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MONITORING THE PROGRESSIVE INCREASE OF THE LONGEST EPISODE OF SPONTANEOUS MOVEMENTS IN GUINEA PIG FETUS

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Abstract - The aim of this work was to determine the changes in the duration of spontaneous movements in the guinea pig fetus after the appearance of its first movements. Every day from the 25th to the 35th gestation day, one fetus from each of twenty pregnant animals was examined by ultrasound. Fetal movements were observed for 5 min. The episode with the longest period of movement was taken into consideration and was recorded as: <1 s, 1-3 s, and >3 s. Days 25 and 26 were characterized by episodes lasting <1 s; days 29 to 31 were dominated by episodes lasting 1-3 s, and days 34 and 35 by episodes lasting >3 s ($\chi^2 = 140.51$ $p < 0.05$). Tracking the dynamics of progressive increases in the longest episode of spontaneous movement could be a useful factor in estimating the maturity and condition of a fetus.

Key words: Guinea pig, fetus, ultrasound, movements, maturation

INTRODUCTION

The postural and locomotor development of the guinea pig takes place entirely prenatally, which is why it is considered a precocial mammalian species. On the other hand, in altricial rodent species like rats and mice, postural and locomotor development occurs postnatally. In humans, the subcorticospinal system that maintains posture develops prenatally, while the corticospinal system that controls posture and locomotion develops postnatally. Therefore, the guinea pig fetus can be a better experimental model than altricial species for researching prenatal motor development by ultrasound examination (Sekulić et al., 2009; Sekulić et al., 2010). Ultrasound examination is a noninvasive method for prolonged and repeated observation of the fetus. The assessment of fetal movements could demonstrate the existence of aberrations in fetal physiological development. A reduction in embryonic activity indicates fetal suffering (de Vries and Fong, 2007).

Previous studies that investigated prenatal motor development in guinea pig fetuses have examined the appearance of basic movements (Sekulić et al., 2009), the possibility of provoking postural reactions in the fetus (Sekulić et al., 2010, 2009), and the appearance of patterns in fetal activity (van Kan et al., 2009). Until now, the duration of spontaneous movements of the guinea pig fetus after the appearance of its first movements has not been examined. The aim of this study was to establish the dynamics of changes

Table 1. Distribution of episodes of the longest episodes of spontaneous fetal motor activity by days of gestation.

Longest period of spontaneous movements	Number of fetuses for each day of gestation with longest episodes of spontaneous fetal motor activity										
	25th	26th	27th	28th	29th	30th	31st	32nd	33rd	34th	35th
<1 s	1	2	1	3	2	1	0	0	0	0	0
1-3 s	0	0	6	7	15	18	16	11	10	3	2
>3 s	0	0	0	0	0	0	1	9	10	17	18

Table 2. Number of females with the first observed fetal movements depending on the day of gestation.

Day of gestation	25	26	27	28	29	30	31	32	33	34	35
Number of females with the first observed fetal movements	1	1	5	6	4	2	1	0	0	0	0

in the longest episode of spontaneous movements in the guinea pig fetus after the appearance of its first movements.

MATERIALS AND METHODS

The animals selected for the experiment were albino guinea pigs (*Cavia porcellus*) obtained from the Department of Biochemistry at the Medical Faculty of the University of Novi Sad. The experiments were approved by the Medical Faculty's Ethics Committee. The guinea pigs were housed in 400 mm x 1000 mm x 300 mm (w x d x h) plastic containers that were organized according to a "harem system" (4 females and 1 male). The animals were fed a standard commercial pellet diet as determined by the Veterinary Institution JSC Subotica in Subotica, Serbia, and were provided with water enriched with vitamin C (30 mg/100 ml) *ad libitum*. Artificial daylight cycles were provided with 12 h of light (08:00-20:00) and 12 h of darkness. Room temperature was maintained at 24±2°C and the air was fully circulated 6 to 10 times per hour. Individual guinea pigs were identified by observing the yellow patterns on their backs. Inspection of the female vaginal introitus was performed on a daily basis and the first day of gestation was determined based on the microscopically confirmed presence of spermatozoids in a vaginal smear. The abdominal regions were shaved after the animals were put into a brief state of narcosis by inhaling ether on the 23rd day of gestation. An ultrasound examination was performed using a Siemens SL 2 apparatus with a 7.5 MHz linear probe. Twenty pregnant animals were included in the study. Six animals had one fetus, 4 animals had 2 fetuses, 4 had 3 fetuses, and 6 had 4 fetuses. Ultrasound examinations were performed each day from the 25th to the 35th days of gestation. Pregnant animals were held with their bellies facing upward by an assistant during the examinations and fetal movements were observed for 5 min. During the course of one day, one fetus in each pregnant animal was examined, regardless of the total number of fetuses present. The episode with the longest burst of spontaneous movement was registered regardless of what type of movement was observed. The duration of spontaneous activity was categorized as follows: <1 s; 1-3 s; and >3 s. The signal from the ultrasound was recorded on DVD and later analyzed.

RESULTS

Table 1 shows the distribution of episodes of the longest episodes of spontaneous fetal motor activity by day of gestation. The day when the first fetal movements appeared was not the same for each pregnant female. The first fetal movements were recorded from the 25th to 31st days of gestation (Table 2). Even if spontaneous fetal activity was observed in one of the females on one day, fetal movement was not always seen on the following day in the same female.

As shown in Table 1, as the gestational age progressed, episodes of spontaneous fetal activity became longer. The chi-squared test showed that the increase in duration of these episodes of longest spontaneous fetal activity had a statistical significance of $\chi^2 = 140.51$; $p < 0.05$. The statistical significance of changes for each duration of movements (<1 s, 1-3 s, >3 s) was separately tested in relation to gestational age with Bendat and Persol's test. This test showed that the decreased frequency of spontaneous movements lasting <1 s after the 30th day of gestation had a statistical significance of $p = 0.009$; $p < 0.05$. For the period between the 25th to 30th day of gestation there was no statistically significant difference in the frequency of spontaneous movements lasting <1 s; $p = 0.6$ $p > 0.05$. The highest frequency of movements lasting 1-3 s was recorded in the period between the 29th and the 31st days of gestation. The distribution of the frequency of spontaneous movements lasting 1-3 s in the period from the 25th to the 35th days of gestation was at the edge of statistical significance $p = 0.048$; $p < 0.05$. The distribution of the longest-lasting total fetal activity (>3 s) was statistically significant $p = 0.0121$; $p < 0.05$.

Movements shorter than one second were isolated movements of individual body segments or generalized movements. Movements longer than one second involved a couple of segments of the body or were generalized.

DISCUSSION

Experiments with embryonic chickens demonstrated that the generator of fetal motor activity that first appears originates in the spinal cord (Hamburger et al., 1966). The same authors demonstrated that the activity of these spinal generators of motor activity was unrelated to external stimulation. In other words, the developing nervous system could generate coordinated activity in the absence of sensory experience or reflexive stimulation (Hamburger et al., 1966). As gestation advances, fetal movements become more frequent and more organized, thus showing a qualitative pattern in the forms of cyclicity and synchronicity (Smotherman et al., 1988).

Ultrasound assessment of fetal movement patterns can be used to evaluate the development of the nervous system. Spontaneous movements in guinea pig fetuses in this study began on the 25th day of gestation. This finding is in accordance with the results of previous studies that investigated the appearance of movements in guinea pig fetuses. Regardless of whether the method of exteriorization and observation of the fetus was in a warm bath (Cerebelle, 1964; Carmichael, 1934) or by ultrasound (Sekulić et al., 2009; van Kan et al., 2009), the first movements appeared sometime around the 25th day of gestation. Findings of the duration of first fetal movements in other studies, whether they involved human or guinea pig fetuses, also demonstrated that these movements last less than one second (van Kan et al., 2009; Lüchinger et al., 2008). Before the present study, the duration of the longest episodes of movements after the appearance of the first spontaneous movements had not been examined in longitudinal studies with either human or guinea pig fetuses. The longest episode of general movements has been examined in human fetuses from 7 to 16 weeks in physiological gestation and among pregnant females with diabetes. A progressive increase in the duration of the longest episode of general movement was demonstrated in both groups. These kinds of results are in accordance with the results obtained in the present study. However, in the group of women suffering from diabetes mellitus, the first generalized movements appeared later and the durations of the longest episodes of general movements were shorter than in the control group (Mulder et al., 1991). This delay exceeded that of early growth delay, suggesting that a delay in the development of the fetal central nervous system also has an influence (Mulder and Visser, 1991). The difference between these two groups was absent 4-5 weeks after the appearance of the first generalized fetal movements (Mulder et al., 1991). A decrease in the number of movements and a decrease in embryonic activity points to a disturbance in the development of the embryo or to its death. In the case of a threatened miscarriage, the fetus is frequently completely inactive (Henner et al., 1979).

Tracking the dynamics of progressive increases in the longest episodes of movements has not until now been employed as a standard indicator of the maturity or condition of a fetus either in human medicine or in studies with experimental animals. This indicator could be included in the existing parameters for evaluating the condition of a fetus, which would enable a comprehensive assessment of the maturity and condition of a fetus.

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