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Ultrasound assessment of the effect of fetal position on supine to prone righting reflex in the guinea pig fetus

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Abstract

The aim of the study was to assess the effect of guinea pig fetal position on the righting reflex from a supine to prone position using ultrasound examination. Experimental and control group were formed, each encompassing 20 pregnant females with 50 fetuses from the 31st to 66th day of gestation. During ultrasound examination, the fetuses from the experimental group were brought into a supine position relative to gravity by placing the dams in the appropriate position. In the control group fetuses were examined for changes in position irrespective of gravity as well as for changes in position after having been brought into a prone position relative to gravity. Comparison of the experimental and the control group showed that experimental group fetuses in the oldest bracket rotated successfully to a prone position more frequently than at other ages. The observed fetal movements indicate that the fetus changes its position using the contact-righting reflex.

INTRODUCTION

The righting reflex involves active returning of the body to a prone position after any changes in its position. Depending on the medium in which we induce this reflex, there are two forms: air-righting and contact-righting. Air-righting reflex can be induced by dropping an experimental animal in free fall on its back. A variant of the air-righting reflex can be induced in water. Contact-righting reflex can be induced by turning an experimental animal to a supine position on a flat surface. Contact-righting reflex can be triggered with tactile and vestibular stimuli. Righting reflex in the air and water is derived from vestibular and visual stimulation *(1, 2)*.

The guinea pig (Cavia porcellus) is a precocial mammal. Gestation in guinea pigs lasts about 66–70 days, and it is known that the guinea pig's contact-righting reflex develops prenatally. The earliest occurrence of the reflex was recorded on the 60th day of gestation among prematurely born pups. Attempts to induce the righting reflex *in vivo* in the guinea pig fetus after the 60th day of gestation were unsuccessful; fetuses kept their initial intrauterine position regardless of changes in maternal posture *(3)*. Yet, X-ray and ultrasound studies of the fetuses of some precocial mammals, such as pony mares and sheep, have shown that the fetuses change their position from supine to prone immediately before or during delivery. In those studies, induction of the righting reflex the supine to prone position was not attempted; spontaneous behavior of the fetus was observed while the pregnant females assumed a quadrupedal standing posture *(4, 5, 6)*.

Study of the righting reflex in intrauterine environment is interesting for several reasons. First, one of the tasks of developmental science is to identify particular interactions between the fetus and the environment that affect differentiation and growth *(7)*. The possibility of inducing the reflex in intrauterine conditions would prove the presence of continuity between intrauterine and extrauterine development, and also show that vestibular, tactile and kinesthetic stimuli have similar characteristics in relation to gravity in both intra- and extrauterine environments. Also, a proportion of successful righting reactions has often been used as a parameter to estimate individual maturity *(2)*. The change of intrauterine position resulting from an induced righting reaction would thus prove the previously stated assumption that fetal behavior may be gravity-dependent *(8)*.

The aim of the present study was to assess the effect of guinea pig fetal position on the righting from a supine to prone position. The premise of the study was that the fetuses passively brought into a supine position relative to gravity would actively turn to a prone position as a result of the righting reflex.

METHODS

Animals and environment

The study subjects were albino guinea pigs (*Cavia porcellus*) obtained from the Department of Biochemistry, Faculty of Medicine, Novi Sad. The experiments with animals were approved by the Ethics Committee of the Institute of Neurology in Novi Sad. The guinea pigs were kept in 400Wx1000Lx300H mm plastic containers in a harem system: three or four females and one male. Pregnant females were moved to 300Wx300Lx300Hmm plastic containers after the 60th day of gestation, where they were kept separate until delivery, and afterwards for the first 15 days with the offspring. The animals had a standard commercial pellet diet and *ad libitum* water enriched with vitamin C (30 mg/100 ml water). Artificial cycles with 12 hours of light (08:00–20:00) and 12 hours of dark were provided.

Experimental design

Inspection of vaginal introitus was performed daily, and the day of vaginal membrane perforation was taken as the first day of gestation. Individual guinea pigs were identified by yellow patterns on their backs. Pregnant guinea pigs with one to four fetuses were included. The numbers of fetuses were determined by ultrasound examination, and the pregnant guinea pigs were randomly selected for the experimental and control group, until each group contained the same number of pregnant females and fetuses. Each group (experimental and control group) comprised 20 pregnant females with 50 fetuses in total, i.e. a total of 40 pregnant females with 100 fetuses were included in the study. The precise number of dams and the number of fetuses tested across groups are shown in Table 1.

Published data show that fetal reflex activity first occurs around the 31st day of gestation *(9)*. Because of that ultrasound examination was started on the 31st or 32nd day of gestation. In the experimental group dams were rotated until the fetuses were brought into a supine position. Each pregnant guinea pig in the experimental group had a total of 18 ultrasound examination sessions during gestation. Ultrasound examination was performed every other day until the 65th or 66th day of gestation.

Control group was formed for two reasons. First, to show that changes in fetal position registered in the experimental group were not a result of accidental fetal movements. Control dams were examined in horizontal supination to investigate the number of spontaneous changes in fetal position irrespective of gravity. The results from this group are hereafter referred to as group A1. Ultrasound examinations were continued while the dams were brought in such a position that the fetus was in a prone position. The reason for this examination was to demonstrate that the registered changes in the fetal position in the experimental group were not caused by maternal handling stress. The results obtained in this way are hereafter marked as control group A2. Each pregnant guinea pig in the control group had a total of 18 ultrasound examination sessions during gestation. Ultrasound examinations started on the 31st or 32nd day and were performed every other day until the 65th or 66th day of gestation.

Ultrasound examination

The shaving of the abdominal region was made during a short-term inhalatory ether narcosis on the 25th day of gestation. The pregnant guinea pigs were supported in a supine position on a 15x30cm board using plastic strips with clasps, fastened over the thoracic area and both hind legs. The strips were pulled through holes in the board near the body of the animal and fastened on the other side of the board (Figure 1). During the preparation of animals for ultrasound examination they were treated carefully, so they were calm during the restraint. Ultra-

TABLE 1

The number of dams and fetuses in experimental and control groups.

Group	Number of dams with one fetus	Number of dams with two fetuses	Number of dams with three fetuses	Number of dams with four fetuses	Total
Experimental	6	4	4	6	20
Control	5	5	5	5	20

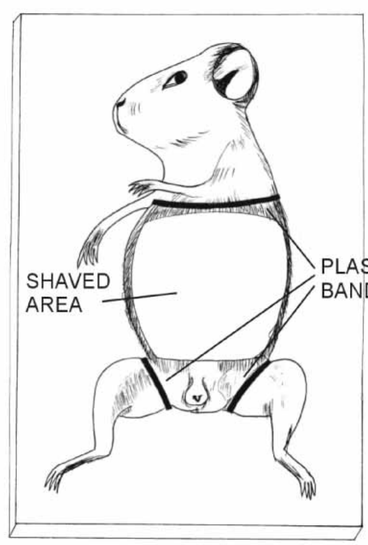
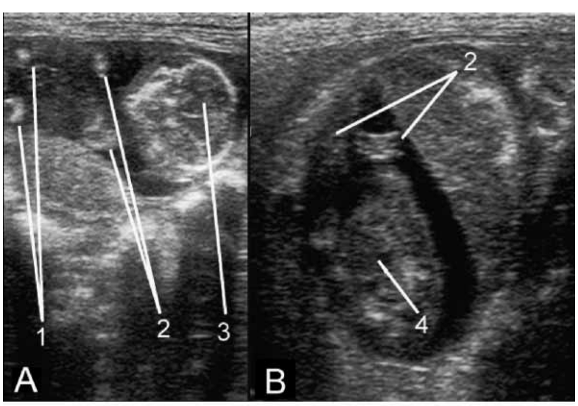


Figure 1. *Guinea pig prepared for ultrasound examination.*

sound examinations were performed with a Siemens SL-2 apparatus with a 7.5 MHz linear probe. Pregnant females from the control and experimental groups were brought into supination by manipulating the board they were fastened to. The number of fetuses, their situses and presentations were determined. The orientation of an individual fetus was determined by tracking along the longitudinal and transversal axes of the fetus with the ultrasound probe. The position of the fetus' trunk was determined on the basis of the positions of its heart and forelimbs and hind legs (Image 1). Then in the experimental group, the board was rotated until the fetus was brought into a supine position relative to gravity and the examination was repeated. In the cases where a fetus was initially observed in a supine position, the pregnant female's position was not changed. In the control group, the dams were first examined in horizontal supination. Then, they were manipulated until the observed fetus came into a prone position. During the examination the following parameters were observed: the absence of any changes in position, rotation to a lateral, prone, supine, head-down or sitting position. The examination session was complete at the moment when a change in the fetus' position was registered, or terminated if no changes were observed during the arbitrarily set period of two minutes. The pressure of the ultrasound probe on the female's body was light, because too much pressure could induce movements of the fetus and changes in the fetal position. In the case a pregnant female carried two to four fetuses, the fetuses were examined separately, with a 10-minute interval between each examination. During the 10-minute interval the pregnant dams were kept in supination.



1. Hind limbs 2. Fore limbs 3. Head 4. Heart

Image 1. *Longitudinal (A) and transversal (B) images of a fetus on the 35th day of gestation.*

In both groups, technical limitations prevented us from examining all fetuses from multiple gestations during one session, but at least one fetus from a multiple gestation was examined during one session. From the 50th day of gestation the fetus kept the same intrauterine place during manipulation with the dams, and this provided the possibility of visualizing the same fetus during consecutive examinations. Until the 40th day of gestation this was not possible because of fetal changes of intrauterine location during manipulation with the dams. The latencies in positive responses could not be observed because of the two-dimensional nature of ultrasound examination and because the examinations were interrupted while the dams were being handled.

Righting reflex among newborns

The presence or absence of the contact-righting reflex on the first day of extrauterine life was examined in both groups by placing newborns supine on a flat surface. Newborn's positioning itself on the plantar side of all four extremities was regarded as a positive response. In order to prevent possible postural correction by visual stimuli, the newborn's eyes were temporarily closed during examination by putting sticking bandages carefully over the animal's closed eyelids.

Data analysis

In the experimental group, rotation of a fetus from a supine to prone, lateral, head-down, and sitting position was regarded as a positive response. The absence of a change in primary position was taken as a negative response. In control group A1, any change in fetal position was considered a positive response. In control group A2, a fetus assuming a lateral, supine, head-down, and sitting position was considered a positive response. A fetus assuming a lateral or prone position was compared between control group A1 and the experimental group. The assumption of a lateral position was compared between control group A2 and the experimental group. In addition, the assumption of a supine position in control group A2 was compared to the assumption of a prone position in the experimental group. Data about overall fetal activity, such as fetus assuming a head down, sitting, supine, prone or lateral position, were compared between the experimental group and each of the control groups.

A test was done to check if the data followed normal distribution (Shapiro-Wilk test). Since the data this wasnot the case, a non-parametric Mann-Whitney test was used. An alpha level of $p<0.05$ was adopted to determine significance in statistical tests. Results obtained from the experimental and control group were compared with regard to the whole gestational period observed, i.e. 31st–66th day of gestation, and each ten-day gestation interval within it: gestation days 31–40, 41–50, 51–60, and 61–66. In addition, the experimental and control group were compared with regard to the number of fetuses.

RESULTS

The most frequent initial position recorded at the beginning of ultrasound examination in both experimental and study groups was lateral (Table 2). In the experimental and control group A1 we did not register rotation into a head down, sitting or supine position. In control group A2, positive responses were related exclusively to the assumption of a supine or lateral position. In this group, rotation from the initial prone position into a head down or sitting position was not registered.

TABLE 2

Position of fetuses prior to rotation.

Initial body position	Experimental group No %	Control group A No %
Lateral	441 78.89	536 79.05
Prone	46 8.22	64 9.43
Supine	38 6.79	41 6.04
Sitting	24 4.29	27 3.98
Upside-down	10 1.78	10 1.47
Total	559 100.00	678 100.00

No = number of scans

For the whole period studied, i.e. from the 31st to the 66th day of gestation, the experimental group fetuses rotated significantly more frequently than the fetuses from control groups A1 and A2 (Figure 2). For group A1, the difference was present with the following comparisons: 1) all positive vs. negative responses showed a significant difference $Z = -4.980$, $p = 0.00$; 2) rotation to a prone position vs. all other responses $Z = -4.816$, $p = 0.00$; and 3) rotation to a lateral position vs. all other responses $Z = -4.747$, $p = 0.00$. For group A2 the results are: 1) all positive vs. negative responses $Z = -5.144$, $p = 0.00$; 2) rotation to a lateral position vs. all other responses $Z = -4.718$, $p = 0.00$; 3) rotation to a prone position in the experimental group and rotation to a supine position in group A2 vs. all other responses $Z = -4.515$, $p = 0.00$.

The incidences of the righting reflex in control groups A1, A2, and the experimental group were compared for each gestation interval. For the experimental group and control groups A1 and A2, the proportion of positive re-

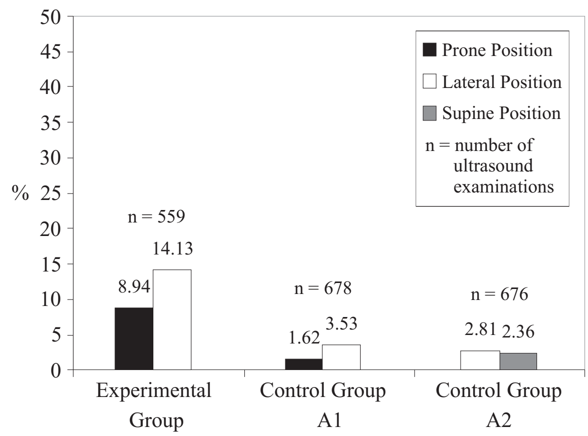


Figure 2. *Percentage of positive responses in the experimental and control groups A1 and A2 for the whole gestation period.*

sponses is presented in Figure 3. Results of the comparison of the experimental group and control groups are presented in Table 3. A statistically significant difference was absent only for rotation to a prone position in the period from the 31st to 40th day of gestation and rotation to a lateral position in the period from the 61st to 66th day of gestation.

The incidences of the righting reflex in the control group and the experimental group with regard to the number of fetuses were also compared. The percentage of positive responses is presented in Figure 4. Results of the comparison of the experimental group and control groups are shown in Table 4. The comparison involving the number of fetuses indicates a greater variability in the number of positive responses.

DISCUSSION

In terms of statistical significance, control groups A1 and A2 showed less fetal rotation from the initial position compared to the experimental group. Statistically significant difference between the experimental group and control group A1 suggests that rotations in the experimental group were not the result of accidental fetal movements. Similarly, the statistically significant difference between the experimental group and control group A2 suggests that rotations in the experimental group were not a result of maternal handling stress. The proportion of successful rotations increased with the advance of gestation. Similar results showing increased incidence of prone rotations among older individuals have been found also in altricial rodents and other species whose ontogeny has been studied, such as the Norway rat, roof rat, field mouse, and cat *(2)*.

The air-righting reflex is the ability of an animal to right itself without the benefit of net external torques. This reflex requires functionality of either the visual or the vestibular system *(1)*. In the guinea pig, the period from the 35th to the 40th day of gestation is characterized by the established link between the sensory epithelium of the vestibular apparatus and the vestibular ganglion *(10,*

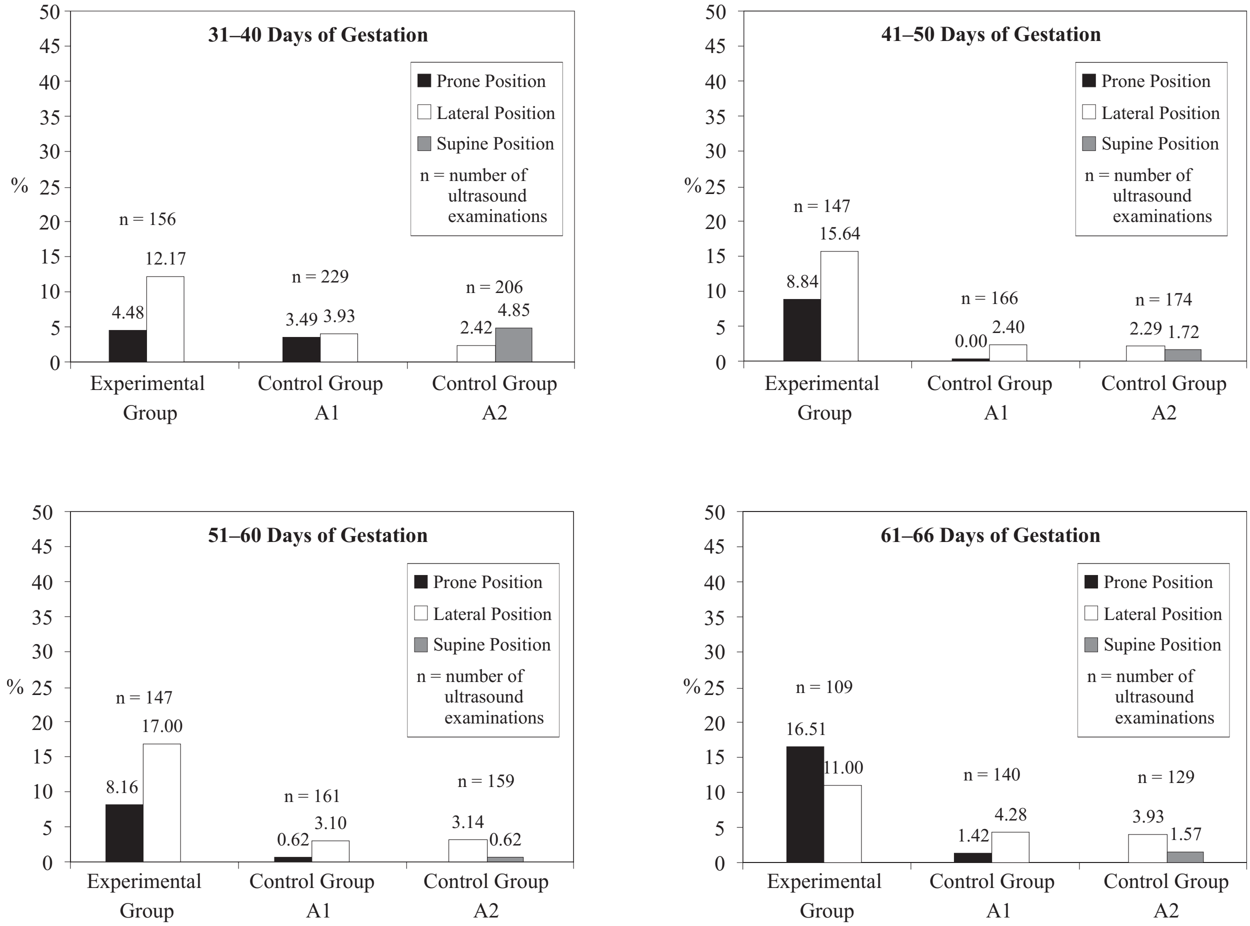


Figure 3. *Percentage of positive responses in relation to the gestation period.*

TABLE 3

Results of Mann-Whitney test comparison between experimental and control group in relation to the period of gestation.

Period of gestation	Control Group A1	Control Group A2
Gestation day 31–40	Z = –0.280 p = 0.820	Z = –0.016 p = 0.989
Rotation to prone	**Z = –2.419 p = 0.023**	**Z = –3.070 p = 0.004**
Rotation to supine	**Z = –2.631 p = 0.009**	**Z = –2.594 p = 0.010**
All positive responses		
Gestation day 41–50	**Z = –3.330 p = 0.014**	**Z = –2.420 p = 0.049**
Rotation to prone	**Z = –3.879 p = 0.000**	**Z = –3.647 p = 0.000**
Rotation to supine	**Z = –4.912 p = 0.000**	**Z = –4.479 p = 0.000**
All positive responses		
Gestation day 51–60	**Z = –2.900 p = 0.026**	**Z = –2.883 p = 0.028**
Rotation to prone	**Z = –3.512 p = 0.001**	**Z = –3.352 p = 0.001**
Rotation to supine	**Z = –4.303 p = 0.000**	**Z = –4.135 p = 0.000**
All positive responses		
Gestation day 61–66	**Z = –4.062 p = 0.000**	**Z = –4.065 p = 0.000**
Rotation to prone	Z = –1.805 p = 0.114	Z = –2.194 p = 0.056
Rotation to supine	**Z = –4.541 p = 0.000**	**Z = –4.742 p = 0.000**
All positive responses		

Results with $p < 0.05$ are printed in bold

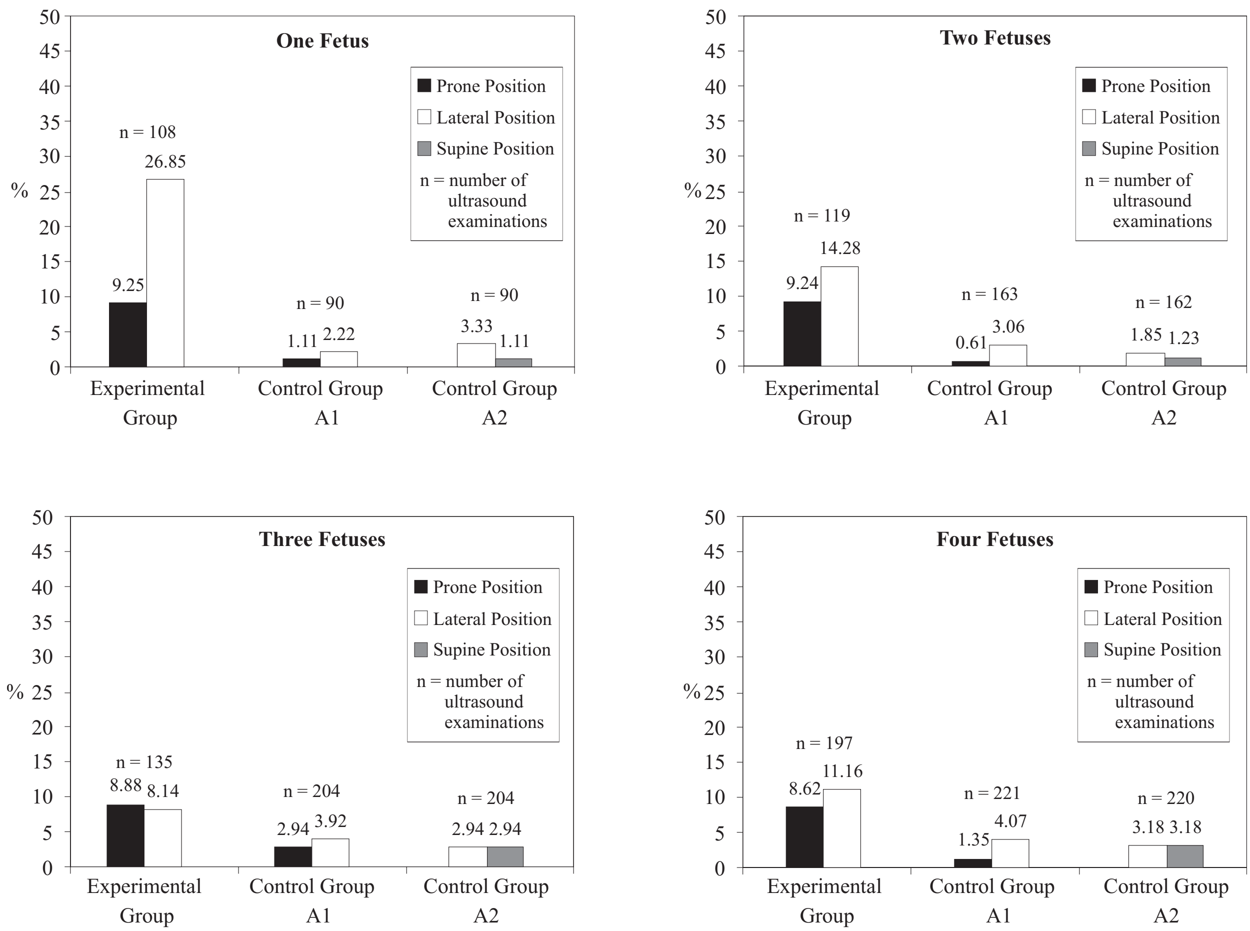


Figure 4. *Percentage of positive responses in relation to the number of fetuses per gestation.*

TABLE 4

Results of Mann-Whitney test comparison between experimental and control groups in relation to the number of fetuses.

Number of fetuses	Control Group A1	Control Group A2
One fetus	Z = –2.063 p = 0.052	Z = –2.063 p = 0.052
Rotation to prone	**Z = –2.803 p = 0.004**	**Z = –2.796 p = 0.004**
Rotation to supine	**Z = –2.783 p = 0.004**	**Z = –2.764 p = 0.004**
All positive responses		
Two fetuses	**Z = –2.558 p = 0.016**	**Z = –2.491 p = 0.016**
Rotation to prone	**Z = –2.460 p = 0.016**	**Z = –2.470 p = 0.016**
Rotation to supine	**Z = –2.449 p = 0.016**	**Z = –2.460 p = 0.016**
All positive responses		
Three fetuses	**Z = –2.214 p = 0.032**	Z = –1.722 p = 0.111
Rotation to prone	Z = –1.359 p = 0.190	Z = –1.353 p = 0.190
Rotation to supine	Z = –1.230 p = 0.286	Z = –1.476 p = 0.190
All positive responses		
Four fetuses	**Z = –2.745 p = 0.004**	**Z = –2.739 p = 0.004**
Rotation to prone	**Z = –2.556 p = 0.009**	**Z = –2.556 p = 0.009**
Rotation to supine	**Z = –2.739 p = 0.004**	**Z = –2.739 p = 0.004**
All positive responses		

Results with $p < 0.05$ are printed in bold

11). Myelination of the vestibular nerve starts at approximately the 40th day of gestation *(10)*. The manifested maturity of the vestibular apparatus indicates that it may initiate the appearance of the vestibular-righting reflex starting from the 40th day of gestation. In the literature available to the authors there is no data about the maturation of proprioceptive and cutaneous receptors in the guinea pig fetus that could initiate the contact-righting reflex.

The observation of fetal movements during rotation to a lateral or prone position suggested the type of righting reflex. Fetuses changed their position by pushing their fore and hind legs or their trunks against the walls of the intrauterine cavity. These movements were accompanied by trunk rotation. We could not observe the whole sequence of rotation to a prone or lateral position due to the two-dimensional nature of the ultrasound examination. Considering that the contact-righting reflex presupposes rotation by pushing oneself against a support *(2)* while in air-righting rotation occurs without net external torques *(1)*, our results suggest that this was most likely a contact-righting form of the reflex. It was not possible to draw a conclusion about whether the righting reflex derived from vestibular or tactile input.

In a single study published on the intrauterine presence of the righting reflex from supine to prone positions in the guinea pig, there were no changes in fetal position on the 63rd day of gestation *(3)*. That study was done with only one pregnant guinea pig with two fetuses, which probably significantly influenced the results. A positive response could not always be induced in our study either, and in this period rotations to lateral and prone positions could be induced in 27.51% of all attempts. On the other hand, in the mentioned study *(3)* the guinea pig was fixed by stretching out and tying all four extremities. It was firmly fastened to a supporting platform on the ventral side. This caused great pressure on the abdomen of the dam and as a result the fetuses had little space for movement and change in position. In our study the righting reflex could be induced in fetuses already from the 41st to 50th day of gestation, while in the earlier study it was observed only on the 60th day of gestation among prematurely born pups *(3),* i.e. 20 days later than the first appearance recorded in the present study. The explanation for this discrepancy may lie in the different methodological approaches used. The premature birth of fetuses may worsen their general condition and significantly reduce their motor capabilities already displayed in the intrauterine environment. Intrauterine movements are facilitated by amniotic fluid buoyancy *(12),* and the contact-righting reflex may consequently be observed at an earlier stage of development.

If the fetus is in a lateral position relative to gravity, gravity could produce an asymmetrical stimulus that may induce the contact-righting reflex. Yet, most fetuses (79%) in the experimental group and control group had a lateral position when examination began. In adult quadrupeds, lateral position relative to gravity represents an inactive posture; their body-on-body reflex is inhibited and occurs only if their head is restrained. During maturation, the body-on-body righting reflex is not inhibited and is present in the newborn of altricial mammal species *(2)*. However, when neonatal rats start going to nipple, they rotate themselves into a supine position and crawl to the nipple on their backs *(13)*. This indicates that the motor centers in the brain stem responsible for the contact-righting reflex are, even at the early stages of the development of this reflex, controlled by the hierarchically higher motor centers that determine goal-directed behavior. The intrauterine lateral position relative to gravity can also be interpreted as an inactive posture. In this way, most fetuses in our study were brought to a supine position from an inactive posture. Since this posture is not an active posture that would require support or correction, the change from a lateral to a supine position generally does not affect the previously passive body posture. This approach can explain the high percentage of negative responses in the experimental group (76.93%). On the other hand, although there is continuity between prenatal and postnatal development, there are both qualitative and quantitative differences between fetal behavior *in utero* and *ex utero*. This is reflected in the fact that *ex utero* fetuses show more motor activity, their behavior is more diverse and their movements have a temporal pattern. A proposed explanation for the differences is the increased restraint *in utero (12),* limiting fetal movement and interfering with the development of the contact-righting reflex. This may also explain the high number of negative responses in our study. It should be noted that the contact-righting reflex was positive in all newborns in both the experimental and the control group. All newborns rotated immediately after being positioned supine, without any latency, touching the surface with the plantar side of all four extremities. In relation to the number of fetuses, the difference between the experimental group and control groups A1 and A2 was less significant, especially in gestations with three fetuses. In humans, gestations with more than one fetus reduce the available intrauterine and intra-abdominal space, and the physiological scope of fetal motility is therefore compromised. This causes the abnormal presentation and situs of the fetus *(14),* as well as congenital deformation of the fetus *(15)*. The appearance of the righting reflex is obviously significantly dependent on the available intra-abdominal space. Another possible explanation is that the lateral position represents a fetal conformation to the available space within maternal abdomen. Changes in maternal position relative to gravity thus do not cause the fetus to change its lateral position. It is also possible that all three factors – inactive posture, restrained movement, and fetal conformation to the physical space within the maternal abdomen – interact, leading to the low incidence of rotations to lateral and prone positions.

The present study shows that fetal behavior may be gravity dependent. The results indicate the presence of continuity not only in the pre- and postnatal behavior of the fetus, but also in the pre- and postnatal effects of gravitational force *(16)*. Likewise, results of this study corroborate the fact that development of the righting reflex is retarded in microgravity *(17, 18)*.

So far, the fetus's condition has been routinely evaluated on the basis of fetal heart rate pattern and on the quantitative assessment of fetal motor activity *(19)*. As noted previously here, the present study shows that fetal behavior may be gravity dependent. The significance of this finding lies in the possibility of developing an examination method for assessing a fetus's condition and nervous system maturity on the basis of its postural reactions. Such an assessment is theoretically possible in human and precocial mammalian fetuses due to their prenatal postural development. In humans, the subcorticospinal system of the brain stem that provides for the upright body posture matures prenatally. In precocial mammals that live in herds, the corticospinal system is also functional at birth *(8)*. Comprehensive study of the quantitative and qualitative characteristics of prenatal maturation of supine to prone righting requires four-dimensional imaging of the fetus.

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