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Evaluation of the Estral Cycle in Rats after Treatment with Dexamethasone for Polycystic Ovaries, Induced by Constant Illumination

Evaluación del Ciclo Estral en Ratones Después del Tratamiento con Dexametasona para Ovarios Poliquísticos, Inducidos por Iluminación Continua

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SUMMARY: The study aimed at obtaining basic information about estrous cycle in rats treated with dexamethasone, for polycystic ovaries, induced by constant illumination. It was used 30 female rats (Rattus norvegicus albinus) from the lineage Wistar, with 90 days years old, divided according the following groups: Group I - rats maintained in a light/dark cycle for 12/12 hours, and after 100 days submitted to the cyclicity evaluation (control); Group II - rats maintained under constant illumination during 100 days and after submitted to the cyclicity evaluation; Group III - rats maintained under constant illumination during 100 days and after treated with dexamethasone for five days, and, then, submitted to the cyclicity evaluation. The results showed that after 100 days of experiment, the animals from group I presented a normal cyclicity, being observed the four phases of the cycle. In the animals of groups II and III, it was observed a higher incidence in the estrous phase, with 85% and 76,5% respectively, characterizing the state of permanent estrous. It was observed the phase of diestrous with 15% in group II, and 23,5% in group III, not being observed the phases of proestrous and metaestrous. After treatment with dexamethasone, it was verified a great reduction in the estrous phases in the animals from group III, what was also observed in the animals from group II, reaching numbers of 34,5% and 20,85%, respectively. Yet, there was an increase in the diestrous phase in group II (64,57%), and group III (75%). It has been noticed in these groups the presence of the proestrous phase with 0,92% in group II and 4,15% in group III, not being observed the metaestrous phase. The treatment with dexamethasone during five days produces, more rapidly, a possible retake of the estrous cycle in rats with ovarian polycystic ovaries.

KEY WORDS: Estrous cycle; Polycystic ovaries; Dexamethasone; Rats.

INTRODUCTION

Rodents are animals from the polyestrous type, i. e., they present five regular and successive estrous cycles, which are manifested through morphological changes in the ovaries, uterus, vagina and mammal glands. (Long & Evans, 1922; Astwood, 1939; Griffith & Farris, 1942; Mandl, 1951; Bertalanffy & Lau, 1963; Mozanska, 1972; Simões, 1979, 1984).

The literature reports that several factors can lead to the interruption of the estrous cycle and, because of this, the permanent estrous in rats is induced. Among these factors, constant illumination, sound, steroid hormones use in newly born, ionizing radiation exposition and pinealectomy are highlighted. (Pfeiffer, 1936; Everett, 1939; Barraclough & Leathem, 1954; Martins *et al.*, 1962; Singh, 1969; Wrenn et al., 1969; Aihara *et al.*, 1988; Pardi, 1992; Patriarca, 1995; Santos *et al.*, 1995).

The state of permanent estrous and, consequently, the loss of cyclic character of sexual activity constitute one of the most interesting phenomena in the endocrinology of reproduction (Rodrigues Lima, 1966). Due to this fact, it is observed an increase in the production of androgens and estrogens in the ovaries of rats (Chiórboli, 1970).

It is known that constant illumination inhibits the synthesis of melatonin through the pineal gland, in which it is produced in the environmental darkness phase, as there is a diurnal harmony in the liberation of melatonin (Buckle, 1983; Neves, 2000).

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When rats are exposed to a continuous luminous stimulus, or pinealectomized, they enter a state of permanent estrous and develop polycystic ovaries (Singh; Prata Lima *et al.*, 1995).

Studies have shown that the glycocorticoid hormones inhibit growth in a general way and its effects can be observed in the reproductive functions. Several authors report that the glycocorticoid, among them the dexamethasone, inhibit the number of induced answers by the estrogens, including the blocking of myotic activity (Szego & Roberts, 1953; Szego & Davis, 1969; Campbell, 1978; Markaverich *et al.*, 1981; Stewart *et al.*, 1983; Bigsby & Cunha, 1988).

According to Queiroz (2004), rats with polycystic ovaries treated during five and ten days and then euthanasiated present a reduction in the amount of ovarian cysts, though without the formation of luteal bodies. However, by administering dexamethasone for a period of 15 days, it was verified a reversion in the polycystic ovaries due to the presence of great amounts of luteal bodies, indicating, thus, a possible retaken of the ovarian cycle. So, before the exposed, the present study aimed at obtaining basic information about the estrous cycle in rats treated with dexamethasone.

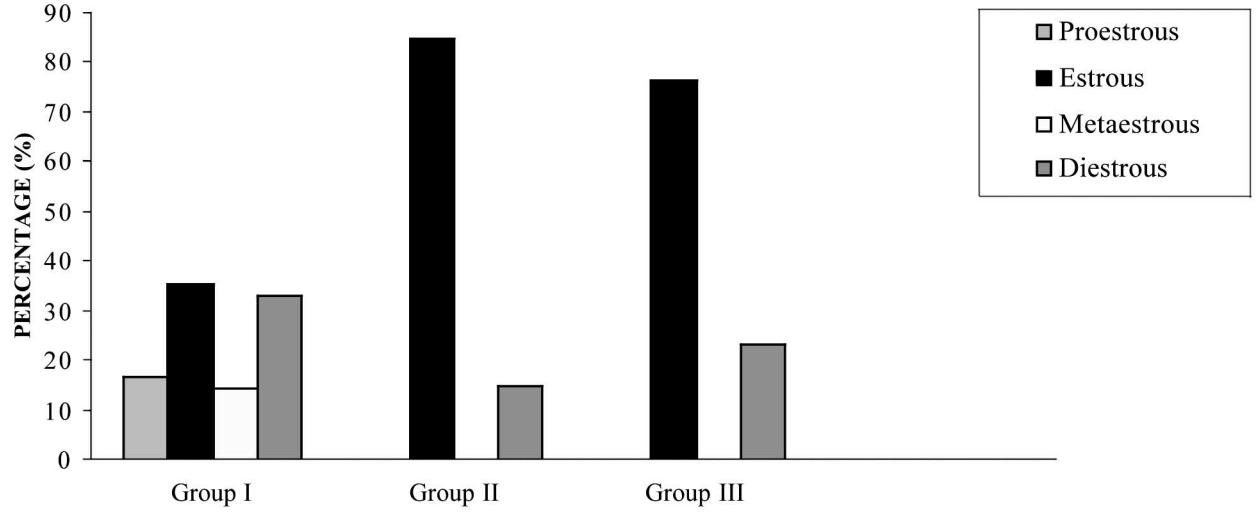
MATERIAL AND METHOD

It was used 30 albino rats (*Rattus norvegicus albinus*), 90 days old, virgin, weighing nearly 200g, from the Wistar lineage, from the vivarium of the Department of Morphology and Animal Physiology from Universidade Federal Rural de Pernambuco. The animals were confined in cages and maintained under food and water ad libitum, temperature of 22º C and artificial lightning using fluorescent lamps (brand name Phillips, daylight model, 40W), which established the photoperiod of 12 hours light and 12 hours dark, considering the period with light from 6 a.m. to 6 p.m. After a period of adaptation, vaginal smears were collected for the determination of the estral cycle. The animals that presented three regular estrous cycles were divided randomly in three groups, each of them constituted by 10 animals:

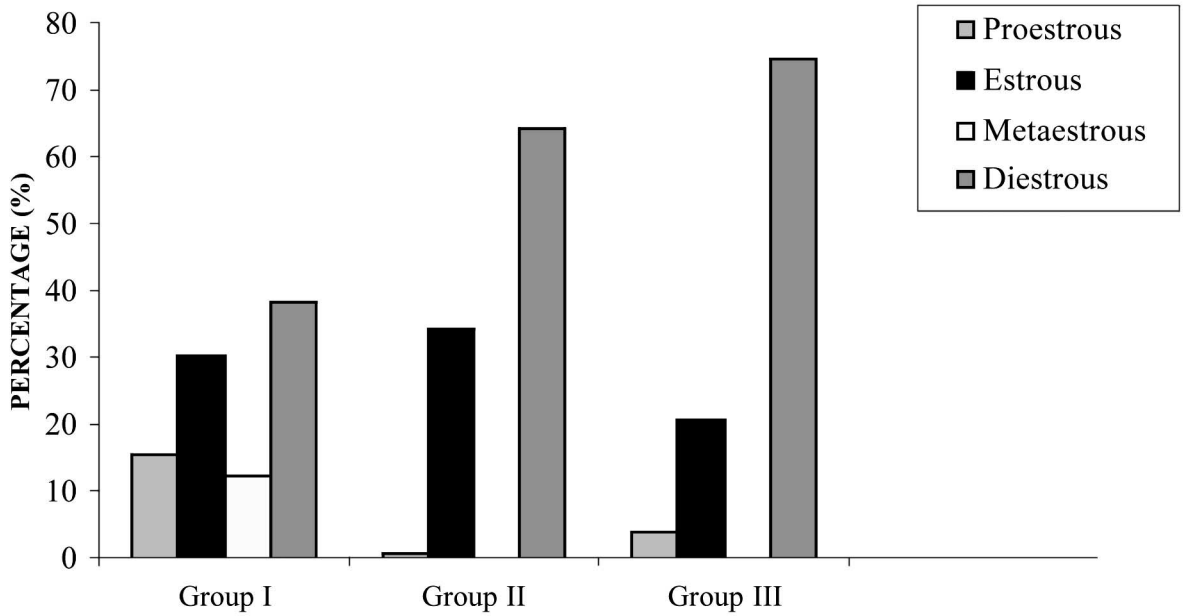
Group I - rats maintained in light/dark cycle (12/12 hours), and after 100 days submitted to the evaluation of cyclicity (control); Group II - rats maintained under constant illumination during 100 days, and then submitted to the evaluation of cyclicity; Group III - rats maintained under constant illumination during 100 days, and then treated with dexamethasone for five days and submitted to the evaluation of cyclicity.

For the constant luminous stimulus, it was used a box of wood measuring nearly 0,5m^3, appropriately dimensioned and ventilated, containing two lamps (Phillips, daylight model, 20W each) which provided 400 Lux in the place occupied by the animals. After 100 days of the experiment, colpocytological exams were done in group I, for the confirmation of cyclicity, and in groups II and III for the confirmation of permanent estrous. After the treatment with dexamethasone, all groups were submitted to exams. The treatment with dexamethasone was done using dexamethasone dissodic phosphate (Decadron ®), in a concentration of 4mg/ml, in which it was administered by means of intraperitoneal injections in a dosage of 0,2ml/day/animal, according to the methodology described by Cruz *et al.* (1996).

Fig. 1. Frequency of the estrous cycle in the animals from experimental groups after 100 days.



Graphic II. Frequency of the estrous cycle phase in the animals from experimental groups after treatment with dexamethasone.



RESULTS

The colpocytological exams showed that the animals from group I presented a normal cyclicity, being observed the four phases of the cycle. Yet, in the animals from groups II and III, it was observed a higher incidence in the estrous phase with a percentage of 85,00% and 76,50%, respectively, characterizing the state of permanent estrous. Also, it was observed in these groups the diestrous phase with a percentage of 15,00% in group I, and 23,50% in group III, not being observed the proestrous and metaestrous phases (Fig. 1.).

The colpocytologic exams after the treatment with dexamethasone showed that the animals from group I also presented a normal cyclicity, being observed the four phases of the cycle. However, the animals from groups II and III were observed as having a stressed incidence in the estrous phase, with a percentage of 34,50% and 20,85%, respectively, and the increase of the diestrous phase with 64,58% for group II, and 75,00% in group III. It was also noticed the presence of the proestrous phase with a percentage of 0,92% in group II and 4,15% in group III, not being observed the metaestrous phase (Fig. 2.).

DISCUSSION

The constant illumination promotes reduction in the levels of melatonin hormones, and elevates the levels of circulating estrogens causing, consequently, the permanent estrous phase. The analysis of the estrous cycle in the animals of groups II and III after 100 days of the experiment showed a higher incidence in the phase of the estrous, reaching more than 70% of the cycle, characterizing the disruption of cyclicity. These results are according to the ones obtained by Singh, Santos *et al.* and Queiroz.

Regarded to the reduction of the estrous phase and increase of the diestrous phase, besides the appearance of the proestrous phase in the animals of group II, observed in graphic 2, this can be related to the retaken of the nocturne release of melatonin in these animals, because, according to Johnson *et al*. (1982), its levels vary according to the different estrous cycle phase of rats, being higher during the metaestrous and diestrous. Besides, the highest incidence of estrous in rodents, produced by constant illumination, can be reverted by transference for shorter photoperiods or by the administration of melatonin (Gittes & Chu, 1965; Teixeira *et al*., 2002). However, it must be

mentioned that in group III (treated with dexamethasone) the reduction in the estrous phase, as well as the increase of the diestrous and proestrous phases was more stressed if compared to group II, indicating a possible retaken of the estrous cycle. This fact must be related to the stimulating action of the dexamethasone over the release of melatonin, as, according to Luboshitzky, *et al.,* (2000) and Barriga *et al.* (2002), it has been observed that rats pinealectomized and treated with melatonin, right after or in the end of a 3 months period, the estrous cycle was regular, indicating a regulatory activity of melatonin in the maintenance of the estrous cycle. This way, the dexamethasone can have exerted an indirect effect to quicken the return of the estrous cycle in these animals.

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RESUMEN: El objetivo del estudio fue obtener información básica del ciclo estral en ratones tratados con dexametasona, en ovarios poliquísticos, inducidos por iluminación continua. Fueron utilizados 30 ratones albinos (*Rattus norvegius albinus*) del linaje Wistar, con 90 días de edad, divididos en los siguientes grupos: Grupo I – ratones mantenidos en ciclo claro/oscuro de 12/12 horas, tras 100 días sometidos a la evaluación de la ciclicidad (control). Grupo II – ratones mantenidos bajo iluminación continua, durante 100 días y luego sometidos a la evaluación de la ciclicidad. Grupo III – ratones mantenidos bajo iluminación continua, durante 100 días, después tratados con dexametasona durante cinco días y sometidos a la evaluación de la ciclicidad. Los resultados mostraron que tras 100 días de pruebas, los animales del grupo I presentaron una ciclicidad normal, siendo observadas las cuatro fases de éste. En los animales de los grupos II y III se verificó una mayor incidencia de fase de estro, con el porcentaje de 85% y 76,50% , respectivamente, caracterizando el estado de estro permanente. Fue observada la fase de diestro en el 15%, en el grupo II, y 23,5% en el grupo III, no siendo observadas las fases de proestro y metaestro. Posterior al tratamiento con dexametasona, se verificó una reducción acentuada en la fase de estro en los animales del grupo III, lo que también fue observado en los animales del grupo II , alcanzando un 34,5% y 20,85, respectivamente. Hubo incluso aumento de la fase de diestro en el grupo II (64,58%), y grupo III (75%). Notamos en esos grupos, la presencia de la fase de proestro en el 0,92% en el grupo II y 4,15% en el grupo III, no siendo observada la fase de metaestro. El tratamiento con dexametasona durante cinco días, produce más rápidamente una eventual vuelta del ciclo estral en ratones con poliquistosis ovárica.

PALABRAS CLAVE: Ciclo Estral; Ovarios Poliquísticos; Dexametasona; Ratones.

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