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Evaluation of anaerobic threshold in non-pregnant and pregnant rats

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ABSTRACT

Several studies present different methodologies and results about intensity exercise, and many of them are performed in male rats. However, the impact of different type, intensity, frequency and duration of exercise on female rats needs more investigation. From the analysis of blood lactate concentration during lactate minimum test (LacMin) in the swimming exercise, the anaerobic threshold (AT) was identified, which parameter is defined as the transition point between aerobic and anaerobic metabolism. LacMin test is considered a good indicator of aerobic conditioning and has been used in prescription of training in different exercise modalities. However, there is no evidence of LacMin test in female rats. The objective was to determine AT in non-pregnant and pregnant Wistar rats. The LacMin test was performed and AT defined for mild exercise intensity was from a load equivalent to 1% of body weight (bw), moderate exercise as carrying 4% bw and severe intensity as carrying 7% bw. In pregnant rats, the AT was reached at a lower loading from 5.0% to 5.5% bw, while in non-pregnant the load was from 5.5% to 6.0% bw. Thus, this study was effective to identify exercise intensities in pregnant and non-pregnant rats using anaerobic threshold by LacMin test.

Key words: Exercise, swimming, anaerobic threshold, lactate, pregnancy, rats.

INTRODUCTION

The knowledge lack regarding the physical exercise practice is evident. In addition, the information is questionable and subject to misinterpretation. It is necessary to standardize the methodology used in physical exercise studies to allow a clearer, more consistent and adequate assessment (Booth et al. 2010). Exercise protocols, both for humans and animals, are importance for future interventions, especially in metabolic diseases that compromise the individual's physical condition (Manchado et al. 2006). The physical exercise recommendation during gestation dates to several years. However, the research results to indicate exercise during the gestational period are inconclusive, since obstetricians have many doubts about the exercise type, intensity and frequency on the maternal and fetal repercussions.

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Several professionals consider aquatic immersion as the best resource to increase the aerobic capacity of the pregnant women and facilitate breathing, avoiding musculoskeletal trauma risk. The positive results of immersion stimulate the adherence of the pregnant women and indication by the professionals who accompany them in prenatal care (Bates and Hanson 1998). In addition, there are other types of recommended lighter exercise, such as walking and cycling (Batista et al. 2003) or those of moderate intensity that contribute to the weight gain of the newborn. However, more intense and high-frequency exercises maintained for long periods of pregnancy may lead to low birth weight infants (Bell 2002). A significant number of studies that involve physical exercise has been performed in laboratory animals, with swimming program (free of load, 1 hour/day during pregnancy) to severely diabetic female rats (Volpato et al. 2009, 2015, Damasceno et al. 2013). At term pregnancy, these rats presented a improved maternal lipid profile, contributing to higher alive fetus number, but this swimming program caused higher fetal anomaly frequency.

For a standardization of exercise intensity, Heck et al. (1985) carried out exercise tests with constant loads in humans and evaluated the blood lactate concentrations throughout the sessions, since it is known that the blood lactate concentration increases exponentially with the physical exercise intensity. However, there is a need to know the transition period from aerobic to anaerobic metabolism (transition zone). This determination is very important for physical evaluation and conditioning, leading to a correct prescription of physical activity (Gobatto et al. 2008). Voltarelli et al. (2002) determined the intensity of physical exercise in male rats by analyzing the blood lactate concentration during the lactate minimum test (LacMin) in swimming exercise. Based on this test, the anaerobic threshold (AT) was identified, which is defined as the point at which a transition occurs between aerobic and anaerobic metabolism. The test has been considered a good indicator of aerobic fitness and has been employed for training recommendations in different exercise modalities (Wasserman and Mcilroy 1964). However, no studies defining the anaerobic threshold in female rats are available. Therefore, the objective of the present study was to determine the anaerobic threshold in non-pregnant and pregnant Wistar rats using LacMin test.

MATERIALS AND METHODS

ANIMALS

This study was approved by Regional Ethics Committee for animal experiments and adhered to the institutional guidelines for the care and treatment of laboratory animals (Protocol Number 1050/2013). Female Wistar rats in reproductive age (three months) were used, weighing about 250 grams (g). The animals were purchased from Biological Research Centre (CEMIB), State University of Campinas (UNICAMP) and were adapted and maintained at the Laboratory of Experimental Research on Gynecology and Obstetrics/UNESP under controlled conditions of temperature (22 ± 2°C), humidity (50 ± 10%), 12 hours light/dark cycle, water and feed were provided *ad libitum*.

SWIMMING PROGRAM – ADAPTATION PERIOD

Approximately at day 80 of life, all rats were submitted to an adaptation period in liquid medium in cylindrical tanks (100 x 30 cm) with shallow water (10 cm of deep) to 31±1°C temperature for 10 consecutive days per 10 minutes.

EXPERIMENTAL GROUPS – PREGNANT (MATING PERIOD) AND NON-PREGNANT RATS

After the adaptation period in water, the rats that were destined to pregnant group (n=5) were subjected to mating period. During this period, the rats selected to compose the non-pregnant group (n=5) continued to be subjected to tanks with shallow water.

SWIMMING PROGRAM

The pregnant and non-pregnant rats remained in contact with shallow water on days 0 to 6 of pregnancy because embryo implantation in rats occurs near the day 5 of pregnancy. Then, all pregnant rats were subjected to the lactate minimum (LacMin) test from the day 7 of pregnancy to avoid potential embryonic losses.

LACTATE MINIMUM (LacMin) TEST

To determine the anaerobic threshold (AT) of pregnant and non-pregnant rats, the LacMin test was performed. This test consisted of an effort exercise test using hyperlactataemia induction, a period of rest, followed by an incremental test. The pregnant rats were subjected to the LacMin test on days 7, 10, 14, 17 and 20 of pregnancy. Similarly, on the respective days of the experiment, the test was applied to non-pregnant rats. Then, the LacMin test was applied five times per rat throughout the experiment.

For the LacMin test, the pregnant and non-pregnant rats were placed in the same cylindrical tanks for the adaptation period; however, the water had a depth of 40 cm. To induce hyperlactatemia, all the rats carried loads comprised of 50% of the body weight, with lead fish sinkers strapped to the chest. With this load, the rats were induced to jump into the water for six minutes (30 seconds of exercise interrupted by 30 seconds of rest, until six minutes were completed). Following, the rats remained at rest for nine minutes. To finish LacMin test, the pregnant and non-pregnant rats were subsequently subjected to the incremental test, which comprised a swimming exercise in which the rats carried at progressive loading of 3.0; 3.5; 4.0; 4.5; 5.0; 5.5; 6.0; 6.5 and 7.0% of their body weights, on days 7, 10, 14, 17 and 20. The exercise lasted for three minutes per load and was followed by tail blood sample collections for the analysis of lactate concentrations for each exchange specific load using strips in Accutrend® Plus (Roche®, Germany) (modified Voltarelli et al. 2002) (Figure 1).

On the days for which the rats did not undergo the LacMin test, they remained in the tank with 10 cm of water at the same temperature to prevent the loss of contact with the liquid medium.

The choice of days 7, 10, 14, 17 and 20 of pregnancy (for pregnant rats) and experimental (for non-pregnant rats) was standardized to identify the loads that reflect the exercise intensities (mild, moderate or intense) for these groups of animals, as well as to identify variations in the anaerobic threshold in accordance with the progressive changes in body weight during pregnancy compared with the non-pregnant rats. Under each load, the respective blood lactate concentration exhibited a polynomial curve of order 2, "U", and the anaerobic threshold was indicated by the lower value of the lactate concentration in the corresponding load.

STATISTICAL ANALYSIS

For results of the LacMin tests during pregnancy (pregnant rats) and the experiment (non-pregnant rats), t Test was used to analyze statistical significance. For all statistical comparisons, $p<0.05$ was considered as statistically significant.

RESULTS

Taking into account the individual data, all the rats submitted to the LacMin test on different experimental days presented curves in the classic "U" format (Figure 2).

The comparison of the mean values of loads related to anaerobic thresholds in LacMin test during swimming training of non-pregnant rats showed no statistically significant differences among the different experimental days (D7, D10, D14, D17, D20) (Figure 3a). The mean values of

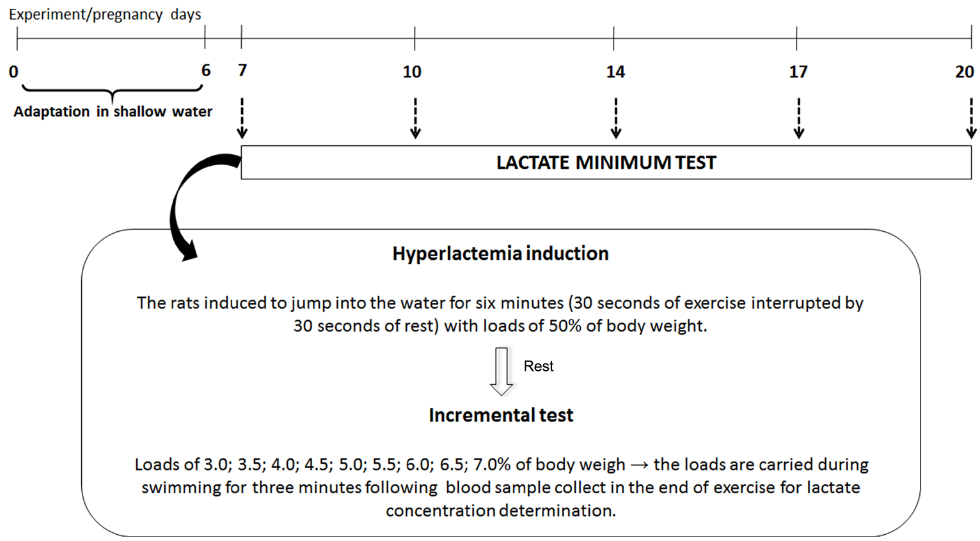

Figure 1 - Experimental design of LacMin test performed in pregnant and non-pregnant rats for swimming protocol.

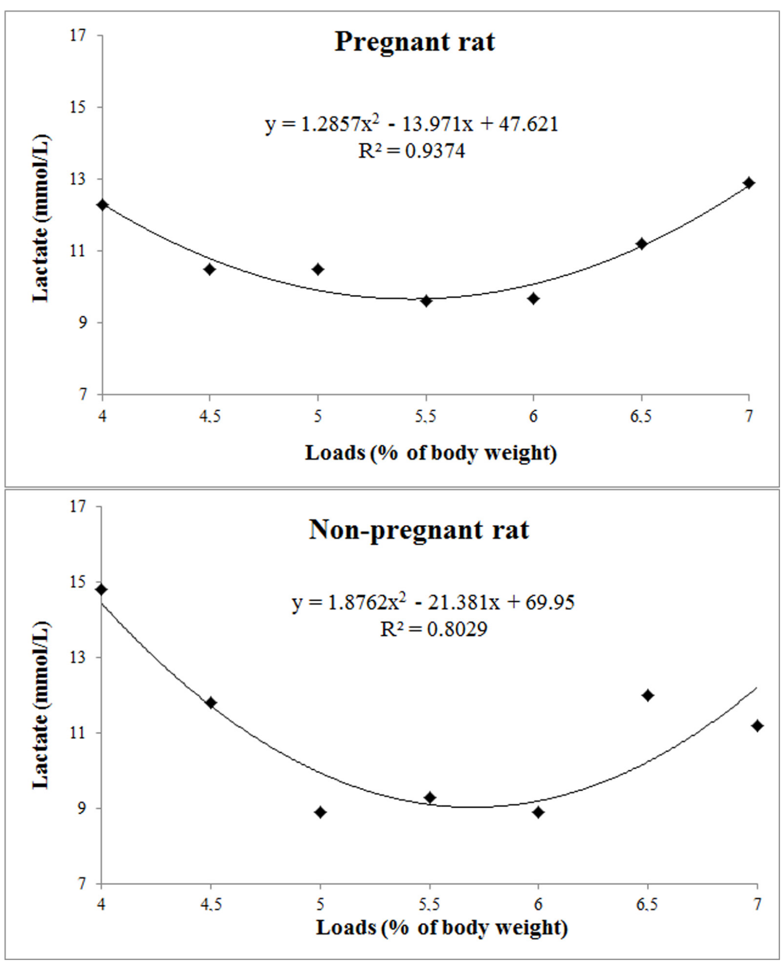

Figure 2 - Blood lactate concentration (mmol/L) and load (% of body weight) of pregnant and non-pregnant rats during lactate minimum (LacMin) test.

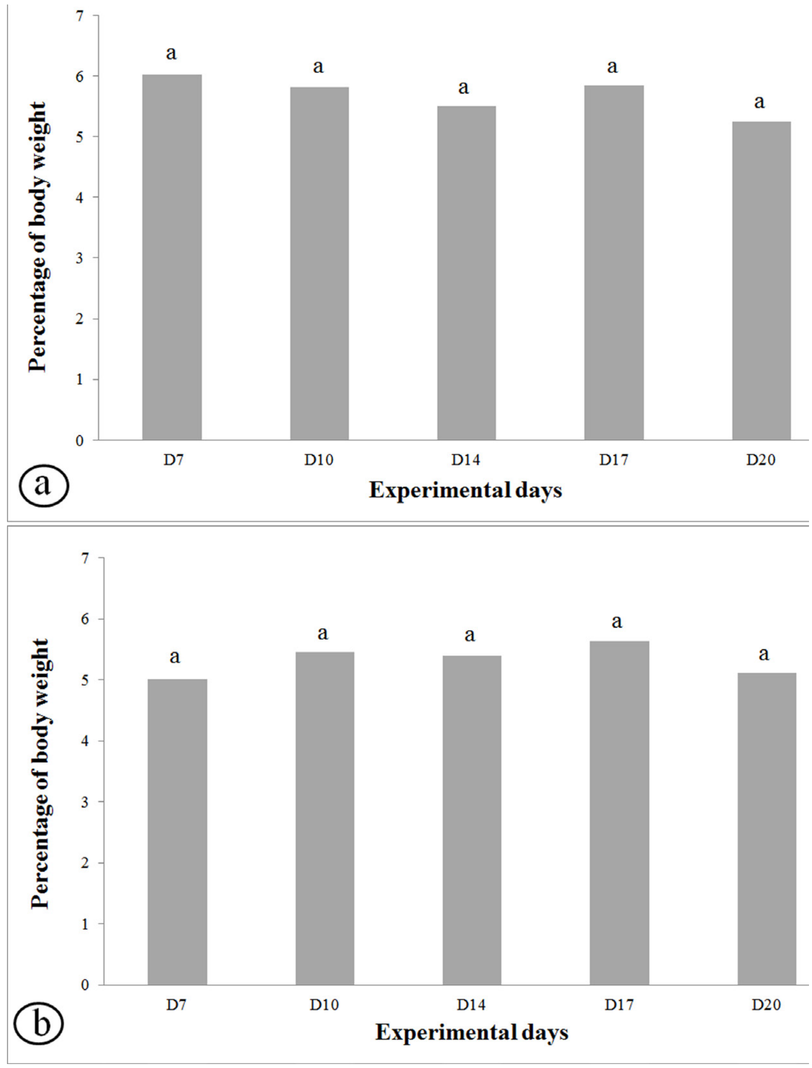

Figure 3 - Load equivalent to anaerobic threshold of non-pregnant and pregnant rats during swimming lactate minimum (LacMin) test in diferente days of experiment (**a**) and pregnancy (**b**).
n=5 animals/group.
Means followed by similar lowercase letters show no statistical difference (ANOVA).

the loads on each pregnancy day (D7, D10, D14, D17, D20) during LacMin test of pregnant rats also presented no statistically significant differences (Figure 3b).

Considering anaerobic threshold on all days of pregnancy of the rats, the mean value of the loads was statistically reduced (5.29 ± 0.87) compared with those of non-pregnant rats (5.78 ± 0.59) in LacMin test (Figure 4).

DISCUSSION

The progressive loads used in this study were based on previous results, which demonstrated the anaerobic threshold in rats occurs in loads varying between 5 and 6% of the body mass (Gobatto et al. 2001, Manchado et al. 2006). The values found for our female Wistar rats were similar to those of male rats of the same lineage, as described by Gobatto et al. (2001), who used the maximal stable

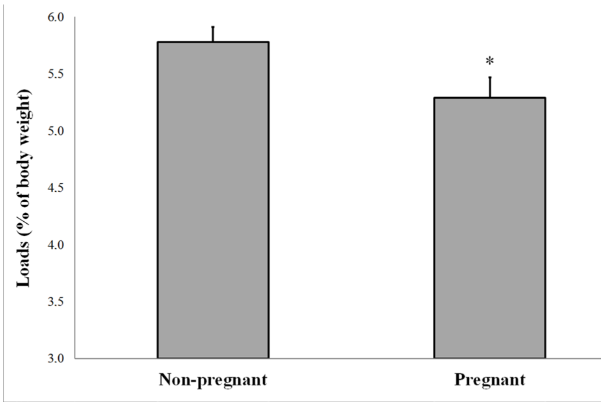

Figure 4 - Comparative analysis of the loads (% of body weight) equivalent to anaerobic threshold of non-pregnant and pregnant rats in all days of experiment, n=5 animals/group. Data presented as mean ± standard error of mean (SEM). *p<0.05 – compared to non-pregnant group (t test).

phase of lactate (MLSS) and obtained a lactate concentration of 5.5 mmol /L at a load equivalent to 5% of body weight.

The curves were U-shaped, corroborating Voltarelli et al. (2002), who verified the lactate response in the incremental test presents a U-shaped kinetic behavior, reducing in initial intensities (the lactate removal was greater than the production) and increasing successively after the corresponding load has been exceeded to anaerobic threshold (the lactate production was greater than the removal). Thus, the effort intensity, equivalent to the lowest lactate value after mathematical adjustment, corresponds to the minimum lactate or anaerobic threshold (LAn), in which the lactate production is in equilibrium with its removal.

There is evidence that the kinetics for the minimum lactate in subliminal loads, that is, in the first loads of the incremental phase after hyperlactacidemia induction, has beeen reduced because it is used as an energy substrate in this predominantly aerobic phase (McArdle 2013). However, some animals of our investigation continued with increasing lactate concentrations even after the beginning of the incremental phase. This non-reduction of lactate concentration may be due to its increased production during to hyperlactatemia induction. Then, this finding might be a characteristic of female rats, and we suggested these animals would require a greater rest time than the nine minutes performed between one test and another. Therefore, the points continued to present high lactate concentration in this phase were withdrawn, since they did not represent the lactate consumption stage, but still synthesis, contrary to kinetics for the mathematical analysis of the polynomial adjustment (de Araújo et al. 2007). Then, the discarded points were those in which lactacidemia continued greater, even in the incremental phase due to the induction magnitude, which is individually observed and thus must be respected in order for the individualization proposal to be maintained.

The studies found in the literature regarding the study of the repercussions of physical exercise different intensities are applied in male laboratory animals. However, the lack of research regarding female animals, especially during pregnancy, is worth noting. Thus, it is necessary to deepen the studies in this subject to standardize an exercise protocol applied to pregnant rats. The anaerobic threshold (AT) of pregnant rats statistically differed from non-pregnant after individual analysis of the data. This shows the threshold was reached at a lower loading (from 5.0% to 5.5% bw) for pregnant rats while the non-pregnant was in a load of 5.5% to 6.0% bw. This finding might be related to the hormonal action during the pregnancy or enzymatic performance, such as lactate dehydrogenase (LDH). Lactate dehydrogenase is an enzyme found in several tissues, which acts on the anaerobic metabolism of carbohydrates and catalyzes from pyruvate to lactate. Serum LDH is elevated at the end of pregnancy and at delivery (Makkonen et al. 1980). However, no differences were found between the pregnant group (9.70 ± 2.42 mmol/L) and non-pregnant group (9.31 ± 2.66 mmol/L) when blood lactate concentrations were analyzed at the respective AT. This fact represents the load mean values of each rat group might be used for exercise application. According to Carvalho et al. (2005), the AT mean of a homogeneous rat group can be successfully used for the application in an aerobic training.

It should be noted the progressive loads chosen for the incremental test (from 3 to 7% bw) were based on articles in the literature, which observed the anaerobic threshold in male Wistar rats occurs with loads ranging from 5 to 6% bw (Gobatto et al. 2001, Voltarelli et al. 2002, Manchado et al. 2006). The interest in verifying the influence of body weight gain during pregnancy of rats in relation to loads equivalent to the AT also occurred. When the LacMin tests were performed at different times of pregnancy or the experiment, it was verified the weight gain did not interfere in the load values observed between the several days studied. In addition, the evaluation of the hydrostatic weighing of the animals, which was performed in partnership with another laboratory, also confirmed these findings (data not shown). This may be justified by the fact that the rats' weight gain occurs due to the lean mass gain from their offspring and not just from maternal adipose tissue.

Our results in pregnant and non-pregnant rats corroborate those found in males (Gobatto et al. 2001, Voltarelli et al. 2002), that is, the AT values are between 5 and 6% bw and blood lactate concentration between 9.0 and 9.9 mmol/L. Then, the LacMin test might be used for identification of the intensity of swimming exercise in pregnant and non-pregnant rats.

Thus, the present study determined the intensities (mild, moderate and intense) of swimming physical exercise for pregnant rats. Considering AT is the transition point from aerobic to anaerobic metabolism, the AT of pregnant rats was in the load of 5% bw. Therefore, the load intensity to work within the aerobic metabolism corresponding to 80% of this anaerobic threshold was standardized for the practice of physical exercise of moderate, which was represented by the load of 4% bw of the animal. As for the mild intensity physical exercise, 20% of the AT was standardized, which is working within the aerobic metabolism, and was represented by the load of 1% bw, equivalent to a minimum load to perform the exercise. For the intense intensity physical exercise, the load of 7% bw - a load higher than the AT - is equivalent to 140% of this threshold. This load was standardized for exercise practice in the anaerobic metabolism. Therefore, our study showed that the LacMin test was effective for assessing exercise intensity in pregnant and non-pregnant rats, showing the importance to analyze anaerobic metabolism of female rats in different situations, especially in pregnancy, before to apply any exercise intensity.

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REFERENCES

BATES A AND HANSON N. 1998. Os princípios e propriedades da água. In: Exercícios aquáticos terapêuticos (Editora Manole). São Paulo, p. 21-28.

BATISTA DC, CHIARA VL, GUGELMIN AS AND MARTINS PD. 2003. Atividade Física e gestação: saúde da gestante não atleta e crescimento fetal. Rev Bras Saúde Matern Infant 3(2): 151-158.

BELL R. 2002. The effects of vigorous exercise during pregnancy on birth weight. J Sci Med Sport 5(1): 32-36.

BOOTH FW, LAYE MJ AND SPANGENBURG EE. 2010. Gold standards for scientists who are conducting animal-based exercise studies. J Appl Physiol 108: 219-221.

CARVALHO JF, MASUDA MO AND POMPEU FA. 2005. Method for diagnosis and control of aerobic training in rats based on lactate threshold. Comp Biochem Physiol A Mol Integr Physiol 140(4): 409-413.

DAMASCENO DC, SILVA HP, VAZ GF, VASQUES-SILVA FA, CALDERON IM, RUDGE MV, CAMPOS KE AND VOLPATO GT. 2013. Diabetic rats exercised prior to and during pregnancy: maternal reproductive outcome, biochemical profile, and frequency of fetal anomalies. Reprod Sci 20(7): 730-738.

DE ARAÚJO GG, PAPOTI M, MANCHADO FB, MELLO MAR AND GOBATTO CA. 2007. Protocols for hyperlactatemia induction in the lactate minimum test adapted to swimming rat. Comp Biochem Physiol A Mol Integr Physiol 148(4): 888-892.

GOBATTO CA, DE MELLO MA, SIBUYA CY, DE AZEVEDO JR, DOS SANTOS LA AND KOKUBUN E. 2001. Maximal lactate steady state in rats submitted to swimming exercise. Comp Biochem Physiol A Mol Integr Physiol 130(1): 21-27.

GOBATTO CA, DE MELLO MAR, GOBATTO FBM, PAPOTI M, VOLTARELLI FA, CONTARTEZE RVL AND ARAÚJO GG. 2008. Avaliações fisiológicas adaptadas à roedores: aplicações ao treinamento em diferentes modelos Experimentais. Rev Mack de Educ Fís e Esp 7(1): 137-147.

HECK H, MADER A, HESS G, MUCKE S, MULLER R AND HOLLMANN W. 1985. Justification of the 4-mmol/L lactate threshold. Int J Sports Med 6(3): 117-130.

MAKKONEN M, PENTTILÄ IM AND CASTRÉN O. 1980. Serum lactic acid dehydrogenase and isoenzymes during pregnancy and labor. Acta Obstet Gynecol Scand 59(2): 97-102.

MANCHADO FB, GOBATTO CA, VOLTARELLI FA AND MELLO MAR. 2006. Non-exhaustive test for aerobic capacity determination in swimming rats. Appl Physiol Nutr Metab 31(6): 731-736.

MCARDLE WD, KATCH FI AND KATCH VL. 2016. Fisiologia do exercício, nutrição, energia e desempenho humano. 8ª ed., Rio de Janeiro: Guanabara, 1120 p.

VOLPATO GT, DAMASCENO DC, KEMPINAS WG, RUDGE MV AND CALDERON IM. 2009. Effect of exercise on the reproductive outcome and fetal development of diabetic rats. Reprod Biomed Online 19(6): 852-858.

VOLPATO GT, DAMASCENO DC, SINZATO YK, RIBEIRO VM, RUDGE MV AND CALDERON IM. 2015. Oxidative stress status and placental implications in diabetic rats undergoing swimming exercise after embryonic implantation. Reprod Sci 22(5): 602-608.

VOLTARELLI FA, GOBATTO CA AND DE MELLO MAR. 2002. Determination of anaerobic threshold in rats using the lactate minimum test. Braz J Med Biol Res 35(11): 1389-1394.

WASSERMAN K AND MCILROY MB. 1964. Detecting the threshold of anaerobic metabolism in cardiac patients during exercise. Am J Cardiol 14: 844-852.