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EFFECT OF VITAMIN E ON OVARIAN ACTIVITY, EMBRYONIC MORTALITY AND PRODUCTIVE PERFORMANCE OF NZW RABBITS DURING SUMMER SEASON

Ahmed, Nagwa A.[1]; A.H. Barkawi[1]; A. Ismail[2] and E.M. Abd El-Kafy[2]

1 Department of Animal Production, Faculty of Agriculture, Cairo University, Giza, Egypt.

2 Animal Production Research Institute, Agriculture Research Center, Dokki, Giza, Egypt

ABSTRACT

Twenty-four New Zealand White does were used in this study that was executed during summer season. Rabbits were housed individually in wire cages and fed commercial pellet diet. Ambient temperature and relative humidity indoors through the experiment averaged 23.4°C and 57.7% at minimum and 32.1°C and 83.5% at maximum, respectively. Does were divided into two equal groups, control (G1) and treated (G2) which was injected intramuscularly with 56 mg vitamin E once a week.

Gestation period, litter size at birth and weaning, pre-weaning mortality % were weekly recorded. Progesterone (P4) and vitamin E concentration were determined in plasma of does through pregnancy. In each parity, three does from each group were sacrificed at 48h post-coitum to study some measurements on does and embryos. In sacrificed does, the weight of ovaries, oviducts and uterine horn weights were recorded. Ovulation rate, number of collected embryos, stage of cleavage, quality of embryos were recorded at 48h post- coitum. *In Vitro* development of embryos through 96h after collection) was also studied.

Vitamin E concentration in plasma of G2 was 2.4 times that of G1 during the 1st three weeks of pregnancy stage, increased to 3.2 at the end of pregnancy term (28 days). P4 concentration increased gradually during pregnancy reaching its peak at mid-gestation. Ovulation rate was not statistically affected by vitamin E treatment or by parity. Total number of collected embryos at 48h post-coitum from G2 was almost double that of G1. The quality of embryo (48h after coitum and 96h after collection) and productive performance (litter size at birth and weaning) were better in G2 than in G1. Vitamin E treatment reduced pre-weaning mortality rate and improved daily gain during suckling period. Body weight gain significantly increased in the 2nd parity at 3rd week during suckling period.

Keywords: Rabbits, hot conditions, vitamin E, ovulation rate, embryonic cleavage and mortality, reproductive performance.

INTRODUCTION

During the last decades, the official administration in Egypt adopted the policy of producing white meat to cover the food gap. Rabbit is one of the options to achieve such goal for their prolificacy and favorable growth rate. This requires an intensive system of production all year round. However, a managerial approach to prevent breeding during summer is highly considered to avoid the loss of offspring due to the unfavorable environmental conditions

(El-Fouly *et al*., 1977 and Ismail, 1988) and low reproductive efficiency of females (Ahmed Nagwa, 2000).

Fertility of doe rabbits is low under high environmental temperature (Matassion *et al*., 1970 and Shafie *et al*., 1984) due to a complex set of physiological process. Such phenomena may be due to the decline in ovulation response (Farrell *et al*., 1968), low fertilization rate (Rathore, 1970) and high embryonic mortality (El-Fouly *et al*., 1977 and Ismail *et al*., 1992a).

Many trails were conducte to overcome the negative effect of hot conditions on reproductive efficiency using vitamins. Vitamin A (Hassanein *et al*., 1995 and Ghaly, 1988), C (Ismail *et al*., 1992a) and E (Ghaly, 1988 and (Ismail *et al*., 1992a) were studied. Available data concerning the role of vitamin E showed contradictory trends, due to the season of treatment and the used dose. This arise a question concerning the actual role of vitamin E on reproductive response and what is the proper does that would be applied for rabbits nutrition under Egyptian summer condition.

In the light of the previous information, the objective of this study was to investigate the effect of vitamin E treatment on ovarian activity, embryonic survivability of the New Zealand White (NZW) rabbits during summer season to achieve better reproductive performance.

MATERIALS AND METHODS

This work was carried out in Animal Production Research Institute, Agriculture Research Center, Ministry of Agriculture, Giza, Egypt and Animal Production Department, Faculty of Agriculture, Cairo University during summer season from June to August 1999.

Animals and Management

Twenty-four New Zealand White does (NZW) aging 6-7 months and weighing 2.7-3.1 kg were used in the present study. Does were housed individually in wire cages with standard dimension of 60x60x35 cm and provided with nest boxes (35x35x35 cm) for kindling and nursing. Cages were equipped with feeding hoppers and have nipples for automatic drink. Rabbits were fed commercial pelleted diet containing 16.4% crude protein, 12.7% crude fiber and 2540 kcal/kg metabolizable energy and 30 mg vitamin E/Kg diet. Does fed *ad libitum* and clean fresh water was made available all the time. The ambient temperature indoors through the experiment averaged 23.4°C at minimum and 32.1°C at maximum with relative humidity was 57.7% and 83.5% at minimum and maximum, respectively.

Does were mated twice within 30 minutes to ensure conception and were abdominally palpated to re-mate rabbits that did not conceive.

Experimental Design

In summer season, does were divided into two equal groups (12 per group), untreated (control G1) and treated (G2). In G2 each doe was injected intramuscularly with 0.5 cm30 corn oil containing 56mg vitamin E Cairo Pharmaceuticals and Chemical Industries Co. Cairo) once a week. Treatment

started two weeks before the first mating and continued to 4th weeks post-partum of the second parity. Throughout two parities, three does from each group were sacrificed at 48h post-coitum, while the other does were kept to the end of gestation period.

In non sacrificed does, blood plasma concentrations of vitamin E and progesterone during pregnancy were determined in samples withdrawn at 0, 7, 14, 21 and 28 days of gestation, litter size at birth and weaning and pre-weaning mortality% were recorded in each parity.

In sacrificed does, weight of ovaries, oviducts and uterine horn were recorded. Number of corpora lutea was counted as an indicator for ovulation rate. After sacrificing, embryos were collected by flushing oviduct using TCM medium. Number of collected embryos, stage of cleavage and quality of embryos were recorded just after sacrificing. Morphology of embryo was evaluated according to Lindner and Wright (1983) and Takedo (1986). Embryos were classified based on their morphological symmetry of blastomers and stage of cleavage into excellent, good, fair and poor.

Embryos were cultured *in vitro* for 96 h after collection (144 h age) to study cleavage rate, viability and measurements of embryos. *In vitro* medium composed of 9.5 g/l TCM-199 plus 2.2 g/l NaHCO3, supplemented with 0.04 g/l sodium pyruvate, 0.07 g/l streptomycin sulfate. 0.03 g/l penicillin G potassium and 10% fetal calf serum. Osmolarity and pH of medium was 305 and 7.8, respectively. Medium was filtered with 0.02 µm Millipore filter.

Embryos were collected and examined using stereo scope (8X). After collection, embryos were transferred to clean medium (100 µl/droplet) under liquid sterile paraffin oil in culture dish (35x10 mm). Embryos were incubated at 38°C under atmosphere of 5% CO_2, 95% air and 70-80% humidity. Embryos were transferred daily to a fresh medium for morphological evaluation and dimension of embryos were quickly measured at room temperature using inverted microscope fitted with calibrated eyepiece micrometer.

Blood sampling and analysis

Blood samples, from each doe, were collected from the marginal ear vein into heparinized syringes at mating (zero day), 7, 14, 21 and 28 days through pregnancy during the experimental period to determine the concentrations of progesterone (P4) and vitamin E in the peripheral blood. Samples were centrifuged at 3000 rpm/15 minutes for plasma separation. Plasma was stored at -18°C until P4 and vitamin E determination.

Radioimmunoassay technique was used for P4 assessment. Ready antibody coated tube kits (coat-A-count) were used according to manufacturer's information, antiserum, at 50% displacement, has values of cross reaction of 100% with progesterone, < 3.9% with pregnenolone and <1% with any of the other steroids. The standard curve ranged between 0.0 and 40.0 ng/ml. Intra assay coefficient of variation was 8.6. Vitamin E in plasma was determined by using HPLC.

Statistical Analysis

Least square means was used to calculate the mean of studied traits using SAS (1990). The T-test were used to detect significant differences between the least square means.

RESULTS AND DISCUSSION

Vitamin E Concentration

Vitamin E concentration in the peripheral blood of NZW does was higher ($P<0.05$) in G2 than in G1 (Table 1), reaching 2.4 times that of G1 during the 1st three weeks of pregnancy, increased to 3.2 at the end of pregnancy (28 days). Low level of vitamin E in G1 may be attributed to the depletion of the vitamin E storage in the liver and body tissues (Yamini and Stein, 1989) as a result of low vitamin E intake as compared to G2.

Parity had no significant effect on vitamin E concentration in the blood of NZW does during pregnancy (Table 1), however, vitamin E level was slightly higher in the 2nd parity relative to the 1st one. Values of vitamin E in the 2nd parity ranged from 0.105 to 0.118 mg/ml vs. 0.075 to 0.108 mg/ml in the 1st parity.

Table 1: Vitamin E Concentration (mg /ml) in blood plasma of NZW Rabbits through pregnancy as affected by Vitamin E injection and parity

Days through pregnancy	Groups		Parities		S.E
	Control	Vitamin E	1st parity	2nd parity	
Zero*	0.053 [b]	0.125 [a]	0.075 [b]	0.106 [a]	0.007
7	0.061 [b]	0.146 [a]	0.090	0.118	0.009
14	0.063 [b]	0.155 [a]	0.106	0.111	0.007
21	0.050 [b]	0.158 [a]	0.103	0.105	0.006
28	0.051 [b]	0.165 [a]	0.108	0.108	0.006

[ab] Means having different superscripts within rows are significantly different ($P < 0.05$).
*** Day zero is defined as the day of mating.**

Reproductive Measurements

1. Reproductive tract

Weights of ovaries as well as oviduct was higher ($p<0.05$) in G_2 than in G_1 by 4.5 and 12%, respectively (Table2). Weight of ovary obtained in this study was nearly close (300mg) to those found by El-Fouly *et al*. (1977). The increase in ovarian weight of G_2 could be attributed to the higher number of corpora lutea (8.7) than in G1 (5.3). Higher weight of oviduct may be related to the expected hyper secretion of cells as response to the higher level of P_4 (Figure 1) as well as due to the number of embryos that may enhance cellular secretion (Beier, 1974).

Weights of uterine horn did not differ between G1 and G2 that may be due to the early time of the test (48h post-coitum) since the proliferation of the endometrium did not reach its maximum. Parity had no significant effect on the weight of the reproductive organs.

Table 2: Characteristics of genitalia of NZW rabbits as affected by vitamin E injection and parity.

Traits	Groups		Parities		S.E
	Control	Vitamin E	1st	2nd	
Reproductive tract weight (mg)					
Ovary	250.5[b]	261.8 [a]	254.5	257.8	2.6
Oviduct	227.5 [b]	267.7 [a]	244.5	250.0	4.3
Uterine horn	6172	6122	6128	6165	81.8
Number of corpora lutea	5.3	8.4	7.0	7.0	0.8

[ab] **Means having different superscripts within rows are significantly different (P < 0.05).**

2. Ovarian activity

Number of corpora lutea at 48h post-coitum was used as an indicator for ovarian activity. In the present study, overall mean of ovulation rate in NZW rabbits (7.0) was in agreement with the findings of El-Fouly *et al*. (1977) but less that than reported by Ismail *et al*. (1992a, 10.5). Ovulation rate was not affected statistically neither by vitamin E treatment nor by parity (Table 2). However, ovulation rate in G_2 was about 1.6 times that of G1 (8.7 vs 5.3) but such difference was not statistically significant, probably due to the small number of does tested in each group (n=3). Low ovulation rate in G1 than in G_2 is mostly due to the effect of hot conditions. Such effect may be alleviated partially with vitamin E treatment. Ismail *et al*. (1992a) found that treated rabbits (25 mg vitamin E/doe/day) had no significant effect on ovarian activity. However, the recovery rate of embryo was higher in G_2 than G_1 (93 vs. 81). Results of ovulation rate as affected by parity agrees with the results of Ismail *et al*. (1992b) and Hassanein *et al*. (1995) reporting no difference in ovulation rate between 1st and 2nd parities.

Quality of embryos

1. Collected embryos (48h post-coitum)

Total number of recovered embryos as well as number of transferable embryos, collected at 48h post-coitum was almost double in G_2 as compared to G_1 (Table 3).

Table 3: Embryo Characteristics, collected 48 h post-coitum ,of NZW rabbits as affected by vitamin E injection and parity.

Embryos	Groups		Parities		S.E
	Control	Vitamin E	1st	2nd	
Transferable[1]	4.3	7.8	6.6	5.9	0.6
Non transferable[2]	0.2	0.2	0.0	0.3	0.1
Degenerated[3]	0.3	0.0	0.2	0.2	0.1

[1]**: Embryos those were in morula stage and evaluated as excellent or good.**
[2]**: Embryos those were in morula stage and evaluated as fair or poor.**
[3]**: Embryos those were stopped at 8-16 cell stage.**

Number of transferable embryos (excellent and good) in both parities was lower in G_1 than that of G_2 (Table 4), where excellent embryos in G_1 were 1.8 and 2.7 times that of G_1 in the 1st and 2nd parity, respectively. These results are in harmony with those found by Rich and Alliston (1970) and Ismail *et al*.

(1992a). Parity had no effect on both number of collected and transferable embryos (Table 3). This result agrees with the findings of Ismail *et al*. (1992b) and Hassanein *et al*. (1995).

Table 4: Quality of collected embryos (48h age) and embryos after *in vitro* culturing (96h age) as affected by vitamin E injection and parity in NZW rabbits.

Embryos Quality	Control group				Vitamin E group			
	E	G	F	P	E	G	F	P
48h age								
1st Parity	1.8 ±0.4	0.8 ±0.2	0.0	0.0	3.3 ±0.6	0.7 ±0.1	0.0	0.0
2nd Parity	1.2 ±0.3	0.5 ±0.1	0.2 ±0.1	0.0	3.3 ±0.6	0.5 ±0.1	0.2 ±0.1	0.0
144 h age								
1st Parity	0.0	0.0	1.8 ±0.4	0.8 ±0.2	0.0	0.6 ±0.1	1.6 ±0.3	1.8 ±0.3
2nd Parity	0.0	0.0	1.1 ±0.2	0.8 ±0.2	0.0	0.5 ±0.1	2.0 ±0.4	1.5 ±0.3

E: excellent, G: good, F: fair and P: poor.

2. In-Vitro Culture

The quality of embryos during in-vitro culture for 96h post-collection (144 h age) is shown in Table (4). It is clear that vitamin E has a promoting role on embryo cleavage. In Vitro cleavage of embryos collected from does of G2 tended to be faster. Thickness of mucin layer and zona pellucida as well as cell mass diameter were higher in G2 than in G1, however the difference was insignificant (Table 5). Parity had no significant effect on the quality of embryos and rate of embryos cleavage, however measurements of embryos in the 2nd parity were greater than in the 1st parity (Table 4 and 5).

Table 5: Embryos diameter (µm) of NZW rabbits as affected by vitamin E and parity.

Embryos	Groups		Parities		S.E
	Control	Vitamin E	1st	2nd	
Mucin layer thickness	81.3	96.9	79.8	98.4	11.8
Zona pellucide thickness	15.8	16.6	15.9	17.1	2.7
Cell mass diameter	97.6	121.1	100.8	117.7	13.5
Embryo with coverings	194.7	234.6	196.3	233.2	34.8

Reproductive Performance

1. Gestation Length

Gestation period averaged 30.4 days with a range of 29-32 days. Gestation period seems to be neither affected by vitamin E treatment nor parity (Table 6). This result is in agreement with most previous studies that reported range of Gestation length ranged 30-35 days in rabbits with an average of 30-32 days (Hassanein, 1980; Azoz, 1996; Khadr, Amina *et al*., 1996 and Ahmed, Nagwa 2000). The slightly shorter gestation period of G2 may be attributed to the higher number of litter size at birth (8.0) as compared to G1 (5.1). This agrees with the result of Afifi and Emara (1985); Hilmy (1991) and Khadr, Amina *et al*. (1996). They found that gestation period decreased linearly with the increase of litter size at birth.

Table 6: Effect of vitamin E injection and parity on reproductive performance in NZW rabbits.

Traits	Groups		Parities	
	Control	Vitamin E	1st parity	2nd parity
Gestation length (day)	30.5±0.4	30.1±0.4	30.5±0.3	30.3±0.5
Litter size at birth	5.1±2.0	8.0±2.3	6.9±1.5	6.2±1.8
Litter size at weaning	2.5±1.0	4.7±1.7	3.8±0.9	3.3±0.9
Pre-weaning Mortality %	34	26	29	31

2. Progesterone (P4) Concentration

Progesterone concentration increased gradually reaching its peak at the mid-term of gestation (Figure 1). Afterwards, it declined gradually to reach the lowest concentration just before parturition. Generally, P_4 concentration in G_2 was higher than in G1 through pregnancy. The increase of P_4 level in does of G_2 is most probably attributed to the higher number of detected corpora lutea (8.6) as compared to that of G1 (5.3) (table 2). This result is supported by the findings of Hillard *et al*. (1973); Ahmed Nagwa *et al*. (1994) and Azoz (1996). They reported that P4 ovarian secretion during the pregnancy is correlated with the number and size of corpora lutea.

2. Litter size and mortality rate

Litter size at birth in G2 was 1.6 times that of G1 (Table 6). Similar trend was observed at weaning. These results agree with the findings of Ismail *et al*. (1992a) and Hassanein *et al*. (1995) reporting more litter size at birth and weaning obtained from does treated with vitamin E compared to untreated does. Pre-weaning mortality % was higher in G_1 than in G_2. The difference in mortality rate between groups due to parity was not significant. These result agree with the result of Afifi and Emara (1985).

3. Body weight gain of bunnies

Data in table 7 showed that body weight gain of bunnies during suckling period was similar in both G_1 and G_2. These results agree with the findings of Ismail *et al*. (1992c). They concluded that weight gain is more affected by litter size rather than vitamin E treatment. There is significant difference in weight gain of bunnies between two parities at 3rd week of age. This result may be attributed to doe milk production that is more in the 2nd parity than in the 1*st* one.

Table 7: Effect of vitamin E injection and parity on body weight gain (g) during suckling period.

Suckling Period	Groups			Rarity		
	Control	Treated	S.E	1st parity	2nd parity	S.E
1st week	42.2	47.5	3.9	46.5	43.4	4.2
2nd week	49.1	50.1	3.9	45.0	54.2	4.3
3rd week	55.4	59.1	4.2	45 [b]	64.58 [a]	4.6
4th week	137.8	161.4	11.5	136.4	160.8	12.6
Total Gain	284.7	318.9	21.0	277.5	325.8	23.0

[ab] **Means having different superscripts within rows are significantly different (P < 0.05).**

GENERAL DISCUSSION

Results of the present study indicated that treated does with vitamin E during summer months may eliminate the effect of hot climatic conditions on reproductive process. Elevation of vitamin E level circulating in the peripheral blood of treated does during pregnancy (Table 1) improved the ovulation rate by 64% than untreated does (Table 2). Vitamin E is thought to have an important rule in increasing the concentration of free FSH and LH in the blood by minimizing the binding of these hormones with their specific proteins in the plasma (Youssif *et al*., 1989), resulting in better ovarian activity response. Treatment of vitamin E, also improved quality of embryos at early stages of cleavage. Number of transferable embryos, collected at 48h post-coitum, was 1.8 times of those collected from untreated does (Table 3 and 4). This indicates that vitamin E may have a vital role in reducing embryonic mortality at early stage of pregnancy (Ismail *et al*., 1992a), through its direct and indirect effects.

At body level, increasing progesterone concentration in the blood of treated does would enhance the genitalia secretion (Figure 1). This may elucidate the increase of oviduct weight of treated does at early stage of embryonic cleavage than untreated ones (Table 2). In addition progesterone activates endometrium proliferation and stimulates production of uteroglobins or blastokinin (Urzuo *et al*., 1970 and Arthur and Daniel, 1972). This has an impact on survivability of embryos in treated does (Table 3). Increasing levels of total protein and cholesterol in the blood, as a response to the increasing of vitamin E (Shetaewi, 1998), stimulates secretion of albumin and globulin in oviducts (Beier, 1974 and Tucker and Schultz, 1977) that are necessarily for embryo cleavage.

At embryo level, vitamin E protects the cell membrane (McDowell, 1989) and enhanced protein synthesis as well as metabolic rate of embryonic cells (Fischer, 1987 and Jang and Fischer, 1988). Experiments of Fischer (1987) indicated that vitamin E also stimulates DNA synthesis by enhancing the rate of cell division causing greater measures of embryo collected from does treated by vitamin E as observed in *in-vitro* culture (Table 5).

Increasing litter size at weaning and reducing pre-weaning mortality of bunnies delivered from does treated by vitamin E may be attributed to its transfer to milk through mammary gland (McDowell, 1989). Vitamin E protects leukocytes and macrophages in the blood of bunnies from phagocytes; by this way vitamin E improves disease resistance and immune response of bunnies (McDowell, 1989). Moreover high intake of vitamin E via milk of does (Ghally, 1988; and Hassanien *et al*., 1995), improves the growth rate and viability of bunnies. The insignificant difference in body weight gain between bunnies that delivered from vitamin E treated does and control ones is most probably due to the more litter size of vitamin E treated does which decrease the share of bunnies in milk intake.

In conclusion, this study indicates that vitamin E could be offered in does rabbits diet particularly during hot months to overcome the heat stress.

However, further studies on large number and for longer period are needed to emphasis the obtained results particularly on embryonic growth performance, biochemical of genitalia secretion and histology of reproductive tract.

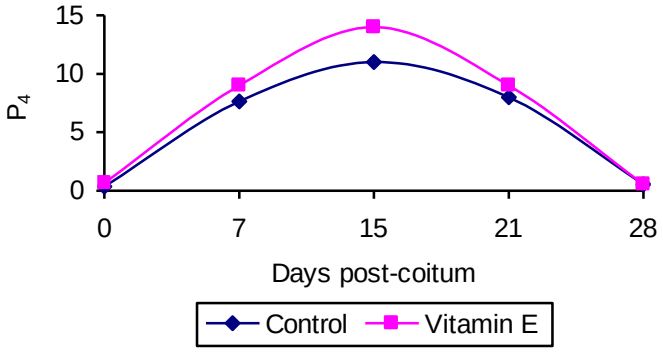

Figure 1: Progesterone Concentration (ng/ml) in blood plasma of NZW rabbits measured through pregnancy as affected by vitamin E treatment.

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تـأثير فيتـامين هــ علـى النشـاط المبيضـى ونفـوق الأجنـة والأداء الإنتـاجى فـى الأرانـب النيوزلندى فى فصل الصيف

نجوى عبد الهادى أحمد[1]، أشرف هشام برقاوى[1]، أحمد أسماعيل[2] والسيد محفوظ عبد الكافى[2]

1 قسم الإنتاج الحيوانى، كلية الزراعة، جامعة القاهرة، الجيزة ـ مصر

2 معهد بحوث الإنتاج الحيوانى، مركز البحوث الزراعية، الدقى ـ مصر

أجرى هذا البحث فى معهد بحوث الإنتاج الحيوانى وكلية الزراعة ـ جامعة القاهرة. وقد تمت هذه الدراسة على 24 أم نيوزلندى وضعت بصورة فردية فى بطاريات وغذيت على عليقة أرانب تجارية، سجلت درجة الحرارة والرطوبة النسبية داخل العنبر فكان متوسط درجة الحرارة الصغرى 23.4°م والعظمى 32.1°م والرطوبة النسبية المصاحبة 57.7% و 83.5% على التوالى. قسمت الأرانب الى مجموعتين متشابهتين حقنت إحداها بفيتامين هـ بمعدل 56 مجم مرة أسبوعيا.

سجلت مدة الحمل وعدد الخلفات عند الميلاد والفطام ونسبة النفوق من الميلاد وحتى الفطام، كما قدر تركيز هرمون البروجسترون وفيتامين هـ فى بلازما الدم خلال فترة الحمل. وقد ذبحت ثلاث أمهات من كل مجموعة على 48 ساعة بعد التلقيح، قيس فيها وزن المبيض وقناة المبيض وقرن الرحم وعدد الأجسام الصفراء، وقدر عدد الأجنة ومرحلة الانقسام الجنينى وجودة الأجنة، كما درس تطور الأجنة خارج الرحم حتى 96 ساعة بعد جمعها من الإناث.

وأوضحت النتائج ارتفاع مستوى فيتامين هـ فى بلازما دم المجموعة المعاملة 2.4 مرة قدر مجموعة المقارنة خلال الثلاثة أسابيع الأولى من الحمل وبلغت 3.2 مرة فى نهاية فترة الحمل (28 يوم)، وزاد تركيز هرمون البروجسترون تدريجيا خلال فترة الحمل ليصل الى أقصى تركيز فى منتصف فترة الحمل، بينما لم يتأثر معدل التبويض، وبلغ عدد الأجنة التى جمعت من مجموعة فيتامين هـ ضعف عددها فى مجموعة المقارنة. كما تحسنت جودة الأجنة نتيجة للحقن بفيتامين هـ وزادت سرعة الانقسام فى الخلايا الجنينية.

وقد أدى الحقن بفيتامين هـ الى زيادة عدد الخلفات عند الميلاد والفطام وانخفاض معدل النفوق وتحسن معنوى فى وزن الجسم أثناء فترة الرضاعة.