

DETERMINATION OF SERUM LEVELS OF ESTROGEN, PROGESTERONE, LH AND FSH OF ATHYMIC NUDE RATS IN THE PROESTRUS AND METESTRUS PHASES OF THE ESTROUS CYCLE

Determinação dos níveis séricos de estrógeno, progesterona, LH e FSH das ratas nude atímicas nas fases de proestro e de metaestro do ciclo estral

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The present study compares serum steroid and gonadotrophic hormones levels of congenitally athymic (rnu/rnu) rats, homozygous for the recessive nude gene with those of their euthymic littermates (+/rnu), heterozygous for the same gene, both of the Rowett strain, in proestrus and metestrus phases of the estrous cycle.

We could demonstrate that steroid levels of rnu/rnu and +/rnu rats are statistically different in the proestrus and metestrus phases of the estrous cycle.

Regarding gonadotrophin levels, no differences between both types of rats could be observed in the studied phases, except for the FSH levels in the metestrus phase.

These differences led us to believe that hormonal disorders occur in rnu/rnu rats possibly due to their congenital athymia and that the low reproductivity of these rats would be also related to hormonal alterations inherent in these athymic organisms.

Keywords: Rats, nude; estrus; LH; FSH; estrogens; progesterone.
Unitermos: Rato, nude; estro; LH; FSH; estrógeno; progesterona.

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Introduction

Serum fluctuations of gonadotrophic and sexual hormones have been studied in the estrous cycle of the normal rats. Experiments aiming at the detection of plasma LH (8, 12, 13, 14, 19, 21) and FSH (8, 9, 11, 14) in normal rats revealed basal levels during the whole cycle with a peak in the afternoon of proestrus.

Regarding sexual hormones in normal rats it is reported low levels of estradiol on the afternoon of estrus with gradual increase reaching maximal levels on the second day of diestrus and remaining elevated until proestrus. In relation to serum levels of progesterone, basal progesterone levels during the whole cycle with a higher peak in proestrus and with lower ones including in metestrus were observed (14).

Since recent studies on hormonal dysfunctions in nude mice reported low estrogen and progesterone levels in these animals (10) and no hormonal dosage studies on athymic rats, of the Rowett strain were found in the literature, considered as not good reproducer, in this colony, we decided to carry

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out this study with the objective to detect serum levels of the gonadotrophic (FSH and LH) and sexual (estrogen and progesterone) hormones of the nude athymic (rnu/rnu) and euthymic(+/rnu) rats in the phases of proestrus and metestrus of the estrous cycle.

Material and method

Forty-eight adult females, of the Rowett strain with ages ranging from 3-5 months, weighing approximately 250 g, from the Pathological Anatomy Laboratory, Instituto Butantã were used in this study and kept in semiconventional conditions of feeding, hygiene and controlled temperature (20° C). Iniatially it was determined duration of the estrous cycle performing sequential colpocytological tests during 3 consecutive cycles which were stained by Harris-Shorr. All the animals presented a 5-day estrous cycle. In the fourth cycle, 6 animals in the proestrus and 6 in the metestrus (3 rnu/rnu and 3 +/rnu) were decapitated and the blood was collected from the abdominal aorta, centrifugated at 5000 rpm for 10 minutes and the supernatant serum separated and conserved at -20° C until hormone assay.

Hormone assays

Serum samples, in duplicate were used for the LH and

FSH levels. Standard curves of LH and FSH, containing 15.6 to 2000 pg per tube respectively were established and LH-Rp3/1 AC was used as reference standard. The limits of detection for both assays was 15.6 pg per tube.

Estradiol and progesterone concentrations were determined in duplicate, by the Delphia immunofluorometric procedure, using a tracer standard estradiol or the progesterone bound Europium .

All samples were determined in the same assay in order to avoid inter-assay variations of the method.

Statistical method

Mann-Whitney's test for independent groups was used for the analysis of the results and for the independent groups (18) when we compared the estradiol, progesterone, LH and FSH values obtained in the different groups of rats (rnu/rnu and +/rnu) at each phase separately. The same test was used to compare the values of phase 2 and 5 for the groups of +/rnu and rnu/rnu rats separately.

A difference was considered to be significant when the double tail probability was <0.05.

Results

Our results are shown in the tables I and II.

Table I - Athymic (rnu / rnu) or euthymic (+ / rnu) rats according to LH (nanogram per milliliter) or FSH (nanogram per milliliter) in proestrus (phase 2) and metestrus (phase 5)

	LH				FSH			
	Phase 2		Phase 5		Phase 2		Phase 5	
	rnu / rnu	+ / rnu	rnu / rnu	+ / rnu	rnu / rnu	+ / rnu	rnu / rnu	+ / rnu
	0,33	0,53	0,52	0,54	5,34	8,56	10,14	21,08
	0,37	0,48	0,70	0,59	17,81	16,93	8,61	20,72
	0,72	0,50	0,46	0,48	5,44	12,00	12,14	19,63
Mi	0,37	0,50	0,52	0,54	5,44	12,00	10,14	20,72
Mann-Whitney Test								
1º) rnu / rnu x + / rnu								
	LH		FSH					
	Phase 2		Phase 5		Phase 2		Phase 5	
	U calculated=3,0		U calculated=4,0		U calculated=3,0		U calculated=0,0* (p<0,05) rnu/rnu < + / rnu	
2º) Phase 2 x Phase 5								
	LH		FSH					
	rnu / rnu		+ / rnu		rnu / rnu		+ / rnu	
	U calculated=3,-0		U calculated=2,5		U calculated=3,0		U calculated=0,0* (p<0,05) Phase 2 < Phase 5	

Table II - Athymic (rnu / rnu) or euthymic (+ / rnu) rats according to estradiol (picrogram per milliliter) or progesterone (nanogram per milliliter) in proestrus (phase 2) and metestrus (phase 5)

Estradiol				Progesterone			
Phase 2		Phase 5		Phase 2		Phase 5	
<i>rnu / rnu</i>	<i>+ / rnu</i>	<i>rnu / rnu</i>	<i>+ / rnu</i>	<i>rnu / rnu</i>	<i>+ / rnu</i>	<i>rnu / rnu</i>	<i>+ / rnu</i>
87,3	149,0	75,1	23,0	46,6	13,1	22,4	31,7
43,3	37,0	63,7	32,0	45,7	7,2	27,9	30,0
45,3	88,0	136,0	26,0	33,9	15,0	12,5	33,2
<i>Mi</i>	45,3	88,0	75,1	26,0	46,6	13,1	22,4
Mann-Whitney Test							
1º) rnu / rnu x + / rnu							
Estradiol				Progesterone			
Phase 2		Phase 5		Phase 2		Phase 5	
<i>U calculated=3,0</i>		<i>U calculated=0,0*</i> <i>(p < 0,05)</i> <i>rnu / rnu > + / rnu</i>		<i>U calculated=0,0*</i> <i>(p < 0,05)</i> <i>rnu / rnu > + / rnu</i>		<i>U calculated=0,0*</i> <i>(p < 0,05)</i> <i>rnu / rnu < + / rnu</i>	
2º) Phase 2 x Phase 5							
Estradiol				Progesterone			
<i>rnu / rnu</i>		<i>+ / rnu</i>		<i>rnu / rnu</i>		<i>+ / rnu</i>	
<i>U calculated=2,0</i>		<i>U calculated=0,0*</i> <i>(p < 0,05)</i> <i>Phase 2 > Phase 5</i>		<i>U calculated=0,0*</i> <i>(p < 0,05)</i> <i>Phase 2 > Phase 5</i>		<i>U calculated=0,0*</i> <i>(p < 0,05)</i> <i>Phase 2 < Phase 5</i>	

Discussion

The importance of gonadotrophic hormones in the follicular maturation and ovulation processes is well known, however levels were not determined in the rnu/rnu and +/rnu rats of the Rowett strain. Thus, we believe that any alteration could explain the low reproductivity observed on rnu/rnu rats.

During the estrous cycle of normal rats plasma LH levels (8, 13, 14, 20) and those of FSH (8, 9, 14) remain low during the whole estrous cycle to reach a peak in the afternoon of proestrus. However, the FSH levels are maintained high until the morning of the estrus (9, 14).

Our results showed that LH levels of rnu/rnu and rnu/+ rats in the phases studied - proestrus and metestrus - did not differ among them and are similar to the results found by Nequin et al. (14). The same occurred in relation to serum levels of FSH except for in the metestrus phase when the levels of this hormone were lower in rnu/rnu than in +/rnu rats. The FSH levels in metestrus of rnu/rnu rats were compatible with the findings by Gay et al. (8). These data suggest that levels of LH and FSH can be obtained as a basal one during the whole cycle. However, the standardization of a fixed period at 01:00 p.m. for the blood collection of the samples did not allow us to detect the increase in the levels of these hormones. It must be emphasized that the FSH has an

important function in the development and follicular maturation (17).

Regarding plasma levels of estradiol in the proestrus phase, the small size of the sample did not allow us to reject the equal hypothesis although the results suggest that the levels of this hormone are lower in rnu/rnu than +/rnu rats. In the metestrus phase, the levels of estradiol were higher in the rnu/rnu rats.

When compared the levels of estradiol in the proestrus and metestrus phase of the rnu/rnu rats, the equal hypothesis also could not be rejected due to the small size of the sample, however, the results suggest that levels of this hormone are higher in metestrus. On the other hand, the levels of estradiol of +/rnu rats were statistically higher in proestrus in relation to metestrus.

Regarding progesterone levels, it was observed that in rnu/rnu rats in the proestrus phase, these levels were higher than in +/rnu while in metestrus the opposite occurred. When the progesterone levels of rnu/rnu rats in the two phases studied were compared, it was observed that these levels were higher in proestrus than in metestrus. In the +/rnu rats the contrary was detected.

In relation to hormonal steroid dosage, the differences found between the two group of rats could explain the low reproductivity of the rnu/rnu rats.

It must be mentioned that the events required for the production of gonadotrophins seem to involve the synergic action of estrogen and progesterone on the hypothalamus signalling the stimulus for the release of LHRH essential for the discharge of ovulatory LH (1, 6). Steroids also seem to act on the hypothalamus or directly on the hypophysis, modifying the sensitivity of the hypophysial response to LHRH. High levels of plasma estrogen act on the hypophysis via a negative feedback blocking a greater release and a fall of FSH levels. Consequently, occurs an increase in release of LHRH which stimulates the elevation of plasma LH and FSH levels (1, 7).

The release of hypophysial LH, between the morning of proestrus and the estrus, required for ovulation, is a result of the increase in hypothalamus excitability by the initial stimulus of progesterone and occurs only after a long period of estrogenic action on the hypothalamic-hypophysial system from diestrus to proestrus (4, 5, 16).

In the same way alterations of the ovarian steroid levels are directly related to gonadotrophin levels. FSH requires a combined action with LH for the secretion of steroids and ovulation (17). The observations reported by *Bélangier* et al. (3) indicate that LH stimulates the increase in ovarian steroid

production. Also the LH released in proestrus activates the secretion of progesterone (2, 15, 17, 10). On the other hand the fall in estradiol levels may be due to the direct or indirect action of the increase in LH levels (20).

Considering that low levels of estrogen and progesterone may be one of the causes of infertility in athymic mice (10) it is possible that the hormonal disorders which occurred in nude rats could also be the reason for their low reproductivity. Although it is needed to mention that in our experiment the size of the sample was small and thus, doubts regarding equal hypothesis occur.

To better understand the hormonal physiology of these athymic rats, further more detailed studies on hormonal levels must be carried out in other phases and periods of the estrous cycle.

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Resumo

O presente estudo compara os níveis sorológicos de hormônios esteróides e gonadotróficos de ratas congenitamente atímicas (rnu/rnu), homozigotas para o gene recessivo nude com os de suas "littermates" eutímicas (rnu/+), heterozigotas para o mesmo gene, ambas da linhagem Rowett, nas fases de proestro e de metaestro do ciclo estral.

Pudemos demonstrar que os níveis de esteróides diferem estatisticamente entre as ratas rnu/rnu e as rnu/+ nas fases de proestro e de metaestro do ciclo estral.

Quanto aos níveis de gonadotrofinas não observamos diferenças entre ambos os tipos de ratas nas fases estudadas, exceto em relação aos níveis de FSH na fase de metaestro.

Essas diferenças nos levam a acreditar que distúrbios hormonais ocorrem em ratas rnu/rnu, como possível decorrência de sua atímia congênita, e que a baixa reprodutividade destas ratas esteja também relacionada às alterações hormonais inerentes a esses organismos atímicos.

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