

The righting reflex from a supine to a prone position in the guinea pig fetus

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Abstract. The aim of this study was to examine the righting reflex from a supine to a prone position in the albino guinea pig fetus. Ultrasound examinations of one-fetus gestations were performed in the period from the 31st to the 66th day of gestation. The experimental and control group each encompassed 6 fetuses. Fetuses were brought into supine positions relative to the gravity vector by manipulating the pregnant females into the appropriate positions. The control group received 15 mg/kg of diazepam intraperitoneally before the examination to show whether changes in fetal position occurred as the result of passive rotation. In the experimental group, each fetus was examined every other day (summary results: absent 69 times, prone position 10 times, lateral position 29 times). In the control group, each fetus was examined every five days (summary results: absent 42 times). The absence of the righting reflex in the control group was statistically significant ($\chi^2 = 18.66$, $df = 1$, $p = 0.000$, $p < 0.05$). The experimental group fetuses assumed a prone position more frequently in the period from the 51st to the 66th day of gestation than in the period from the 31st to the 50th day of gestation ($\chi^2 = 4.17$, $df = 1$, $p = 0.0412$, $p < 0.05$), suggesting maturation of the righting reflex.

Key words: Fetus — Reflex — Gestation — Guinea pig

Introduction

The righting reflex from a supine position to a prone position occurs when an individual returns to standing position after being positioned supine. There are two forms of this reflex: air-righting and contact-righting. A variant of the air-righting reflex may be induced in water. The contact-righting reflex can be triggered with tactile and vestibular stimuli, whereas the air-righting reflex and its water variant are prompted by vestibular and visual stimulation (Watt 1976; Pellis et al. 1989). This reflex is important for a fetus because it enables physiological delivery; it was observed in cattle and sheep that the fetus changes its position from a lateral or supine position to a prone position during delivery (Husa

et al. 1988; Fraser 1989). To induce this reflex, it is necessary to position an experimental animal into a supine position relative to the gravitational vector. Accordingly, a study of the prenatal presence of the reflex would provide evidence that gravity, as an environmental factor, affects fetal development (Sekulić 2000).

Guinea pigs belong to the group of mammals that display precocious development. Gestation lasts an average of 66 days. Ultrasound observations of the guinea pig fetus have shown that fetal movements first occur around the 26th day of gestation (Sekulić et al. 2009). Studies of exteriorized guinea pig fetuses suggest that the fetuses open their eyes between the 46th and the 50th day of gestation. They become covered with fur from the 52nd to the 57th day of gestation. The righting reflex of moving from a supine to a prone position is present from the 60th day of gestation, as is crawling. Standing and walking are present from the 63rd day of gestation (Avery 1928). The period from the 35th to the 40th day of gestation is characterized by an established

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link between the sensory epithelium of the vestibular apparatus and the vestibular ganglion (Heywood et al. 1976; Sobin and Anniko 1983). Myelination of the vestibular nerve begins on approximately the 40th day of gestation (Heywood et al. 1976). The authors of this paper found no data in available literature about cutaneous and proprioceptive receptor maturation in guinea pigs.

Intrauterine examination of the reflex has so far been attempted in a single study in which a pregnant guinea pig with two fetuses was observed on the 63rd day of gestation. No changes were detected with X-rays examination of the two fetuses that had been brought into supine positions during the 15-min observation. A possible explanation for the study's failure is its small sample size. Furthermore, the study's technique of fixing the pregnant female involved pressing the female's abdomen and hence also pressing fetuses, which may have prevented the rotation of the fetuses (Avery 1928).

The aim of this study was to examine the righting reflex from a supine to a prone position in the albino guinea pig fetus. The hypothesis was that when fetuses were brought into supine position, they would return to a prone position.

Materials and Methods

The study subjects were albino guinea pigs (*Cavia porcellus*) obtained from the Department of Biochemistry of the Faculty of Medicine, Novi Sad. The experiments with the animals were approved by the Ethics Committee of the Institute of Neurology in Novi Sad. The guinea pigs were kept in 400 (wide) \times 1000 (length) \times 300 (height) mm plastic containers in a harem system: each container held three or four females and one male. Pregnant females were moved to 300 \times 300 \times 300 mm plastic containers after the 60th day of gestation, where they were kept separate until delivery and, with their respective offspring, for the first 15 days after delivery. The animals were fed a standard commercial-pellet diet and *ad libitum* water enriched with vitamin C (30 mg/100 ml water). Artificial cycles with 12 h of light (08:00–20:00) and 12 h of dark were provided.

Inspection of the vaginal introitus was performed daily, and the day that the vaginal membrane was perforated was considered to be the first day of gestation. The study included only pregnant guinea pigs that bore one fetus each. The number of fetuses was determined by ultrasound examination, and the pregnant guinea pigs were randomly selected for the experimental and control groups. Each group (experimental and control) included 6 pregnant females and 6 fetuses.

The shaving of the abdominal region was conducted during a short-term inhalatory ether narcosis on the 25th day



Figure 1. Guinea pig prepared for ultrasonic examination.

of gestation. The pregnant guinea pigs were supported in a supine position on a 15 \times 30 cm board using plastic strips with clasps that were fastened over the thoracic area and the inguinal region from both sides. The strips were pulled through holes in the board near the body of the animal and were fastened on the other side of the board (Figure 1). Pregnant females were calm during examination and showed no signs of distress due to immobilization.

Ultrasound examinations were performed using a Toshiba Nemio SSA-550A apparatus with a 6–11 Hz linear probe. Pregnant females were brought into supination by manipulating the boards to which they were fastened. The fetuses' orientations were then determined by tracking them with the ultrasound probe along the longitudinal and transversal axes of the fetuses. The position of each fetus's trunk was determined by the positions of its heart, forelimbs, and hind legs (Figure 2). Next, the board was rotated until the fetus was brought into a supine position relative to gravity, and the examination was then repeated. If a fetus was initially observed in a supine position, then the pregnant female's position was not changed.

During the examinations, the following parameters were observed: absence of any changes in position, rotation to

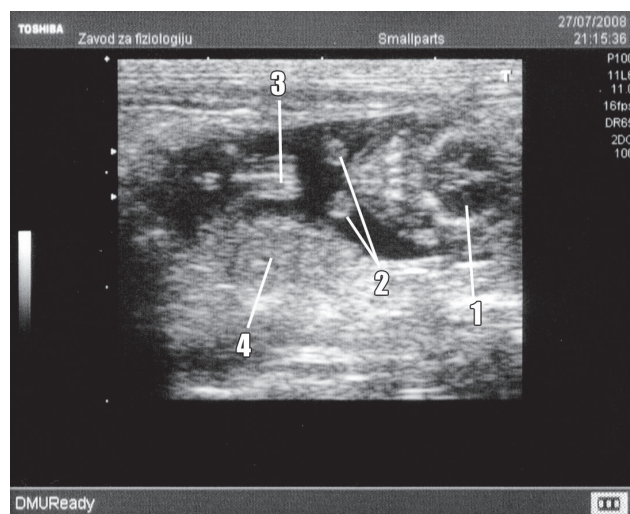


Figure 2. Longitudinal image of a fetus on the 35th day of gestation. 1, head; 2, forelimbs; 3, hind limbs; 4, placenta.

a lateral position, and rotation to a prone position. Rotation to a lateral position was considered an incomplete reflex reaction. The examination was continued until a change in the fetus's position was registered or until the end of the arbitrarily set period of 2 min, whichever came first. The ultrasound examinations began on the 31st day of gestation and lasted until the 66th day of gestation. Each pregnant female in the experimental group had a total of 18 ultrasound examinations during gestation. Examinations were performed every other day.

The control group was formed to show whether changes in fetal positioning occurred as the result of passive fetal rotation. Diazepam has been used to immobilize the human fetus during nuclear magnetic resonance studies (Huisman et al. 2002). In the guinea pig, diazepam passes through the fetoplacental barrier; residual diazepam and its metabolite nordiazepam can be found in the brain of the guinea pig fetus (Ungar and Ecobichon 1986). Diazepam increases the latency of the righting reflex from supine to prone position in adult guinea pig (Scott et al. 1994). For this reason, each animal in the control group received 15 mg/kg of diazepam (Bensedin

10 mg; Galenika, Serbia) intraperitoneally 15 min before each examination. The dosage was determined in a pilot study, when absence of maternal movement and tonus as well as fetal movement was observed only at the dose of 15 mg/kg. Pregnant females weighed from 480 to 750 g each, and the respective administered diazepam dosages ranged from 7.2 to 11.25 mg. In this group, examinations were performed once in every period of 5 days, for a total of 7 examinations during each gestation. Because pregnant females from the control group did not move and were hypotonic for couple hours after examination, in order to avoid their possible injury, they were placed in separate plastic containers measuring 300 × 300 × 300 mm each for the following 24 h.

For statistical analysis, the chi-square test (χ^2 -test) was used. When the expected values were less than 5, Fisher's exact test was used instead of the χ^2 -test. The level of $p < 0.05$ was adopted in order to determine statistical significance.

Results

The results for each gestation period are shown in Table 1. The χ^2 -test shows the statistically significant absence of the righting response (turning to lateral and prone positions) in the experimental group for the entire gestation period ($\chi^2 = 18.66$, $df = 1$, $p = 0.000$, $p < 0.05$). Comparison of the experimental and control group for the period from the 31st day to the 50th day of gestation shows a statistically significant absence of the righting reflex (turning to a lateral or prone position) ($\chi^2 = 8.10$, $df = 1$, $p = 0.004$, $p < 0.05$). This difference was also present for the period from the 51st day to the 66th day of gestation (Fisher's exact test $p = 0.002$, $df = 1$, $p < 0.05$). A comparison of positive responses (turning to a lateral or prone position) in the experimental group between gestation days 31–50 and 51–66 did not show any significant difference ($\chi^2 = 0.76$, $df = 1$, $p = 0.382$, $p > 0.05$). In the experimental group, significantly more fetuses rotated from supine positions to prone positions in the period from the 51st to the 66th day of gestation than in the period from the 31st to the 50th day of gestation ($\chi^2 = 4.17$, $df = 1$, $p = 0.0412$, $p < 0.05$). Turning from a supine to lateral position did not

Table 1. Distribution of responses in experimental (Exp) and control (Con) groups

Fetal responses after being placed in supine position	Gestation days								Total	
	31–40		41–50		51–60		61–66			
	Exp	Con	Exp	Con	Exp	Con	Exp	Con	Exp	Con
Negative response	21	12	20	12	18	12	10	6	69	42
Fetuses turning to prone position	2	0	0	0	3	0	5	0	10	0
Fetuses turning to lateral position	7	0	10	0	9	0	3	0	29	0

decrease significantly as gestation advanced ($\chi^2 = 0.83$, $df = 1$, $p = 0.3616$, $p > 0.05$).

In both the experimental and the control group, the contact-righting reflex was present in all newborns. All newborns rotated immediately after being placed in a supine position, without any latency.

Discussion

Ultrasound observation of the fetuses in this study showed that fetuses change their positions by pushing their legs against the intrauterine walls and by stretching their trunks while leaning against the intrauterine walls. In addition, it was observed that after the 40th day of gestation, the fetus does not float in the intrauterine cavity but rather leans with a great portion of its body against the intrauterine wall. This suggests that the fetus changes its position from a supine to prone using the contact-righting form of the reflex. A more detailed study of fetal movements will be possible only with real-time three-dimensional ultrasound imaging.

Although there was no difference in the frequency of positive responses (rotation to a lateral or prone position) between the observed gestation periods in the experimental group (gestation days 31–50 vs. 51–66), the proportion of successful rotations to prone positions significantly increased in the second part of gestation. This corresponds with the results of an earlier study in which, as mentioned before, this reflex was present after the 60th day of gestation among prematurely born offspring. In the period from the 51st to the 66th day of gestation, compared with the period from the 31st to the 50th day of gestation, there were less frequent lateral rotations, which could also suggest maturation of the reflex. However, the difference was not statistically significant. Similar results showing that the incidence of prone rotation increases among older specimens have also been found in the field mouse, the Norway rat, the roof rat, and the cat (Pellis et al. 1991).

The righting reflex from a supine to a prone position was present in all offspring on the first day of extrauterine life. This finding is substantially different from the intrauterine frequency of the reflex in the period from the 61st to the 66th day of gestation (27.7%). A possible explanation is that fetuses are less mobile than preterm offspring of the same age because increased restraint *in utero* (Robinson and Smotherman 1992). Additionally, the shape of the intrauterine cavity, which remains the same despite any changes in the orientation of the gravitational vector, may favor certain fetal positions.

The complete absence of any change in fetal positioning in the control group suggests that the changes in the experimental group did not result from passive fetal movements

during the manipulation of the pregnant females. In order to achieve extended righting-reflex latency in the adult guinea pig, administration of a new dose of diazepam is needed before every examination (Scott et al. 1994). Daily intraperitoneal administration of diazepam in pregnant rats in the second half of gestation has no effect on the maturation of the righting reflexes in their offspring (Laitinen et al. 1986; Schlumpf et al. 1989). This shows that the administration of diazepam in the control group had only a temporary effect on the inhibition of the righting reflex. The positive contact-righting reflex in all litters from the control group supports this conclusion.

The results of our study demonstrate that fetal behavior may be partly gravity-dependent, as suggested earlier (Sekulić 2000). The results also indicate the presence of the prenatal and postnatal continuity of the influence of gravity. Likewise, the results of this study are in agreement that the development of the righting reflex from a supine position to a prone position is retarded in microgravity conditions (Ronca and Alberts 2000; Walton et al. 2005).

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