



Early Pregnancy Diagnosis by Weight Measurement in the Sprague Dawley Rats: A New Method

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ABSTRACT

Background: An early diagnosis of pregnancy in Sprague Dawley (SD) rats is of great importance for scientific research in reproductive health and perinatology. However, the commonly used methods, vaginal embolus check and vaginal smear for sperms, are not always satisfactory in practical courses. To explore a more convenient method for practice in identifying the pregnant SD rats at an early stage, we hypothesized that differences among weight changes may separate pregnant SD rats from the unpregnant effectively.

Methods: The initial weight (W_0) of SD female rats before mating and the weight of them from day 6 to day 12 after mating were recorded, then gained weight (ΔW) was calculated. Pregnancy was confirmed when female rats gave birth on day 23 or earlier.

Result: In total, weight changes data of 34 pregnant rats and 49 unpregnant rats were collected. Weight gains of pregnant SD rats on the 6th day were found different from those in unpregnant SD rats significantly and a diagnostic model of ΔW on gestation day 6 (ΔW_6) has been set up to distinguish pregnant SD rats with 70.00% sensitivity and 100.00% specificity. Diagnostic data models of $\Delta W_7 \sim \Delta W_{12}$ owned higher sensitivity and specificity respectively. Cut-off values of $\Delta W_6 \sim \Delta W_{12}$ were correspondingly 25.50 g, 23.00 g, 22.50 g, 22.50 g, 31.00 g, 27.00 g and 31.00 g. Practically, diagnostic models of weight gains showed robust potential in affirming the pregnant SD rats at early stage of gestation.

Key words: Early pregnancy diagnosis, SD rats, Weight measurement.

Abbreviations: CI₉₅- 95% Confidence interval, Prgt-rat- Pregnant SD rat, ROC- Receiver-operator characteristic, SD rat- Sprague dawley rat, SPF- Specific pathogen free, Unprgt-rat- Unpregnant SD rat.

INTRODUCTION

SD rats, closed colony animals, were firstly bred from the Sprague Dawley farm in 1925 and characterized by its elongated head, tail body of equal length, high fecundity and faster growth rate than other rat strains (Qin and Tan, 2020). Owing to these advantages, pregnant SD rat is a widely used model for scientific study in obstetrics and embryology nowadays (Davenport *et al.*, 1990; Liu *et al.*, 2006; Salem *et al.*, 2023). Moreover, it plays a fundamental role in some other relevant research like environmental relationships with reproductive health (Ma *et al.*, 2020; Yu *et al.*, 2023; Shuklan *et al.*, 2024). Commonly, there are two ways used to determine conception of rat: embolus check in female rat vaginal orifice (Wolf *et al.*, 2004; Xuan *et al.*, 2016; Yang *et al.*, 2022) and sperm detection in vaginal smear under microscope (Ramesar *et al.*, 2010; Halabi *et al.*, 2021; Zhong *et al.*, 2024). Both methods are carried out the next morning of mating to make an early confirmation of pregnancy. The former approach tells the female rat mates successfully when a white or faint yellow plug is visible, the latter demonstrates the female rat is fertilized by observing sperms on vaginal secretion smear under microscope. However, activities of rats in the cage make searching the plug difficult. On the other hand, small sperm-like odds such as cotton fibers appear easily to be mistaken as real sperms. Meanwhile, sperm cells are often damaged that increases the uncertainty and discovery of sperm cells under microscope doesn't stand for the

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beginning of normal pregnancy because of the fertilization failures. Therefore, the aim of this study is to explore a more convenient, objective and precise method to determine the pregnant rats in the early stage of gestation.

MATERIALS AND METHODS

Animal purchasing and breeding

76 SD female rats about 12 weeks old and 22 SD male rats aging 13 weeks were purchased from Experimental

Animal Center of Southwest Medical University and fed in Specific pathogen free (SPF) animal room of barrier system.

4 or 5 SD rats were bred in one cage with abundant SPF rat breeding feed or maintaining feed and clean water sterilized by high temperature and high pressure. Rats were reared at room temperature 20–26°C, room humidity 40%–70%, with room light and darkness switched in 12 hours cycle as lighting from 8:00 A.M. to 8:00 P.M. Rat padding is changed twice a week (N.G. Mustafa, 2020).

Rats' fertilized time and gained weight calculation

Rats were marked with ear studs showing serial numbers. Weight measurement of female rat, recorded as value $Weight_0$ (W_0), was carried out before mating with male rat. The $Time_0$ (T_0) and the date were recorded when one or two female rats were caged with one male rat. The duration of caged rats spending together was recorded as ΔT and ΔT was controlled to 24 hours as much as possible to increase pregnancy probability but not influence the judgement about the time of mating. Separate time of female and male rats was noted as $Time_1$ (T_1 , $T_1 = T_0 + \Delta T$). $Time_2$ (T_2 , $T_2 = T_0 + \Delta T/2$ or $T_2 = T_1 - \Delta T/2$) was calculated and the corresponding date of T_2 was presumed as the start of gestation day (GD0).

Weight of female SD rats were measured and recorded every day from GD6 to GD12 as $Weight_{6-12}$ (W_{6-12}). Weight changes compared with W_0 were calculated and recorded as gained weight ΔW . For instance, ΔW_6 ($\Delta W_6 = W_6 - W_0$) represented the weight female rats gained on GD6. Weight measurement for SD rats was oriented to time every day to make the experiment be in rhythm. The weight value on platform was recorded when the rat was relative calm with its four pads on platform and the value was rounded off to keep integer. On GD20, female rats with obviously round belly and higher ΔW_{20} ($\Delta W_{20} = W_{20} - W_0$) were selected as possible pregnant rats. Those object female rats were arranged into single cage waiting it to give birth. Conception of the rats were confirmed on GD23 or earlier according to whether there was a delivery.

Statistical analysis

Both pregnant rats and unpregnant rats ΔW_{6-12} data were organized to make normality test respectively by D'Agostino and Pearson test in Graph Pad Prism 9.0.0. The mean value and standard deviation value of each ΔW group, from GD6 to GD12, were attained by using unpaired t test and the grouped comparisons by two-way ANOVA, meanwhile the 95% confidence interval (CI_{95}) of differences between means was shown to help us understand the variation

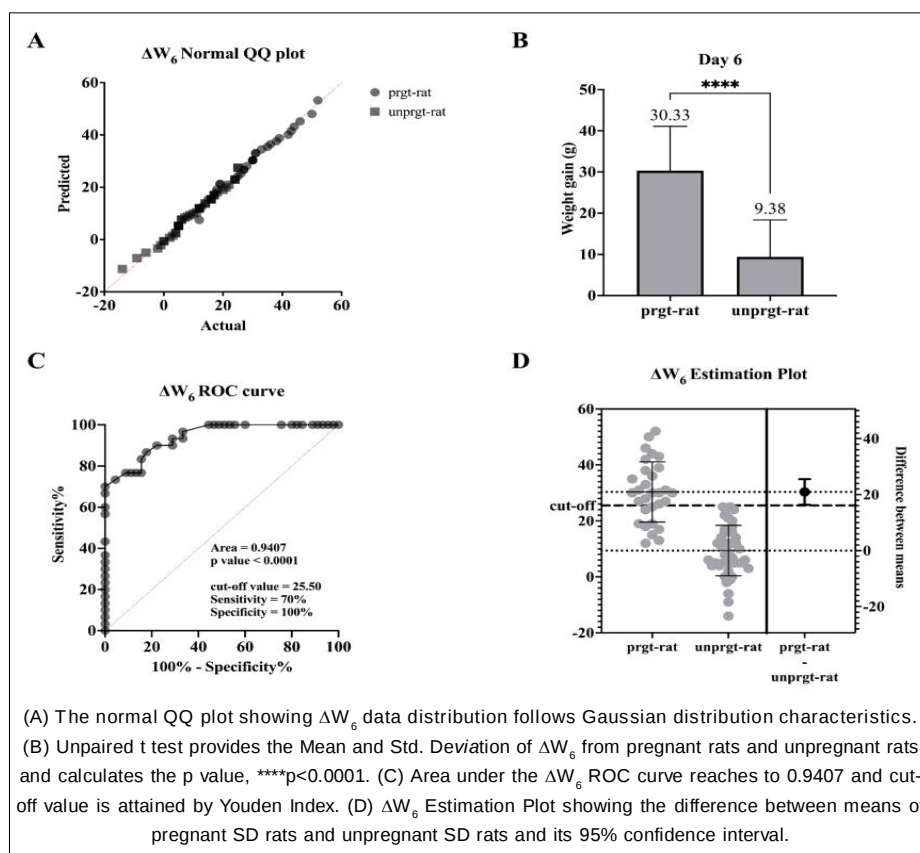


Fig 1: Analysis of ΔW_6 data from the pregnant SD rats (prgt-rat) and unpregnant SD rats (unprgt-rat).

better. The diagnostic potential of each ΔW was tested out by receiver-operator characteristic (ROC) curve. Cut-off value was found *via* calculating the Youden Index of ROC curve data set.

RESULTS AND DISCUSSION

Physiological data of rat weight changes

A pregnant female rat was determined when it gave birth on day GD23 or earlier. The data $\Delta W_6 \sim \Delta W_{12}$ of pregnant rats and unpregnant rats were collected. In this way, 34 pregnant rats weight information and 49 unpregnant rats weight data were gathered. Among 76 female rats, 2 rats were confirmed as pregnant through caesarean section on GD20 and then they quit this scientific research, the other 74 rats gave birth naturally on GD23 without disturbance. The pregnancy course or unpregnant course of a female SD rat was recorded just for one time to assure the independency of each datum. In addition to this, the rats engaged in this study were of physical maturity at 12 weeks and older to present the normal condition.

ΔW_6 diagnostic model for pregnancy

Both pregnant rats and unpregnant rats ΔW_6 data obeyed a normal distribution (Fig 1A). The mean value and standard

deviation value told the average of weight gains on day 6 of pregnant rats was 30.33 g while 9.38 g in unpregnant rats, which showed a statistical difference (Fig 1B). The area under the ROC curve reached to 0.9407, which demonstrated that the ΔW_6 could be a high-performance diagnostic model to help us recognize the pregnant SD rat on GD6 (Fig 1C). Besides, cut-off value helped us understand that a female rat owned 70% probability of pregnancy on GD6 when its ΔW_6 reached more than 25.50 g (Fig 1D). In other words, we could obtain the 70% power to identify the pregnant rats on GD6 by measuring out W_6 and calculating the ΔW_6 .

$\Delta W_7 \sim \Delta W_{12}$ diagnostic models for pregnancy

Similar with ΔW_6 , $\Delta W_7 \sim \Delta W_{12}$ data also followed Gaussian distribution (Fig 2A and 2B). The grouped comparisons by two-way ANOVA were applied to get the p value of each comparison with the 95% confidence interval (CI_{95}) of differences between means (Fig 2C and 2D) and statistical significances were showed in the differences between pregnant rats and unpregnant rats on means of $\Delta W_7 \sim \Delta W_{12}$ (ΔW_7 32.87 g versus 10.15 g, ΔW_8 34.91 g versus 11.00 g, ΔW_9 36.70 g versus 10.91 g, ΔW_{10} 41.23 g versus 10.09 g, ΔW_{11} 44.47 g versus 11.21 g, ΔW_{12} 46.94 g versus 12.37 g, all $p < 0.0001$). The cut-off values of $\Delta W_7 \sim \Delta W_{12}$ on

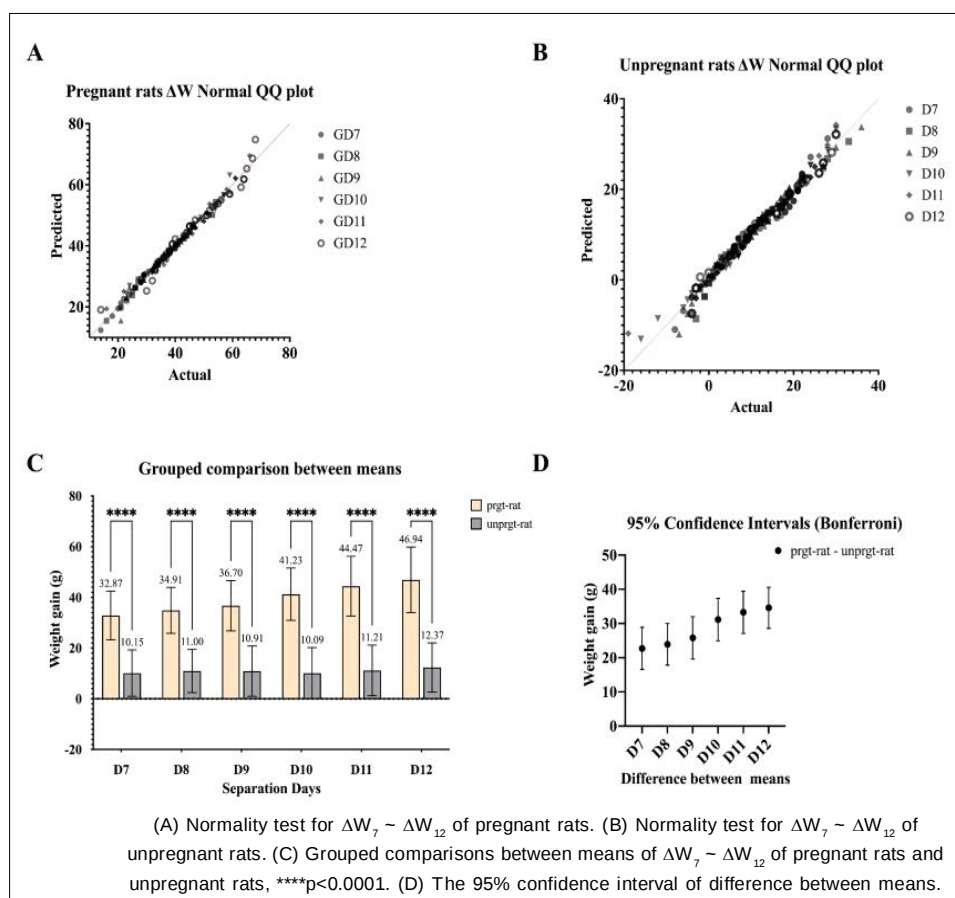


Fig 2: Analysis of $\Delta W_7 \sim \Delta W_{12}$ data from pregnant rats and unpregnant rats.

ROC curve were obtained, Specifically, cut-off value 23.00 g of ΔW_7 granted us 83.87% power to identify the pregnant SD rats on its GD7, cut-off value 22.50g of ΔW_8 owned 93.75% identification power and ΔW_9 22.50 g owned 96.67%, ΔW_{10} 31.00 g conferred 86.67% power, cut-off value 27.00 g of ΔW_{11} conferred 93.33% and ΔW_{12} cut-off value 31.00 g gave 93.75%. Collectively, the models of $\Delta W_7 \sim \Delta W_{12}$ derived from pregnant rats could be in advantage of confirming whether the female rat is pregnant or not from GD7 to GD12.

The hypothesis is that pregnant SD rats may grow faster in weight than unpregnant SD rats on account of the embryo development in uterus. In this research, we confirm that there are statistical significances in differences of means of weight gains between pregnant SD rats and unpregnant SD rats from the 6th day to 12th day after mating. Subsequently, we get seven cut-off values respectively from ROC curve of $\Delta W_6 \sim \Delta W_{12}$ originated from pregnant SD rats and unpregnant SD rats. Each ΔW presents its high potential in distinguishing the pregnant rats and unpregnant rats with its satisfying sensitivity and specificity. Based on these findings, we now have a more efficient and convincing tool to help us pick out the pregnant SD rats at an early stage of gestation for further study about SD rats pregnancy characteristics. The calculated $Time_2$ (T_2 , $T_2 = T_0 + \Delta T/2$ or $T_2 = T_1 - \Delta T/2$), another highlight of this research, is deemed as the time rat fertilized. All the 34 pregnant rats in our study gave birth after 22 or 23 days from $Time_2$, which means $Time_2$ is well tailored to ascertain the beginning of pregnancy.

In summary, this is the first study to utilize weight changes of female SD rats after mating to identify pregnancy. Findings demonstrate a pregnancy confirmation as early as GD6 and physiological data of weight changes during pregnancy of SD rats. Moreover, our findings indicate that SD female rats' $\Delta W_6 \sim \Delta W_{12}$ own potential to perform diagnostic models identifying pregnant rats. This work will strengthen our capacity to obtain pregnant SD rat model for further studies.

Limitations of the study

The weight gains data in the research are collected from 34 pregnant SD rats and 49 unpregnant SD rats. Larger sample size is needed to verify the accuracy of cut-off value from current study. Furthermore, the data of pregnant rats in this study are all primiparous. Whether our study result is applicable for multiparous SD rats needs further study.

CONCLUSION

Weight measurement method brings a great convenience and precision in building pregnant SD female rat model and demonstrates powerful strength in calculating the accurate embryonic age.

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Declarations

Authors' contributions

Qiuyi He contributed to carrying out the research, collecting the data and writing the manuscript. Qingqing Luo applied for the grants, Xiaodong Fu and Qingqing Luo gave instructions on this scientific study and revised this manuscript. Both authors approved the final version.

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Ethics approval and consent to participate

Animal experiments and procedures were approved by the Ethical Review Committee in the Experimental Animal Center of Southwest Medical University (swmu20240051). All applicable international, national and/or institutional guidelines for the care and use of animals were strictly followed. All activities were conducted by following the principles and spirits of 'guiding opinions on treating laboratory animals well' set according to Regulations on the Management of Laboratory Animals (Decree No.2 of the National Science and Technology Commission of the People's Republic of China, 1988).

Availability of data and materials

The data derived from this animal experiment is packaged Additional files, which provide basic information of individual SD rat engaged in this research and analysis data. Anyone who is interested in the files, please contact the authors.

Conflict of interest

The authors declare no competing interests.

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