Maejo Int. J. Sci. Technol. **2026**, *20*(01), 1-15

Maejo International Journal of Science and Technology

e-ISSN 2697-4746
Available online at www.mijst.mju.ac.th

Full Paper

Effects of follow-up frequency on prenatal processes and maternal behaviours in pregnant rats

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Received: 16 May 2025 / Accepted: 1 January 2026 / Published: 5 January 2026

Abstract: This study aims to investigate the impact of follow-up frequency on prenatal processes and maternal behaviours in pregnant rats. A total of 16 primigravida Wistar albino rats were used in the study. The pregnant rats were randomly divided into 4 groups: control group (C), group followed up every 2 days (IG1), group followed up every 4 days (IG2) and group followed up every 6 days (IG3). The daily feed intake, water consumption, weight gain and fasting blood glucose levels of the rats in the groups were determined during the study period. In addition, the birth weight and daily weight gain of the pups were recorded. The open-field test was administered to the pregnant rats and social preference tests were conducted on the maternal rats at post-birth, with locomotor activities evaluated using Python programming. The weight, food and water intake in the IG3 group significantly increased while the IG2 group showed a decrease. The highest offspring weights were recorded in the IG2 group while the lowest were observed in the IG3 group. The longest time spent in the centre of the test maze was recorded in the IG2 group at 39.33±5.50 sec. while the shortest time was recorded in the control group at 21.66±2.88 sec. The follow-up procedure applied every 4 days to pregnant rats was found to decrease food and water intake and weight gain while leading to the highest weights of the offspring, indicating positive effects on behavioural parameters, particularly stress.

Keywords: prenatal process, pregnancy follow-up, social preference test, rat, ultrasonography

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INTRODUCTION

During pregnancy, a process influenced by hormones, physiological and metabolic changes occurs [1]. In the progression of pregnancy process, the health of both the mother and fetus is crucial. Both the mother and the fetus are affected by all positive and negative influences during this period. Under normal conditions, the mother's immune system is sufficient to combat adversities. However, during pregnancy, the physiological and psychological changes in the mother may lead to her immune system being inadequate, depending on the nature and severity of the negative impact [2]. In situations where the mother's immune system is insufficient, negative outcomes such as developmental disorders in the fetus, premature birth and even miscarriage can occur [3]. One of the most significant problems during this period is anxiety, which is an emotional state that causes fear, worry and restlessness, directly affecting the health of both the mother and the newborn [4, 5]. It is known that factors causing anxiety, such as depression and preeclampsia, increase the risk of unplanned cesarean delivery [6]. To prevent such adverse outcomes during pregnancy and to ensure that precautions are taken, periodic checks for both the mother and fetus should be conducted throughout the process [3].

Also known as follow-up, pregnancy checks involve both biochemical and physical assessments of the pregnancy. Biochemical follow-up is conducted to identify hormonal control issues and disorders arising from deficiencies in minerals and vitamins. Additionally, screening tests performed during pregnancy are evaluated within this context [7]. In the physical examinations during pregnancy follow-up, the developmental processes of both the mother and fetus are observed. During this process, physical changes are tracked using sensory observations and various devices [8]. Following the physical changes occurring in the mother during this period is important.

One of the routine procedures in pregnancy follow-up is abdominal examination, which provides information about the health, growth, development and position of the fetus. During the manual examination of the abdominal area, findings such as shape changes, oedema and redness are assessed, and fetal heart sounds are auscultated [9]. Examinations are also conducted using devices such as ultrasound. Ultrasound applications are part of prenatal care used for diagnosing and pregnancy follow-up in developed countries [10]. Prenatal ultrasound is important for diagnosing fetal conditions and following fetal development. Through ultrasound, information such as gestational age, sex, multiple pregnancies and fetal malformation can be identified before birth [11, 12]. The World Health Organisation (WHO) recommends that the first ultrasound should be performed before the 24th week of pregnancy to determine gestational age, detect fetal anomalies, identify multiple pregnancies and enhance the woman's pregnancy experience [13]. Although ultrasound applications in pregnant women may cause anxiety and concern, when the findings indicate normalcy, they can lead to relief and positive thoughts [14]. Pregnancy follow-up is conducted worldwide with the assistance of national and international pregnancy programmes. While follow-up practices differ between countries, the primary aim of these programmes is to assess the health status of the mother and fetus and to establish an obstetric care plan. The recommended frequency of pregnancy follow-up for normal pregnancies varies between countries.

In the United Kingdom it is reported that 10 visits are recommended for uncomplicated nulliparous pregnancies while 7 visits are suggested for uncomplicated multiparous pregnancies [15]. In Australia 10 visits are recommended for an uncomplicated first pregnancy and 7 visits for subsequent uncomplicated pregnancies, with an emphasis on individualising the number of prenatal visits according to the mother’s needs [16]. The WHO has modified the minimum number of visits specified in the basic prenatal care model (FANC - Focus Antenatal Care, 1990) from 4 visits to at least eight contacts ('contacts' instead of 'visits') [17]. In Turkey the Ministry of Health recommends that mothers be followed up four times during the prenatal period [18]. This recommendation appears to align with the WHO FANC model.

WHO envisions a world where "every pregnant woman and newborn receives quality care throughout pregnancy, childbirth and the postpartum period" [19]. To this end, it organises numerous activities and programmes at the international level. While these good practices continue, changing conditions bring about new problems and issues. And every day, new issues are added to maternal health concerns while maternal and infant mortality rates are increasing. WHO and other health organisations are creating programmes to prevent all complications, especially in maternal and infant health by adapting and adopting current and new research findings in the most appropriate way.

Experimental animal studies have become a fundamental component of medical research and have been of vital importance in acquiring basic knowledge in biology. In particular, experimental animal studies have made significant contributions to biomedical and behavioural research [20]. Although some may argue that the models created in many animal studies cannot be applied to humans, it is generally accepted that, due to ethical and moral standards or laws, it is not possible to conduct most experiments on humans initially [21].

In this regard, the present study aims to investigate the impact of follow-up frequency on prenatal processes and behaviours of pregnant primigravida rats, using an experimental animal model developed based on the examination of follow-up frequency applied to pregnant women by WHO and many developed countries. This study seeks to shed light on a situation that is difficult to investigate in humans (by comparing groups of the same age group and gestation period in the same environment and same time frame under different application procedures). This might allow the effect of examination frequency to be revealed.

Pregnancy in humans is divided into three periods, each lasting 12-14 weeks, and each period is called a trimester. The first trimester period in rats generally corresponds to days 4-6 of pregnancy, when implantation occurs. The second trimester period corresponds to days 13-14 in rats, when organs are formed and the heartbeat is detected. The third trimester corresponds to the period between days 19-21, when birth occurs. Although the 21-day gestation period in rats may seem to exclude mammals with longer gestation periods, the stages of pregnancy are similar across all mammals [22, 23]. Although the model organisms in the study are female rats, the results are pioneering, exemplary and inspiring for other mammals with similar physiology and metabolism.

MATERIALS AND METHODS

The study was conducted with the permission of the Animal Experiments Local Ethics Committee of Kirsehir Ahi Evran University, under decision number 23-01 dated 12.12.2024. The procedures used in the study and all methods were performed in accordance with the criteria specified in the Animal Research: Reporting of In Vivo Experiments guidelines [24]. In addition, all procedures performed on live animals in the study were conducted in accordance with the Regulation on the Welfare and Protection of Animals Used for Experimental and Other Scientific Purposes of the Ministry of Food, Agriculture and Livestock of the Republic of Turkey [25]. In the study power analysis (G-Power) was performed using the MINITAB software package, taking similar studies into account. The total number of animals required was calculated as 16, with 4 animals per group, and female Wistar albino rats (primigravida) were used. Throughout the study,

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the rats were kept in a stress-free environment as much as possible at a temperature of 19-21 ºC with a 12-hr light/dark cycle in climate-controlled rooms. The rats were housed in pairs in breeding cages (425 × 265 × 150 mm; floor area 800 cm²) and fed ad libitum standard rodent chow (24% crude protein, 3.94% crude fibre, 5.08% crude fat, 8.8% crude ash, 1.44% lysine, 0.61% methionine, 1.14% calcium, 0.89% phosphorus, 0.28% sodium) and drinking water.

Formation of Study Groups

On the first day of the study, the female rats were placed in the same cage with male rats (2 females to 1 male) for mating. On the sixth day of the study the male rats were separated and the study groups were formed (Table 1).

Table 1. Study groups

Group	Number of Animals (n)	Procedure
C (Control group)	4	Group that receives no follow-up procedures from day 6 of study until birth (15 days)
IG1 (Intervention group 1)	4	Group that receives follow-up procedures every 2 days from day 6 of study until birth (15 days)
IG2 (Intervention group 2)	4	Group that receives follow-up procedures every 4 days from day 6 of study until birth (15 days)
IG3 (Intervention group 3)	4	Group that receives follow-up procedures every 6 days from day 6 of study until birth (15 days)

Study Design and Follow-up Procedures

In the establishment of the study design and follow-up procedures, guidelines such as the Prenatal Care Management Guide, the Labour and Caesarean Section Management Guide, the Postnatal Care Management Guide, and the Emergency Obstetric Care Management Guide currently implemented in Turkey were utilised. A similar model was created for the study based on the follow-up contents specified in these guidelines [26] (Table 2).

Table 2. Follow-up procedures

	Follow-up Procedures	
Step		**Duration (min.)**
1	Use of sensitive scale and measuring instruments to follow-up and control physical characteristics and changes related to pregnancy	2
2	Assessment and inspection of posture, appearance and palpation of sensory organs in pregnant rats	2
3	Measurement of heart rate with stethoscope and body temperature with thermometer	2
4	Detection and pregnancy follow-up using ultrasound imaging	2

Implementation of Follow-up Procedures

The follow-up procedures were performed on pregnant rats by 2 researchers. One of the researchers was a veterinarian. The other researcher had a laboratory-animal-use certificate. An ultrasonography device specifically manufactured for veterinary purposes (Hasvet 838 Veterinary Ultrasound Device, Hasvet®, Turkey) was used to perform ultrasounds on pregnant rats. For this purpose, ultrasound examinations were performed on pregnant rats at intervals specified for each group using a linear probe with a frequency of 50/7.1 MHz via the transabdominal method (2 min.). No analgesic or anaesthetic agents were used during the procedure. Only the areas where the linear probe came into contact with the skin were applied with gel (Lido C Sterile Catheter Gel®, Turkey), which was wiped off and cleaned at the end of the procedure.

Analyses

Physical Analyses

Weight gain: The live weights of pregnant rats in the groups were measured twice using a digital scale on days 6 and 20 of the study. The weight gain of the pregnant rats was calculated as g/100 g/day.

Determination of water consumption: The amount of water consumed by the pregnant rats between days 6 and 20 of the study was measured using a graduated cylinder, and the water consumption was calculated as mL/100g BW/day.

Determination of feed consumption: The amount of feed consumed by the groups was assessed between days 6 and 20 of the study, and the feed consumption was calculated as g/100g BW/day.

Determination of pup birth weight and daily weight gain: At the end of the second hr following birth, the live weights of the newborn pups were measured using a precision scale to determine the birth weights. On the 10^{th} day following birth, the weight measurements of the pups were repeated, and the percentage of live weight gain was calculated.

Biochemical Analyses

Determination of blood glucose levels: On the 11^{th} day of the study a 12-hr fasting diet was applied to the pregnant rats in the groups. Subsequently, blood was drawn from the tail veins and blood glucose levels were measured using a glucometer (Optima blood glucose monitor, Taiwan).

Behavioural Analyses

Open-field test: An open-field test was used to determine the locomotor activity of rats. For this purpose, on the 18^{th} day of the study the locomotor activities of pregnant rats in the groups were determined using an open-field test platform [27]. The test procedure was conducted in a separate test room within the building where the rats were housed. The test was performed using a platform made of opaque material, measuring 100x100x30 cm, with the floor divided into 16 squares. The four squares at the centre of the platform were considered the centre. Experiments were conducted during the light phase, from 8 a.m. to 2 p.m. At the end of each experiment the test platform was cleaned with 70% ethanol. Rats were transferred to the test room 1 hr before the test. They were then placed on the platform and kept there for 60 sec. to acclimate to the environment. After this the rat to be tested was placed on the platform and its movements were observed for 5 min. and recorded with the help of a camera.

In this test the Python programming language, widely used in the field of image processing, was chosen to track the movements of the pregnant rats. By utilising Python's OpenCV library, the video frames provided as input were analysed step by step for object tracking and the position of the rat was updated at the end of each frame. The tracking process within the frames was ensured using the Discriminative Correlation Filter with Channel and Spatial Reliability algorithm. This algorithm provides high accuracy in tracking despite small changes in the image [28].

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The central coordinates of the rat were calculated for each frame to create a movement path, which was then overlaid on the video with line graphics. Throughout the process, the processed frames were displayed on screen simultaneously and recorded as an output video to prepare for the next step. In the following step the path image resulting from the rat's movement was used to calculate the total distance travelled (walking score) solely in pixels.

To achieve this the input image in RGB (red-green-blue) format was converted to HSV (hue, saturation, value) colour space using the OpenCV library. This transformation allowed for the filtering of path lines representing the rat's movements. Appropriate HSV ranges were defined for each colour and masking was performed on the image based on these ranges. This enabled the separation of the path formed after walking from the raw image. After this process the coordinates of each pixel on the resulting path were sequentially collected to create a list. Using this coordinate array, the Euclidean distance was calculated between consecutive points to determine the length of each step.

As a result of this calculation, a pixel-based walking score was obtained directly from the digital image. In the next step the aim was to detect the path formed by the rat's movements on the digital image and export it in a format with a transparent background. To achieve this, the input image in RGB format was converted to HSV colour space using the OpenCV library. Subsequently, thresholding was performed based on the path colour to create a mask. The pixels corresponding to the path colour were then separated using the created masks.

Next, the generated mask was used as an alpha channel to create a new output image in four-channel RGBA (red-green-blue-alpha) format. This resulted in a final image where only the pixels of the path traces were opaque while all other areas were completely transparent. Additionally, by analysing these images the time spent by the rats in the centre and periphery (TSC, TSP), walking score (WS), number of centre visits (NCV), number of rearing movements (NRM) and number of defaecation (ND) were determined, allowing for the identification of behavioural activity differences between the groups.

Social preference test: The study utilised an adapted version of the three-compartment sociality and social novelty preference test platform (80x30x30 cm) used by Kaidanovich-Beilin et al. in their 2011 study [29]. The platform consisted of three chambers with interconnected passages, comprising 37.5%, 25% and 37.5% of the total area. Experiments were conducted during the light phase from 8 a.m. to 2 p.m. on the second day following birth, allowing for the measurement of the rats' interest in their living environment. All compartments of the platform were cleaned with alcohol. In the first compartment, bedding from the rat's own cage was placed while clean bedding was placed in the other compartment. The activity of the rat released into the central compartment was recorded on video for 3 min.. Using the Python programming language, the movement trajectories of the rats in the images were extracted, and preference scores were determined. All compartments and barriers were cleaned with alcohol in preparation for the next social performance test. Using the following formulas, the following values were calculated from the data used in the study:

$$\text{Preference score for own bedding (PSOB)} = (\text{OB} / \text{TD}) \times 100;$$

$$\text{Preference score for clean bedding (PSCB)} = (\text{CB} / \text{TD}) \times 100;$$

where TD = total duration (sec.), OB = time spent in compartment with own bedding (sec.), CB = time spent in compartment with clean bedding (sec.).

Statistical Analyses

The SPSS 22 V statistical software package was used to analyse the data obtained at the end of the study. Initially, the effects of the frequency of observation on certain behavioural and physiological characteristics in pregnant and maternal rats were determined using one-way analysis of variance (ANOVA). In cases where significant differences were found, the Duncan test, a multiple comparison test, was used to identify which specific treatment(s) contributed to the differences. Additionally, a significance level of $p < 0.05$ was accepted for all calculations in the study.

RESULTS

Table 3 shows the live body weight, weight gain, and feed and water consumption of the pregnant rats in the groups. The weight gains among the pregnant rats in the groups show similarity between C, IG1 and IG3 groups while significant differences are found between IG2 group and the others ($p < 0.05$). Additionally, feed and water consumption were measured across the groups with significant differences observed ($p < 0.05$). The observation procedures applied in the current study clearly affect the pregnant rats. The procedure applied to the IG3 group increases the live weights, feed and water consumption of the pregnant rats, whereas a decrease is observed in the IG2 group.

Table 3. Live weight gain, feed and water consumption data of pregnant rats

Group	LW (g)	LWG (g/100 g/day)	FC (g/100 g/day)	WC (mL/100 g/day)
C (Control)	140.00±2.33^{a}	1.46±0.03^{a}	7.52±0.14^{b}	26.82±0.15^{b}
IG1	147.13±0.20^{a}	1.55±0.01^{a}	7.17±0.23^{c}	26.35±1.65^{b}
IG2	152.90±10.42^{a}	0.91±0.20^{b}	7.08±0.05^{c}	22.66±1.57^{c}
IG3	144.90±7.79^{a}	1.58±0.07^{a}	7.83±0.09^{a}	29.91±0.67^{a}

Note: Means indicated by the same letter are not significantly different at $p < 0.05$ level.
C = Control, IG1 = Group subjected to observation procedure every 2 days, IG2 = Group subjected to observation procedure every 4 days, IG3 = Group subjected to observation procedure every 6 days, LW = Live weight, LWG = Live weight gain, FC = Feed consumption, WC = Water consumption

Table 4 shows the live weights and weight gains of the newborn pups. Significant differences are found among the live weights of the newborn pups ($p < 0.05$). The highest pup weight values are observed in the IG2 group while the lowest values are measured in the IG3 group. In addition, the observation procedure applied to the pregnant rats in the IG1 group reduces the weight gain rate of the newborn pups, whereas the procedure applied to the IG3 group increases it ($p < 0.05$).

An open-field test was conducted on the 18th day of pregnancy in order to ascertain the locomotor activity of the pregnant rats. Using the Python programming language, open-field activities and paths followed by the pregnant rats are identified (Figure 1A). Significant differences of the calculated times spent by the pregnant rats in the centre of the test platform are identified ($p < 0.05$) (Figure 1B). The longest time spent in the centre is 39.33 ± 5.50 sec. in the IG2 group while the shortest time is 21.66 ± 2.88 sec. in the C group. As for the time spent in the periphery of the platform, the IG1, IG3 and C groups show similarities while significant differences are found between IG2 and the others ($p < 0.05$) (Figure 1C).

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Table 4. Weight gains of pups

Group	**LWP** (g)	**WGP** (%)
C (Control)	4.40±0.43^{ab}	22.45±0.78^{b}
IG1	4.26±0.40^{ab}	19.77±1.02^{c}
IG2	4.73±0.25^{a}	20.54±1.41^{bc}
IG3	4.05±0.11^{b}	24.99±1.02^{a}

Note: Means indicated by the same letter are not significantly different at p < 0.05 level.
C = Control, IG1= Group subjected to observation procedure every 2 days, IG2= Group subjected to observation procedure every 4 days, IG3= Group subjected to observation procedure every 6 days, LWP = Live weight of pups, WGP= Weight gain in pups

The walking scores of the pregnant rats determined in pixels using the Python programming language show significant differences among the groups ($p < 0.05$) (Figure 1D). The highest walking score value is in the IG2 group while the lowest is in the IG3 group. The numbers of visits made by the rats to the centre of the platform are similar among the IG1, IG3 and C groups while significant differences are observed between IG2 and the others ($p < 0.05$) (Figure 1E). Similarly, the numbers of rearing movement of the rats on the open-field test platform are found to be insignificant among the IG1, IG3 and C groups while significant differences are noted between IG2 group and the others ($p < 0.05$) (Figure 1F). Additionally, the numbers of defaecation of the pregnant rats during the test are recorded (Figure 1G). There is no defaecation in the IG2 group.

The activities of the mother rats determined on the second day following birth using a social preference platform were tracked using the Python programming language (Figure 2A). Preference was measured using soiled bedding from the area where the rats lived and clean bedding placed in the 2 compartments of the test platform. Significant differences are found in the rates of preference for their own bedding among the rats ($p < 0.05$) (Figure 2B). The highest preference rate for their own bedding is observed in the IG2 group at 19.36 ± 0.72% while the lowest rate is recorded in the C group at 8.90 ± 0.34%. The preference rates for clean bedding are found to be 15.73 ± 0.32% in IG3 group, 29.55 ± 0.85% in the IG1 group, 48.32 ± 4.14% in the C group, and 51.00 ± 3.60% in IG2 group (Figure 2C). Significant differences are noted among the preference rates for clean bedding across the groups ($p<0.05$). Additionally, when the rats' walking scores on the social test platform are determined in pixels and then evaluated using the Python programming language, the intervention groups differ from the control group but are similar among themselves (Figure 2D).

Figure 3 shows blood glucose levels of the pregnant rats, which demonstrates that the differences among the groups are insignificant and that the follow-up procedures applied in the study do not affect the blood glucose level.

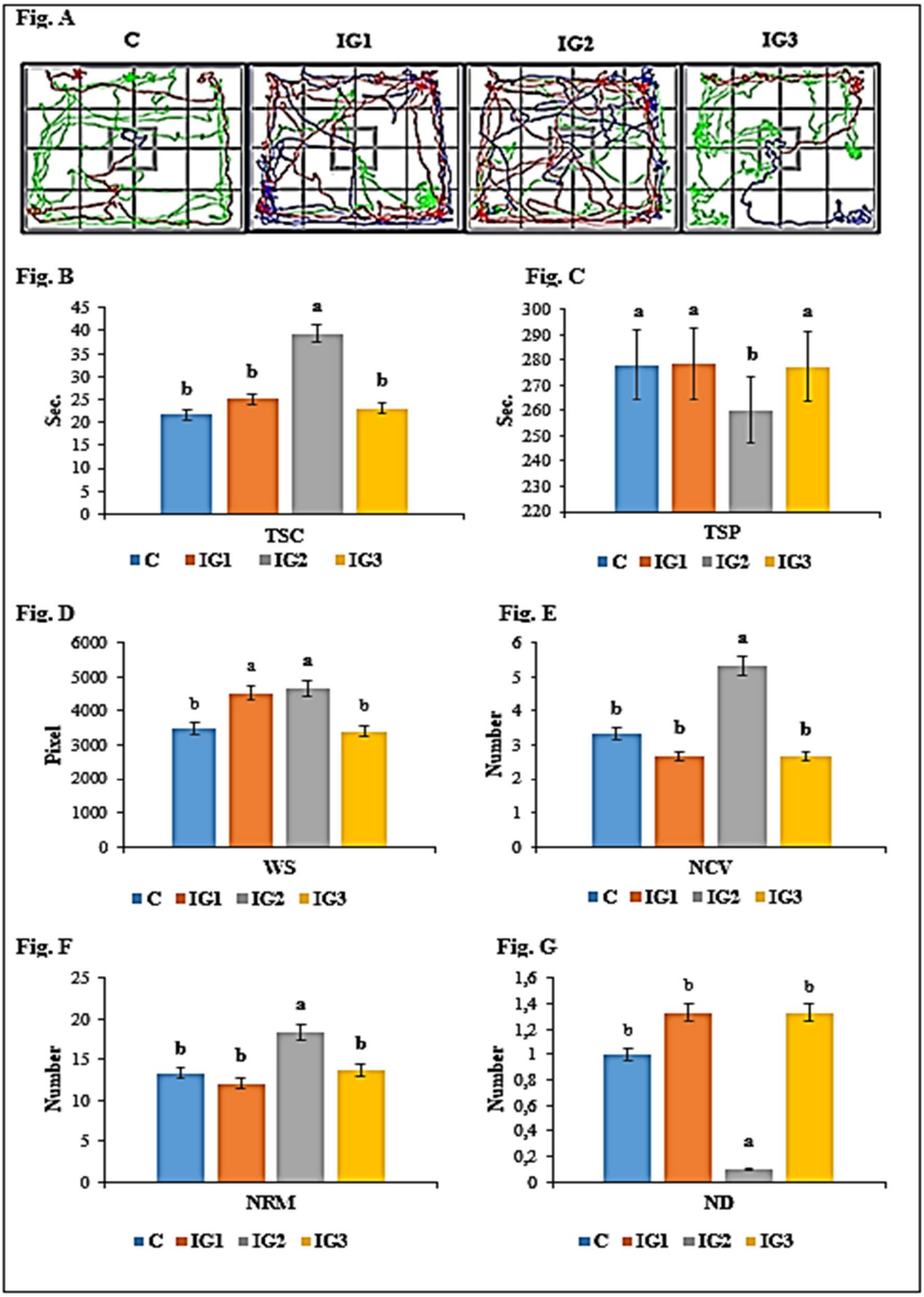

Figure 1. Open-field activity values of pregnant rats

Fig. A= Paths followed by rats on open-field platform; Fig. B= Time spent in the centre; Fig. C= Time spent in the periphery; Fig. D= Walking scores; Fig. E= Centre visits; Fig. F= Rearing counts; Fig. G= Defaecation counts. C= Control, IG1= Group subjected to observation procedure every 2 days, IG2= Group subjected to observation procedure every 4 days, IG3= Group subjected to observation procedure every 6 days; TSC= Time spent in the centre, TSP= Time spent in the periphery, WS= Walking score, NCV= Number of centre visits, NRM= Number of rearing movements, ND= Number defaecation.

Note: Means indicated by the same letter are not significantly different at $p < 0.05$ level.

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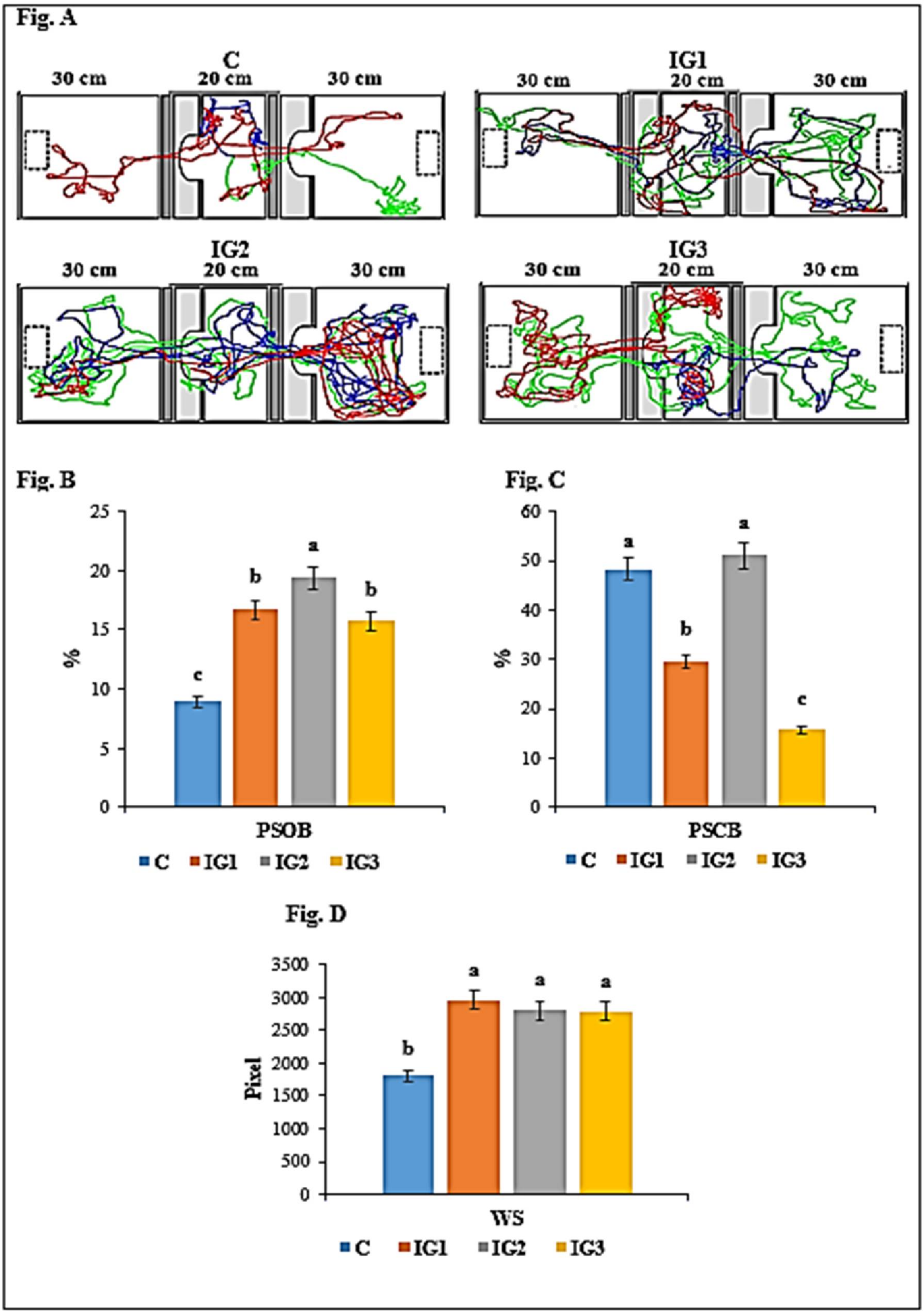

Figure 2. Social preference activity values of mother rats

Fig. A= Social preference paths; Fig. B= Preference for soiled bedding; Fig. C= Preference for clean bedding; Fig. D= Walking scores. C= Control, IG1= Group subjected to observation procedure every 2 days, IG2= Group subjected to observation procedure every 4 days, IG3= Group subjected to observation procedure every 6 days, PSOB= Preference score for own bedding, PSCB= Preference score for clean bedding, WS= Walking score.

Note: Means indicated by the same letter are not significantly different at $p < 0.05$ level.

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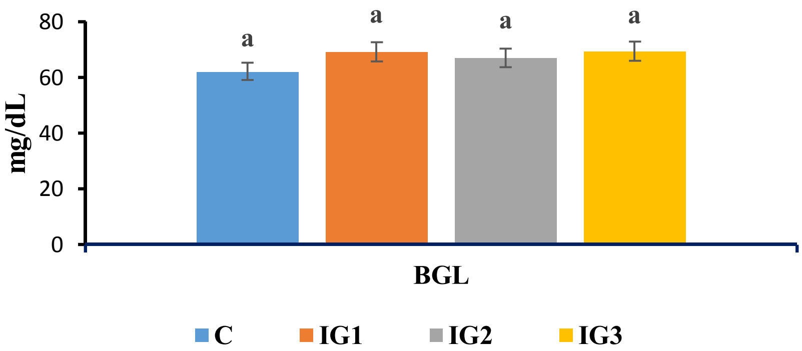

Figure 3. Blood glucose levels (mg/dl) of pregnant rats

C= Control, IG1= Group subjected to observation procedure every 2 days, IG2= Group subjected to observation procedure every 4 days, IG3= Group subjected to observation procedure every 6 days; BGL= Blood glucose level.
Note: Means indicated by the same letter are not significantly different at $p < 0.05$ level.

DISCUSSION

Prenatal stress refers to the exposure of a woman to stress factors that indirectly affect the fetus through maternal stress during pregnancy [30]. Adult rats exposed to varying ultrasound frequencies (22-40 kHz) experience a state of stress. When exposed chronically for approximately three weeks, as previously shown, the rats develop a depressive-like state [31].

The developing fetus during the prenatal period is affected by examinations and checkups in various ways. In particular, the fetus, which is sensitive during this period, is susceptible to effects such as ionising radiation. Therefore, the frequency of follow-up and the methods used during the prenatal period need to be optimised [32]. This is crucial because any adverse outcomes during this period will affect both the mother and the newborn.

Routine follow-up of pregnant women during the prenatal period is essential. These check-ups are important for ensuring controls such as weight gain, immunisation and vitamin and mineral support and for the healthy progression of the process [33]. Knowing women's pre-pregnancy weight and weight changes during pregnancy is crucial for following up on fetal development and identifying potential risks. Excessive weight gain during this period can lead to serious issues such as eclampsia, preeclampsia, oedema and obesity, and consequently, the morbidity and mortality rates for both mother and fetus may increase [34]. In the present study the live weight gains and feed and water consumption of the pregnant rats in the groups were determined and significant differences were found between the intervention groups ($p<0.05$). The lowest daily live weight gain was recorded in group IG2. The highest feed and water consumption among pregnant rats occurred in group IG3 while the lowest consumption was in group IG2. The follow-up procedure applied to the pregnant rats every 4 days resulted in a reduction in live weight gain, feed and water consumption.

Additionally, the study found that the highest live weights of the newborns were in group IG2 while the lowest pup weights were observed in group IG3. The highest values of feed/water consumption and live weight gain of pregnant rats were observed in group IG3. Maternal factors such as birth weight, number of offspring, age, nutrition, obesity and stress affect intrauterine growth during pregnancy [35]. Patin et al. [36] reported in their study that offspring born to stressed mothers may experience adverse effects such as mortality and growth retardation. In the present study the low pup weight values and open-field test parameters observed in the IG3 group are consistent with these findings. This is because the time spent in the centre of the platform is inversely related to stress. Pregnant rats in the IG3 group spent less time in the centre compared to the other groups, which serves as an indicator of their heightened stress levels.

Pregnancy is a special period marked by joy and emotional well-being. However, pregnant women are also prone to various mental health issues during this time [37]. The prevalence of anxiety among pregnant women worldwide ranges from 11.4% [38] to 63% [37]. Anxiety also negatively impacts maternal, fetal, neonatal and child health during the prenatal, postnatal and childhood periods [39]. In the current study the locomotor activity of pregnant rats was assessed using TSC, TSP, WS, NCV, NRM and ND data through an open-field test platform and Python programming. The highest TSC, WS, NCV and NRM values, along with the lowest TSP, were recorded in group IG2.

Defaecation did not occur in the IG2 group. This is an indication that their stress levels were low. In the open-field test, an increase in the time spent in the platform centre indicates lower stress while a decrease indicates the presence of anxiety [40]. Thus, the findings show that the animals in the IG2 group were less affected by the examination procedure and the application caused less stress in the animals in this group compared to the other groups. This is critically important for the health of the mother during pregnancy and consequently for the long-term well-being of the offspring. A similar study conducted on animals reported that stress has specific and lasting effects on caregivers and mothers who have previously experienced stress exhibit an overly protective parenting style compared to mothers with no history of stress [41]. Additionally, it is known that stress or depressive states in the mother before and after birth result in the offspring raised in this maternal environment exhibiting depressive-like behaviours [42]. If this negative stress situation persists, the relationship between the mother and the offspring may be affected and various problems may be encountered in the transmission of maternal characteristics such as warming, breastfeeding, licking and grooming that must be taught from mother to offspring across generations [43].

CONCLUSIONS

As a result of the investigation of the effects of pregnancy examination frequency on maternal behaviour and offspring of pregnant rats using an experimental pregnancy model, it can be concluded that the examination procedure applied every 4 days should be most seemly. Using the rat experiments as model, follow-ups for pregnant women should be conducted every trimester and also close to the delivery date. In cases of high-risk pregnancies requiring closer monitoring, the frequency of follow-ups should be increased, taking care not to cause undue stress to the pregnant woman. It is also believed that the results of this study, conducted on pregnant rats may benefit other animal studies and researches on mammals.

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