirths) were recorded. Post-natal mortality rate and live body weight were recorded weekly for young until weaning at 4 weeks of age. Statistical analysis was conducted by analysis of variance using SAS Package 1990.

Results and Discussion

Ovulation Rate

Average ovulation rates, determined by counting corpora lutea on day 12 of pregnancy, are presented in Table 1. Statistically, there were no significant differences in ovulation rate between groups. No effects on ovulation rate were noted in vitamin-supplemented does and there were no harmful effects of high ambient temperatures of the summer season on ovulation rate. Seasonal effects on ovulation rate are not clear. No variation was found in the ovulation rate of rabbits due to season of the year. A slight increase in ovulation rate was noted in groups 2 (A), 3 (C), 5 (A+C), 7 (E+C) and 8 (A+C+E) compared to groups 1 (control), 4 (E) and 6 (A+E). The best combination affecting ovulation rate during the summer season was group 5 (A+C), where the number was very high (13.2 ± 1.43). These results are similar to findings of Kamar *et al.* (1983) and Ismail (1988).

Number of Implantation Sites

The mean number (\pm SE) of implantation sites are shown in Table 1. Differences due to vitamin supplementation were not statistically significant. This result can be attributed to the mild heat stress all the animals were under. The effect of vitamin supplementation on implantation site was not clear, perhaps due to the dosage used. The number of implantation sites showed a trend similar to that of ovulation rate. The time at which rabbits are exposed to heat, ambient temperature, duration of exposure and type of exposure (constant or fluctuating) are important factors. Present results were in agreement with Sod-Moraiah (1971) and El-Sobhy (1981).

Pre-Implantation Survivability

Early embryonic survivability, expressed as percentage of number of implantation sites related to number of corpora lutea, is presented in Table 1. The role of vitamins in early embryonic survivability, especially when given in doses above requirement during the summer season, is not clear. Early embryonic survivability followed a trend similar to that of number of implantation sites. It was noted that early embryonic survival was less pronounced with constant, severe heat exposure than with circadian fluctuating and mild heat exposure; this result was in agreement with Rich and Alliston (1970a) and Ismail (1988).

Post-Implantation Survivability

The mean number of viable embryos percentage of embryo survival (number of live embryos/number of corpora lutea) and post-implantation survivability expressed as percentage of number of live embryos/number of implantation sites are presented in Table 1. ANOVA revealed that number of viable embryos was not significantly influenced by vitamin

Table 1. Mean (\pm SE) numbers of corpora lutea, implantation sites, preimplantation survivability (%), number of surviving embryos, percentage of embryo survival and post-implantation survivability.

Treatment	Corpora Lutea (C.L.)	Implantation Sites (IS)	Pre-Implantation Sites (PIS), %	Survival Embryos	% of Embryo Survival	% Post-Implantation Survivability
1. Control	10.75 \pm 0.25	9.75 \pm 0.63	90.69	8.25 \pm 1.38	76.74	84.62
2. Vitamin A	12 \pm 0.89	11.4 \pm 0.51	95	11.2 \pm 0.58	93.33	98.25
3. Vitamin C	11.25 \pm 0.25	10.25 \pm 0.63	91.11	10 \pm 0.5	88.89	97.56
4. Vitamin E	10.25 \pm 20.02	9.75 \pm 1.85	95.12	9.75 \pm 1.85	95.12	100
5. Vitamins A+C	13.2 \pm 1.43	11 \pm 2.12	83.33	10 \pm 2.19	75.76	90.91
6. Vitamins A+E	9.75 \pm 1.91	8.5 \pm 1.39	87.18	8.5 \pm 1.39	87.18	100
7. Vitamins C+E	12.25 \pm 0.48	11.5 \pm 0.29	93.88	10.5 \pm 0.87	85.71	91.3
8. Vitamins A+C+E	12 \pm 0.91	10.35 \pm 0.85	85.42	10.25 \pm 0.85	85.42	100

supplementation during the summer season, although the number of live embryos was low in the control group as compared other groups. Percentage of embryo survival was also low in the control group as compared to the treatment groups, with the variation greater in this stage of pregnancy than earlier (pre-implantation survivability; Table 2). Post-implantation survivability was lower in the control group than in other groups and followed a similar trend as percentage of embryo survival. These results support previous reports indicating that embryo survival decreases as pregnancy advances due to cumulative harmful effects of unfavorable ambient temperatures upon post-implantation survival. The effect of vitamin supplementation at this stage of pregnancy (12 day) was not statistically significant, perhaps due to the ambient temperatures which were not severe. In general, these results are in agreement with Hanada *et al.* (1983), Kamar *et al.* (1983) and Ismail (1988).

Litter Size

Average litter size (\pm SE)/doe is shown in Table 2. Differences in litter size due to vitamin supplementation were not statistically significant between groups including the control group. Results indicated that vitamins C and E had an important role and favorable effect on reproductive performance, protecting fetuses against resorption and decreasing early embryonic mortality resulting from heat stress of the summer season. Litter size was high (10 ± 0.85 , 9.7 ± 1.02 and 9.4 ± 0.55) in vitamins C, E and C+E groups, respectively, compared to the control or vitamin A group or any other vitamin combination. The role of vitamin E in increasing fertility, protecting fetuses against resorption, and decreasing abortions, stillbirths and neonatal death is well known (Ringler and Abrams, 1970; DiPalma and Ritchie, 1977; Bendich and Langseth, 1989; Yamini and Stein, 1989). Vitamin E also has a role in disease resistance and increasing immunity response (Reddy *et al.*, 1987b). Vitamin E increases immunity, perhaps due to its role in synthesis of vitamin C (Scott *et al.*, 1982). The role of vitamin C in reproduction is not well known but during adverse environmental conditions and physiological stress it increases immune response and body temperature regulation, especially with heat stress or elevated environmental temperatures (Pardue and Thaxton, 1986; McDowell, 1989), as noted in the present study. Although vitamin A is important for cellular differentiation, fertility and maintenance of pregnancy (Cheeke, 1987; Rachman *et al.*, 1987; McDowell, 1989), it has a teratogenic effect and is toxic when given in high doses to animals, causing reproductive failure such as abortion, fetal resorption, small litter size, etc. (Cheeke *et al.*, 1984; Moghaddam *et al.*, 1987; Deeb *et al.*, 1992). In the present study, the dosage used was not high; two times the requirement showed unfavorable effects on reproductive performance and did not improve the number of litter size/doe or protect fetuses against resorption of heat stress through the summer season compared to vitamins C or E and seemed like an extra stress factor. Vitamin A masked the favorable effects of vitamins C and E when combined to C or E or with C and E together (A+C+E). This effect may have been due to the vitamin A level (in the diet and supplement) which approached toxicity level or to levels of vitamins C and E which were not effective enough to overcome the double stress. In the vitamin A groups (A, A+C, A+E and A+C+E), fetuses were under two kinds of stress (high level of vitamin A and heat stress) and they were more sensitive to these kinds of stresses, so fetal resorption increased and litter size decreased. Excess vitamin A may disturb the function or absorption of other fat-soluble vitamins (Holander, 1981) or might be due to the antagonistic effect

Table 2. Means (\pm SE) of litter size, birth weight, weaning weight, mortality at birth and at 1st week and percentage of mortality at birth, at 1st week and total mortality rate during the suckling period.

Treatment	Litter Size	Birth Weight	Weaning Litter Per/Doe	Weaning Weight	Mortality at Birth		Mortality at 1 Week		Total Mortality, %
					Means \pm SE	%	Means \pm SE	%	
1. Control	(19) 7.42 \pm 1.08	(141) 54.27 \pm 3.35	(60) 3.16	404.93 \pm 28.44 ^a	0.74 \pm 0.25 ^{ab}	9.93	2.79 \pm 0.82 ^{ab}	37.59	57.45
2. Vitamin A	(9) 8.22 \pm 1.01	(74) 52.20 \pm 4.4	(12) 1.33	558.24 \pm 40.75 ^a	1.89 \pm 0.82 ^a	22.97	3.89 \pm 1.16 ^a	47.3	83.78
3. Vitamin C	(9) 10 \pm 0.85	(90) 52.55 \pm 4.64	(57) 6.33	428.12 \pm 36.91 ^b	0.11 \pm 0.11 ^b	1.11	2.11 \pm 1.17 ^{ab}	21.11	36.67
4. Vitamin E	(10) 9.7 \pm 1.02	(97) 48.61 \pm 3.31	(68) 6.8	409.43 \pm 37.58 ^b	0.6 \pm 0.5 ^{ab}	6.19	1.4 \pm 0.64 ^{ab}	14.43	29.9
5. Vitamins A+C	(15) 7.73 \pm 0.88	(116) 60.71 \pm 3.31	(67) 4.47	460.00 \pm 26.94 ^{ab}	0.8 \pm 0.48 ^{ab}	10.34	1.47 \pm 0.5 ^{ab}	25.86	42.24
6. Vitamins A+E	(8) 8.25 \pm 1.21	(66) 54.34 \pm 4.6	(44) 5.5	385.82 \pm 27.32 ^b	0 ^b	0	1.25 \pm 0.65 ^b	15.15	33.33
7. Vitamins C+E	(10) 9.4 \pm 0.55	(94) 47.93 \pm 3.53	(71) 7.1	380.89 \pm 12.65 ^b	0 ^b	0	0.64 \pm 0.45 ^b	7.45	24.47
8. Vitamins A+C+E	(13) 7.7 \pm 0.71	(100) 57.42 \pm 3.17	(64) 4.92	471.76 \pm 45.48 ^{ab}	0.15 \pm 0.15 ^{ab}	2	2 \pm 0.71 ^{ab}	26	36

* Figures in parentheses represent number of animals.

** Within each column, any two means having at least one similar letter are not significantly different.

towards vitamins C and E as reported by Nieman and Obbink (1954; Ismail *et al.*, 1992b,c). Litter size in the vitamin A groups (A, 2; A+C, 5; A+C+E, 8) was lower than the number of live embryos at day 12 of pregnancy compared to other groups (see Tables 1 and 2), indicating that there was additional embryonic mortality in these groups occurring during the later stages of pregnancy where early rabbit embryos were directly affected by increases in maternal body temperature accompanying thermal stress (Alliston *et al.*, 1965; Ismail, 1988), in addition to accumulation of vitamin A in the tissues during the latter stages of pregnancy. Near parturition, body stores of vitamin A are released into the blood, subsequently increasing embryonic mortality. Such effects may not become apparent until late stages of embryonic development. Present results are in agreement with Arthur and Leslie (1965) and Ismail *et al.* (1992c).

Birth Weight and Weaning Weight

Average birth weight and weaning weight (\pm SE) are presented in Table 2. There were no significant differences in birth weight between all groups. This result was expected because there were no significant differences in litter size. One of the most important factors affecting litter weight at birth is litter size. There is a negative correlation between birth weight and litter size ($\gamma = -0.365 \pm 0.037$), but no appreciable difference in birth weight between litters of 5, 6 and 7 (Afifi *et al.*, 1977; Khalil *et al.*, 1987; Ismail (1988) and Ismail *et al.*, 1992c). The high ambient temperatures may have masked any favorable effects of vitamin supplementation on embryonic growth and birth weight. There was a significant difference ($P < 0.05$) in weaning weight between groups. Tukey's test revealed a significant increase in weaning weight in group 2 (A) compared to other groups, except for groups 5 (A+C) and 8 (A+C+E). This result was expected where these groups recorded the lowest litter size at weaning (1.33, 4.47 and 4.92 doe in groups A, A+C, and A+C+E, respectively). These results are in agreement with Afifi *et al.* (1977), Khalil *et al.* (1987), Ismail (1988) and Ismail *et al.* (1992c) who showed that body weight in rabbits at weaning increases as number of kits at weaning decreases. In the present study, the fact that the control group did not show any significant increase in weaning weight in spite of a low litter size (3.16/doe at weaning) is an indicator of the unfavorable effect of the summer season or heat stress on growth rate and body weight. Vitamin supplementation had a slight favorable effect on growth through heat stress where groups 3 (C), 4 (E), 6 (A+E) and 7 (C+E) recorded a high litter size at weaning and reasonable weight. The weaning weight is more affected by litter size than by vitamin supplementation (Afifi *et al.*, 1977; Khalil *et al.*, 1987; Ismail, 1988; Ismail *et al.*, 1992c). Vitamin C may have a beneficial effect by counteracting the harmful effect of excess vitamin A or by increasing immune response toward stress and adverse environmental conditions (Ismail *et al.*, (1992b,c).

Postnatal Mortality Rate

Average postnatal mortality (\pm SE) per/doe and rates (%) at birth, week 1 and total mortality rates from birth until weaning at 4 weeks of age are presented in Table 2. There were significant ($P < 0.05$) differences in mortality at birth between treatments. Tukey's test revealed that mortality in group 2 (A) at birth was significantly higher (22.97%) and significantly different from groups 3 (C), 6 (A+E) and 7 (C+E), which recorded the

lowest mortality rates (1.11, 0 and 0%, respectively). There was no significant difference between groups 1 (control), 2 (A), 4 (E), 5 (A+C) and 8 (A+C+E) and no significant difference between groups 1 (control), 3 (C), 4 (E), 5 (A+C), 6 (A+E), 7 (C+E) and 8 (A+C+E). The high mortality rate at birth in group 2 (A) might have been due to heat stress in addition to the high level of vitamin A. Present results indicate that vitamin A was the major factor affecting stillbirths and mainly responsible for the increase in mortality rate at birth and its effect was greater (alone) than heat stress in the present study. The beneficial effects of vitamin C and E were observed in decreased stillbirths. These vitamins alone or in combination with vitamin A resulted in lower mortality rates which ranged from 0 to 10% versus to 22.97% in group 2 (A). This significant effect of vitamins C and E can be attributed to increased immune response and disease resistance during physiological stress and adverse environmental conditions (Reddy *et al.*, 1987b; McDowell, 1989; Ismail *et al.*, 1992c) in addition to the antagonistic effect of these vitamins towards the harmful effect of hypervitaminosis A (Nieman and Obbink, 1954; Ismail *et al.*, 1992b,c). Present results indicate that the effect of vitamin E on the harmful effect of hypervitaminosis A and heat stress on embryonic mortality (stillbirths) was high compared to vitamin C and this can be attributed to its role in reproduction plus the ability to synthesize ascorbic acid. Mortality rates (%) during the first week of age were very high (above the normal range of 17-27%) in groups 1 (control) and 2 (A) (37.59% and 47.3%, respectively), within normal range in groups 3 (C), 4 (E), 5 (A+C) and 8 (A+C+E) and under that in groups 6 (A+E) and 7 (C+E). Increased mortality rate during the first week of age compared with the subsequent weeks during suckling period in all groups can be attributed to the unfavorable environmental conditions of the summer season, low resistance to microbial infection, starvation and the high level of vitamin A in the milk, in which vitamin A concentrations increase from 4-25 times those of milk as a result of the accumulation in the tissues during the latter stages of pregnancy. Near parturition body stores of vitamin A are released into the blood to meet the high demands required for milk. Vitamin A levels in the milk start to decline gradually and by the third to tenth day return to normal level (Arthur and Leslie, 1965). Total mortality rates through the suckling period showed the same trend, with group 2 (A) recording a higher rate (83.78%), then the control group (57.45) and group 7 (C+E) with the lowest rate, 24.47%. These results confirmed the important role of vitamins C and E in protecting against physiological stress, adverse environmental conditions, and harmful effects of high vitamin A and increased disease resistance and immunity responses subsequently improved survivability in all treatment groups compared to the control group or group 2 (vitamin A alone). These results are in agreement with Partridge *et al.* (1981), Cheeke *et al.* (1984), Damodar and Jatkar (1985), Roedecha and Chanpongsang (1986), Ismail (1988), Yamini and Stein (1989), Deeb *et al.* (1992) and Ismail *et al.* (1992c).

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