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El Darawany A.A.

in

Testik A. (ed.), Baselga M. (ed.).
2. International Conference on Rabbit Production in Hot Climates

Zaragoza : CIHEAM
Cahiers Options Méditerranéennes; n. 41

1999
pages 113-117

TERATOGENICITY AND SEXUAL DEVELOPMENT OF EXOGENOUS MELATONIN IN RABBIT OFFSPRING

A.A.El-Darawany
Department of Animal Production, Faculty of Agriculture
Zagazig University, Zagazig, Egypt

SUMMARY- An investigation was carried out to study the teratogenic effect and sexual development of melatonin treatment on Bauscat rabbit offspring. Results obtained revealed that pregnant doe rabbit injected with 50 or 75µg/kg body weight suffered from low implantation sites and the high percentage of resorbed foeti. Concerning the foetal malformation, the foeti were malformed in their skull, vertebral column, ribs and pelvic bones, as well as, fore and hind limbs. However, melatonin injection with 25µg/kg for doe did not prove teratogenic effect on rabbit embryos. The highest frequency of overdue does showed after injection with any dose of melatonin. The mother's melatonin treatment can act on offspring sexual development.

Key words: Melatonin, malformation, embryo, sexual development

INTRODUCTION

Embryo is the first stage of individuals and consequently population development. The melatonin passes from the mother to the fetus, because melatonin readily crosses almost every biological barrier, including the placenta (Reppert *et al.,* 1979 and Lopez *et al.,* 1995). However, the teratogenic effect and sexual development of melatonin treatment on rabbit embryo was not studied. Therefore, the main objective of this investigation was the evaluation of a melatonin treatment on rabbit offspring.

MATERIALS AND METHODS

This work was carried out in San El-Hagar Agriculture Company farm, in San El-Hagar area of Sharkeya Province, Egypt. The present investigation was carried out on forty Bauscat does at their fourth parity and aged 8-10 months. The animals were reared under similar environmental conditions (spring season, starting April 1997). The animals were lighted 12-14 hours daily throughout the experimental period. The average of maximum and minimum ambient temperature indoors was 26.3±1.3 and 19.1±0.9°C respectively. Animals were fed *ad libtum* on a commercial pelted rabbit diet containing 180g/kg crude protein 140g/kg crude fiber and 10.88MJ/kg of digestible energy. Fresh water was provided from automatic nipple drinkers. The does were divided into equal four groups (10 does for each group). Melatonin was dissolved in a minimum of absolute ethanol and diluted in 0.9% Nacl (1:9 v/v). The first group received ethanol/saline alone as control. The other three groups were treated daily with a s.c. injection of 25,50 and 75µg melatonin/kg body weight respectively. Melatonin daily treatment was given at 07.00 hours. All groups were injected for five successive days during the organogensis period of their foeti, e.g. form the 9^{th} to 13^{th} days of pregnancy (Wilson, 1969). Twenty females (5 does for each group) were sacrificed at 28^{th} day of gestation. The uteri were dissected out, morphologically examined and opened. The foeti were collected, counted, weighed and the body length was measured from the middle of the horizontal line between the two ears till last coccygeal vertebrae along the vertebral column. The resorption sites were also counted as described by Cook and Weather (1968). The foeti were stained with alizarin red "S" as explained by Steptes and Schnell (1964). Skeletal abnormalities of the foeti in comparison with the skeletal normalities as control were recorded. The other twenty females (5 does for each groups) were allowed delivery. After parturition, does were classified according to the gestation length to: preterm = 26 to 29 days, full-term = 30 to 32 days and overdue does = 33 days or more. The litter size estimates were classified to males and females by examining the external organs of their genital tract throughout the first week after birth. Pups remained with mother until weaning on day 25 (birth = day 0). Blood samples were collected into

glass tubes from the marginal ear vein of all male and female offsprings on: infantile period (between 10 and 30 day, animals being collected on day 25), juvenile or prepubertal period (extends from month to three months, animals being collected on day 75), pubertal period (extends from three months to four months, animals being collected on day 105) and at sexual maturation (animals being collected on day 150). The blood samples were kept at 4°C for 6 hours in a refrigerator. Sera were separated by centrifugation for 20 min. at 1000g and stored frozen at –20°C until analyzed. Serum concentration of LH and FSH were determined in all samples by radioimmunoassay kits produced by Diagnostic Product Corporation (Los Angles). The sensitivity of the assay was 18 pg LH/ml and 13 ng FSH/ml. Intra-and inter assay coefficients of variation were 3.8 and 9.0% in LH respectively and 2.6 and 9.5% in FSH respectively.

Statistical analysis was conducted according to Snedecor and Cochran (1982). Proportion data were analysis with Chi-square test (Fleiss, 1981).

RESULTS

Table 1 shows, the foetal changes in pregnant does exposed to melatonin treatment for 5 successive days from 9th to 13th day of gestation. It could be seen that melatonin treatment (50 and 75µg) significantly (P<0.01) decrease the implantation sites, but, the resorbed foeti means and percentages and foetal weight and length were in contrast significantly (P<0.01) increased as compared to the other groups. The malformation in skeleton was recorded in Table 2. Melatonin treated does with 50 and 75µg/kg produced malformation in skull, vertebral column, ribs and limbs. The highest percentage of malformation in such an experimental group was found in the limbs.

Table 1. The foetal changes at 28th day gestation period in pregnant does daily treated with melatonin from 9th to 13th days of gestation

Variable	Control	Melatonin groups µg/kg		
		25	50	75
No. of does	10	10	10	10
Implantation sites	9.2^{a}±0.13	9.0^{a}±0.13	5.2^{b}±1.6	3.3^{c}±1.8
Resorbed foeti: Mean	0.5^{a}±0.01	1.0^{a}±0.08	4.2^{b}±1.1	6.2^{c}±1.3
%	6.3	8.5	52.3	62.7
Foetal weight (gm)	56.2^{a}±4.2	58.8^{a}±5.3	65.6^{b}±8.2	68.1^{b}±6.2
Foetal length (cm)	9.3^{a}±0.18	9.8^{a}±1.8	11.2^{b}±2.6	12.1^{b}±1.3

Means in the same raw having the same letter did not differ significantly, otherwise they differ (P<0.01).

Table 2. Foetal skeletal changes at 28th day of gestation in pregnant does daily treated with melatonin from 9th to 13th days of gestation.

Variable	Control	Melatonin groups µg/kg		
		25	50	75
No. of foeti	73	66	47	39
Malformed in:				
Skull No.	-	-	11.0	18.0
%	-	-	23.4	46.6
Vertebral column No.	-	1.0	8.0	16.0
%	-	1.5	17.0	41.0
Ribs No.	1	1.0	12.0	13.0
%	1.4	1.5	25.5	33.3
Limbs No.	3.0	2.0	25.0	28.0
%	4.1	3.0	53.2	71.8

Table 3 summarizes the reproductive performance for all group. The highest percentage of delivery rate was found in control group then followed treated does with 25µg melatonin. The highest proportion of perterm parturition does was found in control does but, full term parturition does was found in control does and treated does with 25µg melatonin but the highest proportion of overdue does was found in treated does with 50 and 75µg melatonin. The litter size at birth was higher ($P<0.01$) in control does and treated with 25µg melatonin. Treated does with 75µg melatonin was accompanied with higher percentage of males than females when compared to others. Mean pup weight at birth was higher ($P<0.01$) in treated does with 50 and 75µg melatonin than others. The proportion of stillbirth, pre-weaning and total mortality were higher ($P<0.01$) in treated does with 75µg melatonin then followed with 50µg melatonin than others.

Table 3. Reproductive performance of Bauscat does as affected by injection with melatonin from 9^{th} to 13^{th} day of gestation period

Variable	Control	Melatonin groups µg/kg		
		25	50	75
No. of does	15	15	15	15
Delivery rate	87	80	75	75
Classification of does proportion of:				
Preterm	0.15	0.08	0.10	0.11
Full-term	0.69	0.67	0.26	0.29
Overdue	0.16	0.25	0.64	0.60
Litter size at birth:				
Means	$7.8^{a}\pm0.6$	$7.1^{a}\pm0.3$	$5.1^{b}\pm0.8$	$3.2^{c}\pm1.6$
Male %	51.1	50.8	52.1	53.2
Female %	48.9	49.2	47.9	46.8
Pup weight at birth (gm)	$61.8^{a}\pm3.6$	$63.2^{a}\pm5.2$	$68.3^{b}\pm6.1$	$70.2^{b}\pm3.8$
Proportion of:				
Stillbirths	$0.12^{a}\pm0.01$	$0.16^{a}\pm0.03$	$0.22^{b}\pm0.10$	$0.32^{c}\pm0.09$
Pre-weaning mortality	$0.06^{a}\pm0.02$	$0.12^{b}\pm0.05$	$0.23^{c}\pm0.12$	$0.41^{d}\pm0.08$
Total mortality	$0.18^{a}\pm0.03$	$0.28^{b}\pm0.13$	$0.45^{c}\pm0.13$	$0.73^{d}\pm0.19$

Means the same raw having the same letter did not differ significantly, otherwise they differ ($P<0.01$).

Serum LH levels of control offspring males and females (figure 1 and 2) exhibited great variability throughout the postnatal development, with low concentration during the infantile period (403 ± 82.1 and 561 ± 101.75 pg/ml in males and females respectively) and increased basal secretion during juvenile period (1300 ± 97.21 and 2871 ± 216.1 ng/ml in male and female respectively). These values returned to lower levels during pubertal period of the sexual development. At the beginning of sexual maturition (150 days), higher LH serum values were observed (1116 ± 183.1 and 2911 ± 290.1 ng/ml in males and females respectively). In the all males and females offspring of melatonin treated rabbits, the peak in the juvenile period was significantly higher ($P<0.01$) than levels found at other period studied. The peak of LH males and females offspring during the juvenile period was significantly higher ($P<0.01$) than that of the control group and the values remained elevated during pubertal period. Thus the decrease of LH appeared to be delayed until the beginning of the sexual maturation (150 days), when significantly lower value than in control groups males or females.

Serum FSH concentrations (figure 3 and 4) from the infantile period until the beginning of the sexual maturation also showed variations having significantly higher ($P<0.01$) values at the juvenile phase in the four groups to all other time points studied in males and females offspring. Serum FSH levels in the all offspring of melatonin treated rabbits were reduced significantly ($P<0.01$) at all period studied when compared to control group.

DISCUSSION

The clear teratogenic effect of melatonin treatment (50 or 75µg) on rabbit embryos may be due to melatonin passes through placental barrier and causes its effect in F_1 generation. The skeleton malformation may be due to unbalance hormones or enzymes concerned. Reppert *et al.* (1979) who showed that melatonin readily crosses almost every biological barrier, including the

placenta. Moreover, it increased the foetal weight and length might be due to lowering in the implantation sites in melatonin treated rabbits. On the other hand, using of 25µg melatonin for animal treated did not yield teratogenic effect in rabbit embryo's since 25µg decreased the concentration of teratogenic substances.

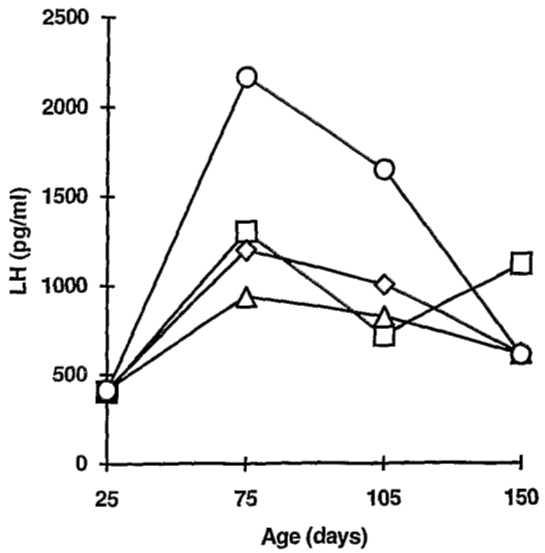

Fig. 1 Serum Luteinizing hormon (LH) levels of male rabbit offsring of control (□), 25µg/kg melatonin treated (Δ), 50µg/kg, (◊) 75µg/kg (o).

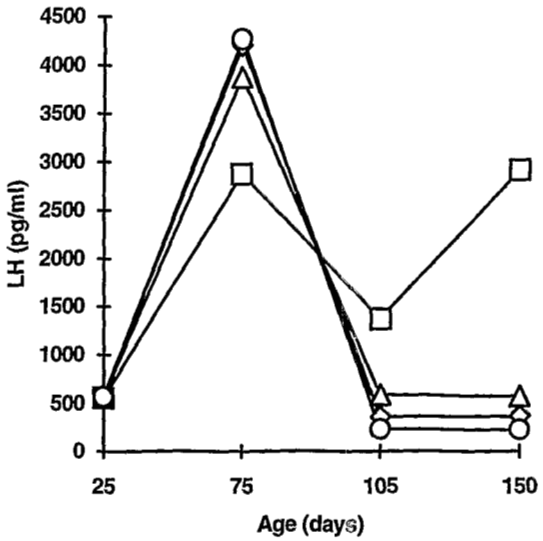

Fig. 2 Serum Luteinizing hormon (LH) levels of female rabbit offsring of control (□), 25µg/kg melatonin treated (Δ), 50µg/kg, (◊) 75µg/kg (o).

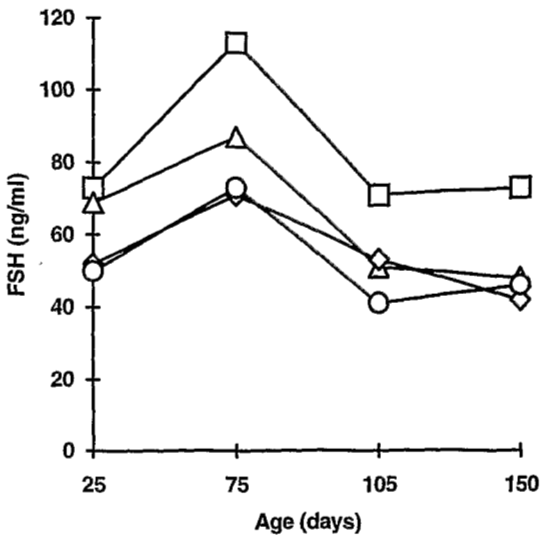

Fig.3 Serum follicle stimulating hormone (FSH) levels of male rabbit offspring of control (□), 25µg/kg melatonin treated (Δ), 50µg/kg, (◊) 75µg/kg (o).

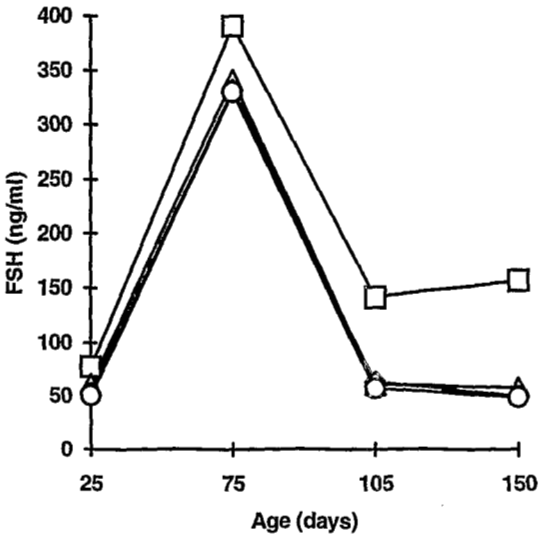

Fig.4 Serum follicle stimulating hormone (FSH) levels of female rabbit offspring of control (□), 25µg/kg melatonin treated (Δ), 50µg/kg, (◊) 75µg/kg (o).

Our results show a different gestation length between control and melatonin treated does, which could be considered as an indicator that melatonin secretion is involved in the endocrine control of parturition. This is in agreement with Lopez *et al.,* (1995) in rat. Moreover, the highest frequency of overdue does after treatment with 50 or 75µg melatonin might be due to, a lowering implantation sites which lead to decrease litter size at birth (El-Darawany, 1994 and Farghaly, 1996). Post-mature litter had a higher proportion of pups born dead, which could be of significant economic importance in commercial units. Our results show a high dose of melatonin, having an effective role on rabbit sex ratio, this may be due to the affinity of ova to Y spermatozoa. However, a different pattern of LH secretion in the males or females offspring of melatonin treated does was observed at 75 and 105 days of age, LH levels were still elevated, suggesting that prenatal melatonin treatment affects the normal development of the neuroendocrine-reproductive axis. The increase of serum LH at maturation males or females offspring control was also observed. This is probably the result of the disappearance of direct central nervous inhibition of LH secretion (Docke *et al.,* 1980). On the other hand, melatonin injection during pregnancy were inhibitory to FSH levels during the infantile, juvenile, pubertal period and maturity as compared to control rabbits, this would affect the sexual maturation of the rabbit by reducing FSH stimulation of leydig cells (Odell and Swerdloff, 1974). Sexual development in male and female control rabbits progressed rapidly, as shown by the significantly increased LH and FSH levels at different period studied as compared to melatonin treated rabbits. It could be concluded that melatonin should not be used in organogensis period during pregnancy due to its teratogenic effect on rabbit embryo and increased proportion of pup born dead, as well as, melatonin injection during pregnancy influence the ontogeny of the hypothalamus-pituitary-gonadal axis during intrauterine life. These changes results in alterations in gonadotropin secretion of the male and female rabbits during sexual development.

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