[BIO16]The effect of palm vitamin E on fetal and newborn development in rats

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Introduction

Palm Vitamin E is an extract of palm oil, which constitutes 25% of tocopherol and 75% of mixed α, δ, γ tocotrienol (Cottrell, 1991). A previous study has found that the feeding of high dosages of palm vitamin E between the range of 250-2500mg/kg body weight (b.w.) did not produce toxicological effects on mice and *Sprague Dawley* rats (Oo *et al*., 1992). A study by Rajikin *et al.* (2001) suggested that a dose of 60mg/kg b.w did not influence the rat gestational period and the number of pups born.

However, thus far there has been no report of toxicological evaluation of tocotrienol intake on fetal and newborn development in rats. The present study, therefore, was undertaken to investigate the effect of palm vitamin E on the newborn rats following maternal feeding with palm vitamin E.

Materials and Methods

Reproductive Performances

The ethical approval for this study was received from Universiti Kebangsaan Malaysia and University of Malaya research committee. *Sprague dawley* female rats (190-230g) were divided into 5 groups: Control, 100mg/kg b.w., 250mg/kg b.w., 500mg/kg b.w. and 1000mg/kg b.w. palm vitamin E and mated with proven fertile males. The presence of mucus plug indicated that the rats have been mated and the day of which the mucus plugs were detected was considered as Day 1 of gestation. The rats were fed orally with palm vitamin E using esophageal tube from 1st through the 13th day of gestation. On the 13th day of gestation, pregnant rats of all groups were sacrificed and number of corpora lutea, implantations and resorptions were recorded/ counted.

Development of the Newborn Rats

The developments of the newborn rats was observed on postnatal day 3 (PD 3) and PD 7 from another set of animal of the same treatment. Gestational periods, number of pups delivered, weight of the newborn, gross development and assessment of the external abnormalities were observed. All the data were given as mean ± standard error mean (SEM).

Results

Reproductive Performance

Palm vitamin E up to 1000mg/kg b.w. did not show any adverse effect on the female fertility with the evident that corpora lutea released are comparable to the number of implanted embryos (fig 1). Maternal intake of palm vitamin E does not induced variations in the number of implantations and percentages of resorption. The percentages of resorption in the rats treated with palm vitamin E and control group is within the normal range of resorption rate in *Sprague dawley* rats (fig 2).

Development of the Newborn Rats

The study showed there was no difference in the duration of the gestation period between the treated groups compared to the control group (Table 1). There was also no difference in the number of pups delivered between the treated groups compared to the control group (Table 2). No indications of abnormalities were observed in the newborn delivered either from the mother rats treated with palm vitamin E or control. Body weight similar between palm vitamin E of the newborn rats on PD 3 and PD 7 were groups compared to the control group. Assessment of growth development showed no significant differences were observed in their body parameter at PD 3 and PD 7 compared to the control group (Table 4).

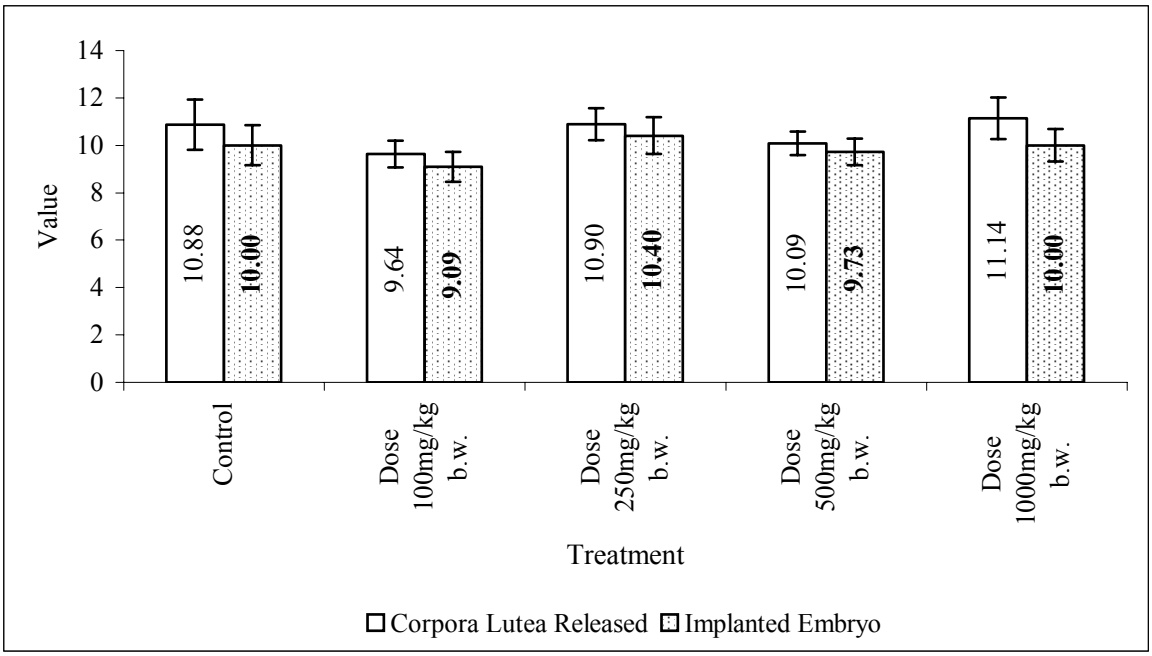

FIGURE 1 Effect of palm vitamin E on number of implanted embryos and number of corpora lutea released in rats treated with different doses of palm vitamin E

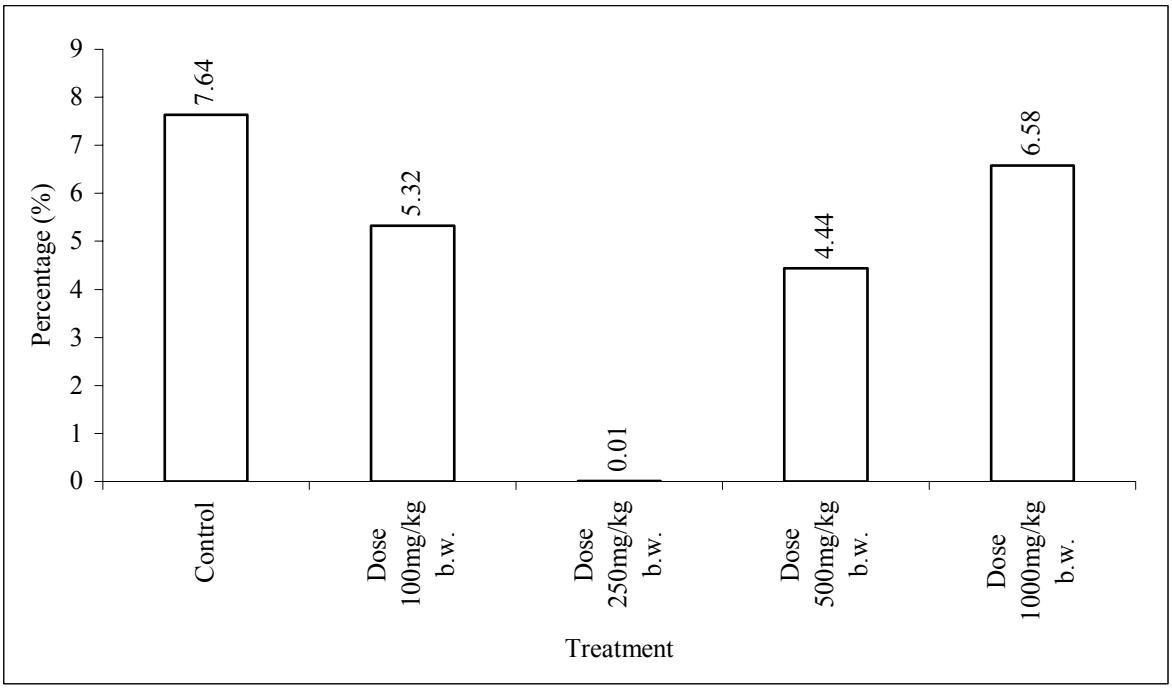

FIGURE 2 Percentage of resorption in the animal treated with different doses of palm vitamin E. Data are presented as resorption per implantation (%).

Discussion

Reproductive Performance

1. Corpora Lutea

Corpora (body) lutea (yellow) is named by Marcello Malphigi (1628-1694) and described precisely by Regneir de Graaf (1641-1673) through encountered of globular shape of coitus bodies in the rabbit's ovary. The corpus luteum persisted even after labour (Niswender *et al.* 2000). De Graaf discovered that the number of corpora lutea

is in concomitant with the number of pups born (Short, 1977).

During degeneration and regression of corpus luteum, reactive oxygen species such as superoxide, hydrogen peroxide and lipid peroxide are produced (Sawada & Carlson, 1989). The presence of these reactive agents is harmful to the function of cell, membrane and enzyme (Pryor, 1982) and in this case, the presence of the agents can leads to the failure of luteal function, damaging the luteal cell membrane and interference of progesterone production (Gatzuli *et al.,* 1991). It has been discovered that the vitamin E level is increased in the ovary as the corpus luteum develops and regresses (Aten *et al.,* 1992). Vitamin E acts as a defence agent, protecting components in the ovary from free radical activities. It also prevents peroxidation of lipid during steroidogenesis (Aten *et al.,* 1994). Therefore, vitamin E is important for maintenance of normal ovarian function.

TABLE 1 Average gestation period from rat treated with different doses of palm vitamin E.

Treatment Dose Palm Vitamin E	**Average Gestation Period (Average± SEM) Day**
Control	22.200 ± 0.200
Dose 100mg/kg b.w.	22.222 ± 0.222
Dose 250mg/kg b.w.	21.889 ± 0.200
Dose 500mg/kg b.w.	21.889 ± 0.200
Dose 1000mg/kg b.w.	21.950 ± 0.153

TABLE 2 Number of pups delivered from rat treated with different doses of palm vitamin E.

Treatment Dose Palm Vitamin E	**Number of Pups Delivered (Average± SEM)**
Control	9.571 ± 0.948
Dose 100mg/kg b.w.	9.667 ± 0.726
Dose 250mg/kg b.w.	8.000 ± 1.195
Dose 500mg/kg b.w.	8.125 ± 1.109
Dose 1000mg/kg b.w.	8.368 ± 1.920

Base on the data obtained, palm vitamin E administered to female experimental animals does not interfere with the process of follicular phase (before ovulation) and luteal phase (after ovulation) during ovarian cycle. Furthermore, it gives extra protection to the ovary from free radicals activities and

prevents lipid peroxidation in maintaining normal ovarian function.

2. Implantation

In general, it is shown that the exposure of experimental dose does not have any adverse effect towards the implantation process as that there is no significant difference ($p>0.05$) in comparison to that of the control. Study by Kaempf-Rotzoll *et al*. (2002) discovered that α-tocopherol plays a role in the process of implantation via the action of α-TPP (α-tocopherol transfer protein). The finding of Hurley and co-workers (1983) revealed that the administration of vitamin E (22312.5 IU/kg diet) does not influence the number of implantation in pregnant experimental animals supplied with low vitamin E diet. The same finding is also obtained by Norfilzar *et al.* (2001), that is, the exposure of 60mg/kg palm vitamin E does not interfere with the implantation as well as embryos developmental process.

The failure of pregnancy is demonstrated by the absence of implantation site on the uterus (Harazono *et al.,* 1996). Study by Feussner *et al.* (1992) showed that rabbits with incidence of early abortion have little implantation site.

Palm vitamin E perhaps plays a role in preparation of the uterus for implantation. This is in concomitant with the finding of Ledee-Bataille *et al*. (2002), which they discovered that administration of vitamin E (1000 IU/day) for sixty days increases pregnancy rates of subject with thin uterus endometrium wall by increasing the thickness of the endometrium and prepares it for implantation.

Based on the data obtained from the study, it is discovered that experimental doses do not have an effect on the number of implantation. It shows that the administration of experimental dose of palm vitamin E does not cause failure or miscarriage in the pregnant rats.

3. Resorption Rate

The normal range of resorption of rats' embryos is eight to ten. Hence, from the range given, it is concluded that administration of experimental dose of palm vitamin E has no effects on the process of embryos development. Furthermore, the experimental dose has the potential to decrease the occurrence of resorption of the embryos.

Administration of vitamin E is crucial in the reproduction process for continuation of pregnancy and for nourishment of pregnant dams (Machlin, 1984). In rats, the occurrence of resorption is related to the abortion of foetus. Foetus failed to survive at the implantation site will then be absorbed by the maternal uterus wall (Melby & Altman, 1974). Viana and co-worker (1996) discovered that the administration of vitamin E and butylated hydroxytoluene decreases the rate of anomalies and resorption of embryos of type II diabetic rats. Research by Cegerberg and co-workers (2001) revealed that administration of both vitamin E and C also decreases the rate of abnormalities and resorption of diabetic rats' foetus. Martin and co-workers (1977) discovered that over-consumption of vitamin E does not have any impact on the resorption rate on pregnant rats or the number of pups born.

Development of the Newborn Rats

1. Gestation Period

As of the results, it is suggested that the experimental dose of palm vitamin E has no adverse effect on embryogenesis and implantation process during pregnancy. The prolong duration of pregnancy that occurred could be the in relation with the delay of implantation process or during the cleavage phase (Card & Mitchell, 1979; Hammer & Mitchell, 1979). Experiment with lower dosage of palm vitamin E (60mg/kg b.w.) has been reported to have no effect on the duration of the pregnancy of the rat (Rajikin *et al.,* 2001).

2. Number of Pups Delivered, Abnormalities and Growth Development

As of the results, it is suggested that the experimental doses of palm vitamin E exposed do not have any effect on the number of pups delivered. The similar result was reported by Norfilzar *et al.* (2001), which the administration of 60mg/kg b.w. of palm vitamin E did not exhibit any adverse effect on embryos or the number of pups born. The result acquired is in concomitant with the previous finding, of which the palm vitamin E exposed during pregnancy does not affect the development of the foetus.

Observation made toward the development of the pups shows that the experimental doses of palm vitamin E given to the pregnant dams do not affect the body weight and level of development (crump rump length, the upper and lower limbs, the tail and the circumference of the head) at PD 3 and PD 7. Study by Martin & Hurley (1977) demonstrated that exposure of vitamin E (22.5 to 2252 mg/kg/day) on pregnant rats has no abnormal or teratogenic effects on the pups born.

The role of vitamin E in prevention of abnormalities on pups is from the stage of embryogenesis. If there were interference on embryogenesis, the percentage of abnormal pups born would be high. There are numerous researches regarding the action of vitamin E on the protection of the embryos from the free radicals and oxidative pressure. These free radicals have the potential to attack the embryos' chromosomes that leads to malformation of the pups (Young, 1992).

Study by Rajikin and co-workers (2001) revealed that the administration to pregnant rats of the palm vitamin E (60mg/kg b.w) has the protective effect against nicotine (source of free radicals). This has increased the status of fertility and percentage of the normal pups born from the nicotine exposed dams. It is known that vitamin E is a potent anti-oxidant, protecting the embryos from free radicals (Aruoma, 1999).

In summary, palm vitamin E in this study (100 to 1000mg/kg b.w.) is crucial in the reproduction and development of the pups in general.

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