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Polycystic Ovarian Syndrome: temporal characterization of the induction and reversion process in an experimental model

Síndrome do Ovário Policístico: caracterização termporal do processo de indução e reversão em um modelo experimental

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Abstract

Numerous experimental models have been developed for the study of the polycystic ovarian syndrome in the rat. In the present study, the syndrome was induced by exposure to constant light. The process was evaluated during its induction and also during its reversion. The estral cycle was analyzed through the vaginal cytology; reproductive parameters were evaluated by mating, as well as the ovarian morphology. All the animals developed the syndrome after 13 weeks of permanent light. The histologic characteristics of the ovaries, at week 15, were similar to those observed in the polycystic ovarian syndrome in human and other species. After regression of the syndrome, there was not difference in any of the evaluated reproductive parameters, when compared with the control group.

Key-words:
Histopathology.
Ovary.
PCOD.
Polycystic ovarian syndrome.
Rat.

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Introduction

The follicular cysts affect approximately 7 to 13 percent of dairy cows during post-partum. As a result, the interval parturition-conception becomes significantly longer.[1] This causes important losses in the dairy industry and in the cattle production in general. On the other hand, the cysts are also accompanied by the expression of receptive sexual behavior (*estrus*). This represents an additional cost for the fruitless artificial inseminations.[2]

In this sence, an animal model suitable to investigate the causes and the pathophysiology of the polycystic ovarian syndrome (POS) should have a clearly defined reproductive cycle. The ideal POS model should also have well-known anatomical, biological and biochemical features.[3] The laboratory rat (*Rattus norvegicus*) has been the subject of numerous studies. For that reason, it is the species on which histologic and physiologic aspects of reproduction are better characterized.

Numerous experimental models for POS have been developed in rats. The following hormonal substances, among others, have been employed to induce that syndrome: estradiol valerate, dehydroepiandrostenerone (DHA), and neonatal androgenization.[4,5,6,7]

In spite of their effectiveness, all these

hormonal treatments cause a sudden appearance of polycystic ovaries due to disturbances in the metabolic and physiologic processes. Thus, these models do not reproduce faithfully what happens in the real syndrome. The continuous administration of estrogens, like the valerate of estradiol, induces a progressive degeneration of the arcuate hypothalamic nucleus, which impairs the study of the hypothalamo - hypophyseal system.[5,8] The treatment with androgens (DHA) can interfere with liberation of luteinising hormone (LH) in the central nervous system. Therefore, this model may introduce variables that are not present in the spontaneous disease.[8,9] In fact, androgenized rats showed morphologic and endocrine patterns, which are not totally correlated with those of the POS. Consequently; androgenization could not be an appropriate model for the study of this illness.[10]

A relatively simple method to induce polycystic ovaries consists on exposing mature rats to an environment with constant light.[11] It induces cysts in a gradual form similar to what can it turns become the POS.

The last model that is also the least invasive of all the ones developed until now[12,13]. It can be used to investigate the etiology, pathogenesis and therapy of the POS. In fact, there are numerous endocrinologic and biochemical studies in polycystic ovaries induced by constant light.[14,15,16,17] There are marked discrepancies among different authors regarding the necessary exposure time to constant light to induce this syndrome and the reported periods varying from 7 to 14 weeks. Although the reversibility of the syndrome is mentioned in this model once eliminated the condition of constant light, there are not specific studies that endorse this fact.

The constant light-POS-model could provide a useful tool to test therapeutic and preventive measures for a serious reproductive disease in many animal species, including livestock. The present experience was conducted to contribute new information regarding the induction period and the reversion process.

Materials and Methods

Animals and treatment

All the procedures were carried out according to the Guide for the Care and Use of Laboratory Animals.[18] Wistar rats were provided by the Center for Experimental Biology and Laboratory Animals, Faculty of Veterinary Sciences, UNL. Before the experiment, the animals were kept with a controlled cycle of light-darkness (lights on between the 6:00 a.m. and the 8:00 p.m.), and to a temperature of 20-24º C with free access to water and commercial balanced food (Balanceados Costantino, Córdoba, Argentina). Fluorescent tubes were used to obtain an intensity of 350 lux to 1 meter on floor.[19]

Fifty-five female virgin puber rats were used. They were 8 weeks old with a weight of 160 g (+/- 20). The regularity in their estrous cycle was evaluated by colpocytology, carrying out daily vaginal smears during two weeks previous to the beginning of the experience.

Thirty of these animals were placed in the mentioned conditions except for the cycle of light that was extended to 24 Hs (continuous light), where they remained during 15 weeks. Then, the light was decreased again to a 14 daily hour period, to evaluate the processes of reversion of the syndrome, during 15 weeks. A parallel group of 25 animals, under normal environmental conditions, were used as controls.

Determination of the ovarian cycle

Vaginal changes reflect the presentation and regression of POS. Twenty of the thirty animals maintained under continuous light, were identified individually and examined daily during all the experience by means of vaginal smear according to Montes and Luque.[17] In this procedure, vaginal washing were examined under a microscope for the relative abundance of nucleates epithelial cells, cornified cells and leucocytes. Cycles with duration of 4 to 5 days were considered

regular. The observation of cornified cells in the smears during a minimum of 10 serial days was defined as persistent vaginal cornification (PVC).

Evaluation of the reproductive aptitude

The reproductive aptitude of the animals was analyzed after 15 weeks of continuous light and other 15 weeks in a normal cycle of light. Twenty females were mate with males of proven fertility. Because the estrous cycles were irregular, was difficult to define the stadium of the cycle. To overcome this trouble, each female was housed permanently with a male and daily vaginal smears were conducted. The animals that showed sperms were separated in individual cages. A group of animals of the same age was maintained under normal environmental conditions during all the experience. They were employed as controls and were matted similary.

The reproductive performance of both groups was compare, evaluating the following parameters: days of mating (defined as the interval since they were placed with the male until they showed sperms), percentage of fertile copulations (defined as those in which there were births), size of the litter, and percentage of weaned offspring's. The reproductive records of our files were used as reference values, considering only the values of the first pregnancy.

All the data are expressed as the media +/- standard deviation. The different groups were compared by means of a variance analysis (ANOVA) and the test of multiple ranges of Duncan.

Tissue sampling

Three animals maintained under continuous light were sacrificed at the end of the period of permanent light and 3 at the end of the experience. The ovaries were dissected and they fixed in 10% buffered formalin during 12 Hs, washed in buffer saline phosphate (PBS) and processed for inclusion in paraffin.[21] Four μm thick sections were mounted in glasses previously treated with VECTABOND (Vector Labs, Burlingame, USA) and stained with hematoxylin and eosin.

Results

Some animals showed irregular cycles at the second week of exposition to constant light. In the week 3 this was the case in 100%. The firsts animals with PVC were observed in the week 4, reaching 100% in the week 13 (Figures 1 and 2).

When one returned to conditions of normal light (14 hours per day), a persistence of the PVC was observed during the first week. It decreased gradually until disappearing in the week 13. In the week 15, it was observed that only 15% of the animals had returned to a regular cycle. When these animals were mated, they needed a longer mating period in comparison with the average of all the animals in the files of our Center. Anyhow, no differences were observed with the control group.

The offspring by parturition was

Table 1
Reproductive parameters evaluated after regression of polycystic ovarial condition. Animals that were exposed to permanent light (treated) are compared with the control group and also with the reproductive files of the Center

	Controls	Treated	Laboratory Records
Copulation days	3.29 +/- 1.26	4.15 +/- 1.69 [a]	2.51 +/- 1.14 [b]
% of fertile copulations	80	85	89.92
Litter size	6.75 +/- 2.86 [a]	6.35 +/- 2.86 [a]	8.55 +/- 1.91 [b]
% of weaned animals	93.67 +/- 9.84	91.21 +/- 10.67	96.11 +/- 6.99

Average +/- standard deviation.
Different letters means significant differences ($p<0,05$)

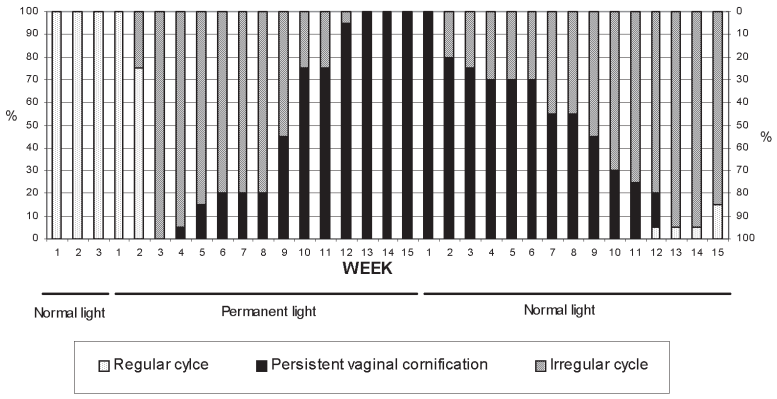

Figure 1
Regularity of the estrous cycles along the experiment. It was evaluated by means of the vaginal cytology. The results are expressed as percentage of animals in each condition

significantly reduced in both the exposed animals and in the control group when compared with previous registrations of the Center. Differences were neither observed in the percentage of fertile copulations or in the percentage of weaned animals (Table 1).

The ovaries of the animals sacrificed after the exposition during 15 weeks to continuous light showed a thick tunica albuginea, scarce primary and growing follicles and numerous atretic follicles. The tertiary follicles were considerably distended and cystic (polycystic ovaries) (Figure 3). The granulosa and theca cells appeared normal. Some stroma cells were hypertrophied. Corpora lutea were not observed. The ovaries corresponding to the animals sacrificed after the period of regression showed a high number of atretic follicles. No other alterations were evident.

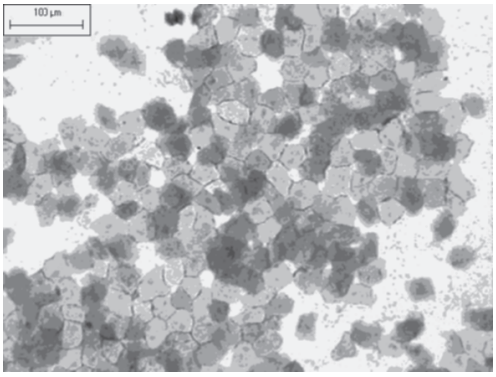

Figure 2
Cytological features of a vaginal flush of a rat with persistent vaginal cornification

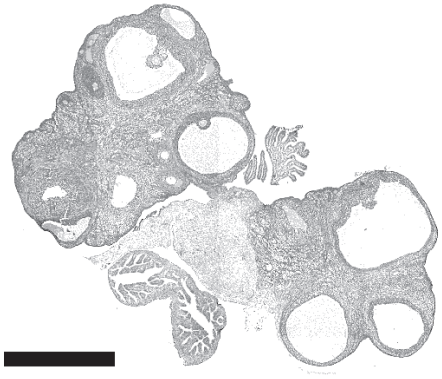

Figure 3
Ovarian histology of a rat after being exposed during 15 weeks to permanent light. There are clear cystic follicles and luteal tissue is absent. (BAR = 1mm)

Discussion and Conclusions

Follicular cysts have been widely studied particularly in relation to their diagnosis and treatment.[22,23,24,25,26,27,28] Nevertheless, relatively little is known about the mechanisms that cause their development.[17,29,30,31,32] The follicular cysts etiology and pathogenesis were the subject for numerous hypotheses since the middle of the XIX century. However, they are not still completely clarified at the present

time, neither in the bovine nor in other animal species.[33,34,35]

The cysts are more frequent in dairy cows and in a smaller proportion in beef cattle. A constitutional weakness and the high yield of milk are in the first place among the endogenous predisposing factors and they are more commonly observed in cows from 5 to 7 years of age and rarely in heifers. The illness generally appears in animals with high production, in the pick of the lactation, and 1 to 4 months after the parturition.[36,37,38]

Among the exogenous causal factors, it has been mentioned inadequate nutritional management (excessive use of concentrates), insufficient feeding and the incorrect supply of minerals and vitamins. Ovarian cysts are much more frequent in intensive farming. Other factors included housing of the animals with lack of exercise and deficiencies in illumination, as well as seasonal and climatic influences.[39,40]

Estrogenic substances are considered precipitating causes. According to Dolezel, Cech and Zajic[41] the administration of synthetic progestational hormones in the proestrus, as frequently do to synchronize the cycles for artificial insemination, could also cause ovarian cysts.

The POS has been defined as a progressive endocrinopathy characterized by disturbance in the hypothalamo – hypophyso – gonadal system that result in failures of the reproductive cycle. This disturbance can lead to a suppression of the synthesis and cyclic liberation of LHRH. In this regard, our results would indicate that in the rat initially it takes place a phase of irregular cycles. This observation agrees with other authors.[42]

On the other hand, it has been known for more than 60 years that the vaginal epithelium of the rat shows histological variations in response to the stimulation for ovarian steroids.[20,43] Our findings of persistent vaginal cornification agree with high levels of estrogens, which are characteristic of follicular cysts. It was corroborated by the histological study.

According to these results, the rats should be it a minimum period of 13 weeks (91 days) of exposition to continuous light to develop the POS. It has already been communicated that rats maintained with constant light developed PVC approximately after 100 days, with atrophic and cystic ovaries, and without corpora lutea.[3,44] However, our induction period was shorter than the one defines by.[45] He reported that rats maintained in constant light during 56 days possessed ovaries with mature follicles and corpora lutea; while they were necessary 250 to 300 days to obtain polycystic ovaries.

The morphological features of the ovaries in this model seem to be similar to those found in the polycystic disease in humans and other species. According to the literature, they would conserve the ability to respond to the stimuli of the FSH/LH, and LHRH.[3,45]

There were no differences, in the evaluated reproductive parameters, between the group exposed to permanent light and the control group. The differences among the data of both groups, in comparison with the files of our Center, could be due to other factors such as the age of the animals at the moment of their first mating.

Although irregularities in the estrous cycles were observed, probably caused by high levels of estrogens, the reproductive capacity of the females was conserved. Therefore, we can assume that the disturbances were reversible. Thus, this animal model could be useful for the evaluation of the induction and regression processes of the polycystic ovarian syndrome.

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Resumo

Numerosos modelos experimentais têm sido desenvolvimos para o estudo da síndrome do ovário policístico em ratos. No presente estudo, a síndrome foi inducida por exposição à luz constante. O processo foi avaliado durante sua indução e inclusive durante sua reversão. O ciclo estral foi analisado através de citologia vaginal; parámetros reprodutivos foram avaliados por acasalamento, bem como a morfologia ovariana. Todos animais desenvolveram a síndrome depois de 13 semanas de luz permanente. As características histológicas dos ovários, na semana 15, foram similares àquelas observadas na síndrome do ovário policístico em humanos e outras espécies. Após a regressão da síndrome, não houve diferenta em nenhum dos parámetros reprodutivos avaliados, quando comparados com o grupo controle.

Palavras-chave:
Histopatologia.
Ovário.
PCOD.
Síndrome do ovário policístico.
Ratos.

References

1.GARVERICK, H. A. Ovarian follicular cysts in dairy cows. **Journal of Dairy Science**, v. 80, p. 995-1004, 1997.

2.WEBB, R. et al. Dynamics and etiology of ovarian follicular cysts in post-partum dairy cattle. **Reproduction in Domestic Animals**, v. 33, p. 285-288, 1998.

3.MAHAJAN, D. K. Polycystic ovarian disease: animals models. **Endocrinology and Metabolism Clinics of North. America**, v. 17, p. 705-732, 1988.

4.ANDERSON, E.; LEE, G. Y. The polycystic ovarian (PCOD) condition: apoptosis and epithelization of the ovarian antral follicles are aspects of cystogenesis in the dehydroepiandrosterone (DHEA)-treated rat model. **Tissue and Cell**, v. 29, p. 171-189, 1997.

5.BRAWER, J. R.; MUÑOZ, M.; FAROOKHI, R. Development of the polycystic ovarian condition in the estradiol valerate-treated rat. **Biology of Reproduction**, v. 35, p.647-655, 1986.

6.KNUDSEN, J. F.; COSTOFF, A.; MAHESH, V. B. Dehydroepiandosterone-induced polycystic ovaries and acyclicity in the rat. **Fertility and Sterility**, v. 26, p. 807-817, 1975.

7.MAHESH, V. B.; MILLS, T. M.; BAGNELL, C. A.; CONWAY, B. A. Animal models for study of polycystic ovaries and ovarian atresia. In: MAHESH V. B.; DHINDSA, D. S.; ANDERSON, E.; KALRA S. P. **Regulation of ovarian and testicular function**. New York: Plenum. 1987. p. 237-258.

8.MARTINI L. New findings in neuroendocrinology. **Acta Physiologica of Latin America**, v. 32, p. 205-218, 1982.

9.KALRA, S. P.; KALRA, P. S. Neural regulation of luteinizing hormone secretion in the rat. **Endocrine Reviews**, v. 4, p. 311-351, 1983.

10.JONES, H. M.; VERNON, M. W.; RUSH, M. E. Systematic studies invalidate the neonatally androgenized rat as a model for polycystic ovary disease. **Biology of Reproduction**, v. 36, p. 1253-1265, 1987.

11.CRITCHLOW, B. V. The role of light in the neuroendocrine system. In: NALBANDOV, A. V. **Advances in neuroendocrinology**. Urbana, USA: University of Illinois Press, 1989.

12.SINGH, K. B. Induction of polycystic ovarian disease in rats by continuous light. **American Journal of Obstetrics & Gynecology**, v. 104, p. 1079-1083, 1969a.

13.SINGH, K. B. Persistent estrus: An experimental model of the polycystic ovary syndrome. **Obstetrics and Gynecology**, v. 24, p. 2-17, 1969b.

14.BREDSHAW, B. A.; CRITCHLOW, V. Pituitary concentration of LH in the three types of "constant estrus rats". **Endocrinology**, v. 78, p. 1007, 1966.

15.LOWTON, I. E.; SCHWARTZ, N. B. Pituitary ovarian function in rats exposed to constant light: A cronological study. **Endocrinology**, v. 81, p. 497, 1967.

16.TAKEO, Y.; KIHNO, J.; HOCANO, M. Ultraestructural evidence for estradiol synthesis in the ovary of persistence estrus rats exposed to continuous illumination. **Acta Anatomica**, v. 127, p. 161, 1986.

17.YOSHIOKA, K.; IWAMURA, S.; KAMOMAE, H. Changes of ovarian structures, plasma LH, FSH, progesterone and estradiol-17 beta in a cow with ovarian cysts showing spontaneous recovery and relapse. **Journal of Veterinary Medicine Science**, v. 60, p. 257-260, 1998.19.BELHORN, R. W. Lighting in the animal environment. **Laboratory Animal Science**, v. 30, p. 440-450, 1980.

18.NATIONAL RESEARCH COUNCIL. **Guide for the**

care and use of laboratory animals. Washington: National Academy Press, 1996. 125 p.
20.MONTES G. S.; LUQUE, E. H. Effects of ovarian steroids on vaginal smears in the rat. **Acta Anatomica**, v. 133, p. 192-199, 1988.
21.WOODS, A.; ELLIS, C. R. **Laboratory histopathology**. A Complete Reference. London: Longman Group Limited, 1994.
22.AGARWAL, S. K.; SHANKAR, U. Clinical response, related progesterone profile and subsequent fertility after gonadotrophin releasing hormone administration in crossbred cows with ovarian cyst. **Indian Veterinary Journal**, v. 75, p. 127-130, 1998.
23.CALDER, M. D. et al. Administration of progesterone to cows with ovarian follicular cysts results in a reduction in mean LH and LH pulse frequency and initiates ovulatory follicular growth. **Journal of Animal Science**, v. 77, p. 3037-3042, 1999.
24.CHRISTOPHER, C.; DEVANATHAN, T. G. Incidence of cystic ovarian degeneration in crossbred cows. **Indian Veterinary Journal**, v. 74, p. 184, 1997.
25.DOUTHWAITE, R; DOBSON, H. Comparison of different methods of diagnosis of cystic ovarian disease in cattle and an assessment of its treatment with a progesterone-releasing intravaginal device. **Veterinary Records**, v. 23, p. 355-359, 2000.
26.NANDA, A. S.; WARD, W. R.; DOBSON, H. Lack of LH response to estradiol treatment in cows with cystic ovarian disease and effect of progesterone treatment or manual rupture. **Research in Veterinary Science**, v. 51, p. 180-184, 1991.
27.OPSOMER, G. et al. Post-partum anoestrus in dairy cows. **Veterinary Quarterly**, v. 18, p. 68-75, 1996.
28.SHARMA, S. P. Surgical treatment of unilateral cystic ovary in a Friesian cow - Clinical case report. **Industrial Veterinary Journal**, v. 75, p. 161-162, 1998.
29.HEUER, C.; SCHUKKEN, Y. H.; DOBBELAAR, P. Postpartum body condition score and results from the first test day milk as predictors of disease, fertility, yield, and culling in commercial dairy herds. **Journal of Dairy Science**, v. 82, p. 295-304, 1999.
30.KESLER, D. J.; GARVERICK, H. A. Ovarian cysts in dairy cattle: a review. **Journal of Animal Science**, v. 55, p. 1147-1159, 1982.
31.RIBADU, A. Y. et al. Lack of LH response to exogenous estradiol in heifers with ACTH-induced ovarian follicular cysts. **Journal of Veterinary Medicine Science**, v. 61, p. 979-981, 1999.
32.RIBADU, A. Y. et al. The role of LH pulse frequency in ACTH-induced ovarian follicular cysts in heifers. **Animal Reproduction Science**, v. 64, p. 21-31, 2000.
33.BARBERI, R. L. Polycystic ovarian disease. **Annual Review Medicine**, v. 42, p. 199-204, 1991.
34.HAMILTON, S. A. et al. Characterization of ovarian follicular cysts and associated endocrine profiles in dairy cows. **Biology of Reproduction**, v. 53, p. 890-898, 1995.
35.MORI, Y.; HASUMI, F.; ITO, A.; SHIINA, K.; HIRAKAWA, S. Collagen and glycosaminoglycans in the human ovarian capsule with polycystic ovarian disease. **Gynecology and Obstetric Investigation**, v. 18, p. 244-251, 1984.
36.GASSE, H. et al. Place of follicle-lutein cysts in the estrus cycle of cows: histological, cytological and hormone analysis studies. **Zentralblatt Veterinarmed**, v. 31, p. 548-556, 1984.
37.GROHN, Y. T.; HERTL, J. A.; HARMAN, J. L. Effect of early lactation milk yield on reproductive disorders in dairy cows. **American Journal of Veterinary Research**, v. 55, p. 1521-1528, 1994.
38.GROHN, Y. T.; EICKER, S. W.; HERTL, J. A. The association between previous day milk yield and disease in New York State dairy cows. **Journal of Dairy Science**, v. 78, p. 1693-1702, 1995.
39.ERB, H. N.; MARTIN, S. W. Interrelationships between production and reproductive diseases in Holstein cows. Age and seasonal patterns. **Journal of Dairy Science**, v. 63, p. 1918-1924, 1980.
40.GRÛNERT, E.; BERCHTOLD, M. **Infertilidad en la vaca**. Buenos Aires: Editorial Hemisferio Sur, 1988.
41.DOLEZEL, R.; CECH, S.; ZAJIC, J. Follicular development during the progesterone therapy of ovarian acyclicity and ovarian cysts in cows. **Veterinary Medicine**, v. 43, p. 145-151, 1998.
42.SINGH, K. B. **Effect of continuous light on the reproductive cycle of the female rat**. Morphology and morphogenesis of the polycystic ovary syndrome. **Dissertation (M.Sc**.) - University of Kansas, USA, 1966.
43.LONG, J.; EVANS, H. The oestrus cycle in the rat and its associated phenomena. **Memorial** University of California, v. 6, p. 1-48, 1922.
44.MARIC, D. K.; MATSUYAMA, E.; LLOYD, C. Gonadotrophin content of pituitaries of rats in constant estrus induced by continuous illumination. **Endocrinology**, v. 77, p. 529, 1965.
45.FISKE, V. M. Effect of light on sexual maturation, estrus cycles and anterior pituitary of the rat. **Endocrinology**, v. 29, p. 187, 1941.
46.BALDISSERA, S. F. et al. Proposal of an experimental model for the study of polycystic ovaries. **Brazilian Journal of Medical Reserch**, v. 24, p. 747-751, 1991.