ORIGINAL ARTICLE/
PŮVODNÍ PRÁCE

# Electrogastrography in experimental pigs

## Methodical design and initial experience

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**Summary**

Background: *Surface electrogastrography (EGG) is a non-invasive method for clinical assessment of gastric myoelectrical activity. The aim of this study was to elaborate methods for EGG in experimental pigs.*
Material and methods: *Eight mature female pigs 4–5 months old entered the study. EGG was recorded using a Digitrapper EGG (Synectics Medical AB, Stockholm). Three separate measurements were performed in each animal. The first, a 30-minute EGG recording was done as a "basic measurement" (fasting condition under general anaesthesia). The second 30-minute measurement was carried out immediately after intragastric administration of 100 mg itopride (a prokinetic drug). The third 30-minute EGG recording was accomplished immediately after intragastric volume challenge (360 mL of water per animal).*
Results: *The normal dominant frequency in pigs was 3.3 ± 0.5 cycles/min. Mean dominant frequencies of gastric slow waves after volume challenge (4.4 ± 0.8 cycles/ min) were significantly higher compared with the basic measurement (p = 0.014) and recording after itopride (p = 0.030). There was a non-significant trend for correlation between the animals' weight and the mean dominant frequency of gastric slow waves after volume challenge (r = 0.613; p = 0.106).*
Conclusions: *EGG in experimental pigs is feasible. Consistent data were obtained in all but one animal. The mean dominant frequency in pigs is comparable with that found in humans. This study is provides basis for further preclinical projects.*

**KEY WORDS:** ELECTROGASTROGRAPHY, EXPERIMENTAL PIG, ITOPRIDE, VOLUME CHALLENGE, DOMINANT FREQUENCY ANALYSIS

**Souhrn**

***Elektrogastrografie u experimentálního prasete. Metodika vyšetření a prvotní zkušenosti***

Východiska: *Povrchová elektrogastrografie (EGG) je neinvazivní metoda pro klinické hodnocení myoelektrické aktivity žaludku. Cílem této studie bylo vypracování metodiky EGG u experimentálních prasat.*
Materiál a metody: *Do studie bylo zahrnuto osm mladých dospělých samic prasete Sus scrofa f. domestica (starých 4–5 měsíců). EGG bylo natáčeno pomocí zařízení Digitrapper EGG (Synectics Medical AB, Stockholm). U každého zvířete byly provedeny tři oddělené záznamy EGG. První 30minutový záznam byl proveden jako „bazální" (nalačno v celkové anestezii). Druhá 30minutová EGG byla provedena bezprostředně po intragastrickém podání 100 mg prokinetika itoprid. Třetí 30minutový záznam byl pořízen bezprostředně po intragastrické volumové zátěži (360 ml vody pro každé zvíře).*
Výsledky: *Normální dominantní frekvence u prasete byla 3,3 ± 0,5 cyklů/min. Průměrná dominatní frekvence pomalých žaludečních vln po volumové zátěži (4,4 ± 0,8 cyklů/ min) byla signifikantně vyšší ve srovnání s bazálním záznamem (p = 0,014) a EGG po itopridu (p = 0,030). Zaznamenali jsme nesignifikantní trend ke korelaci mezi průměrnou dominantní frekvencí pomalých žaludečním vln a hmotností pokusných zvířat při EGG po volumové zátěži (r = 0,613; p = 0,106).*
Závěry: *EGG je u experimentálních prasat uskutečnitelná. Konzistentní data byla získána u 7/8 pokusných zvířat. Průměrná dominantní frekvence u prasat je srovnatelná s hodnotami u zdravých dospělých lidí. Tato studie je východiskem pro budoucí preklinické projekty.*

**KLÍČOVÁ SLOVA:** ELEKTROGASTROGRAFIE, EXPERIMENTÁLNÍ PRASE, ITOPRID, VOLUMOVÁ ZÁTĚŽ, ANALÝZA DOMINANTNÍCH FREKVENCÍ

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Surface electrogastrography (EGG) is a non-invasive method for clinical assessment of gastric myoelectrical activity [3,9,11,13]. Neuromuscular activities of the stomach generate electrical phenomena termed "gastric slow waves". Gastric myoelectrical activity is made up of two types of electrical signals called slow waves or electrical control activity and superimposed spikes also called electrical response activity [6,7,12]. The slow waves are believed to originate proximally from the greater curvature of stomach by the cells located adjacent to the junction between the fundus and the body and propagate through the muscular syncytium in a rhythmic fashion towards the antrum. The exact source of the origin of these slow waves is still not known but the interstitial cells of Cajal located in this area have been implicated. The slow waves represent a cyclic phenomenon which sets the maximum frequency limit with which the gastric contraction can occur in any given interval. Spike potentials (or electrical response activity) on the other hand constitute the other component of gastric myo-electrical activity and seem to be necessary for antral contractions. They develop when a suitable stimulus triggers high-frequency depolarisation activity of the membrane potential surpassing the pacemaker threshold owing to activation of ion channels and pumps simultaneously by neural and humoral stimuli. The slow waves of the gastrointestinal tract mainly function to trigger the onset of a spike to elicit smooth muscle contraction, which provides the essential power of motility [6,7,11,33]. The major parameters of EGG and gastric emptying measures (e.g. half-life of elimination in $^{13}$C-octanoic acid breath test) mutually correlate in healthy humans [5].

The small adult pig can be used in various preclinical experiments as an omnivorous representative due to its relatively very similar gastrointestinal functions in comparison to man [21]. However, there are some distinct differences in the anatomy and physiology of the stomach between humans and pigs [4,23,25]. The porcine stomach is pouch-shaped, gastric cardia is close to the pylorus, so the endoscopic approach to the duodenum is rather hooked. A special transverse pyloric fold (torus pyloricus) serves as a "gatekeeper" [23]. Gastric emptying of pigs is much slower, implemented through small separated separated bursts. There are significant remnants of food in the porcine stomach even after 36–48 hours of fasting [23,34].

Fig. 1.
Electrogastrography in experimental pigs. General arrangement of electrode placement for EGG recording.

To the best of our knowledge, there are no reports on EGG in pigs in the available literature. The aim of this study was to set up and elaborate method for EGG in experimental pigs, as a basis for future pharmacological studies.

## MATERIAL AND METHODS

Eight mature female pigs (Sus scrofa f. domestica), hybrids of Czech White and Landrace breeds, weighing 30.5 ± 2.6 kg (4–5 months old), entered the study. They were fed twice a day (standard assorted food A1) and were allowed access to water ad libitum.

Surface cutaneous EGG was recorded using a Digitrapper EGG (Synectics Medical AB, Stockholm, Sweden) in the morning after 24-hour fasting.

All EGGs were carried out under general anaesthesia. Intramuscular injection of ketamine (20 mg per kg; Narkamon, Spofa, Praha, Czech Republic) was used as an introduction. Repeated doses of this drug were administrated intramuscularly when appropriate. Due to initial technical problems with EGG recording, animal #1 received two doses of initial anaesthesia (within 45 min) because the start of basic recording was delayed for one hour.

All animals were lying in a right lateral position during the EGG recording. The epigastric area was shaved before application of electrodes to decrease impedance in signal conduction through the skin. Electrode placement always began with placing the first electrode roughly within 5 cm of the xyphoid process in the centre and then subsequently placing the other two roughly at a distance of 10 cm from the central electrode on left and right hypochondrium, respectively (Fig. 1). After connecting the device, the recording was commenced and the animals were closely monitored for any flinging movement and muscle tremor with a close eye on the screen to make note of any such events for future reference during the process of analysis. In the case of any such unforeseen event of increased limb shaking and signs of restlessness in the animal, the recording was paused and the animal was administered an intramuscular bolus of anaesthesia. Barring one or two such events, most of the recording was performed in a peaceful manner.

Three separate measurements were performed in each animal. The first, a 30-minute EGG recording was performed as a "basic measurement" (fasting condition under general anaesthesia). The second 30-minute measurement was carried out immediately after intragastric administration of 100 mg itopride, a prokinetic drug (Ganaton tbl., Abbott Laboratories). The third 30-minute EGG recording was accomplished immediately after intragastric volume challenge (360 mL of water per animal).

Running spectral analysis (based on Fourier transform) was used for evaluation of the EGG. The results were expressed as running spectrum percent activity. All possible artefacts (especially motion ones) were removed before final evaluation.

## STATISTICAL ANALYSIS

Data were statistically treated by means of descriptive statistics, non-paired t-test, Mann-Whitney rank sum test and Pearson product moment correlation using SigmaStat software (Jandel Corp., Erkrath, Germany).

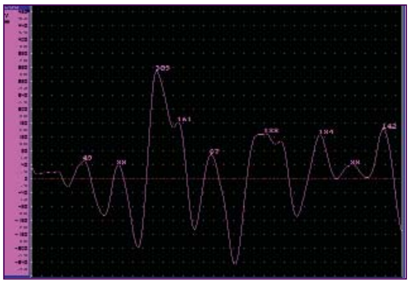

Fig. 2.
EGG rhythm of three cycles per minute at online recording.

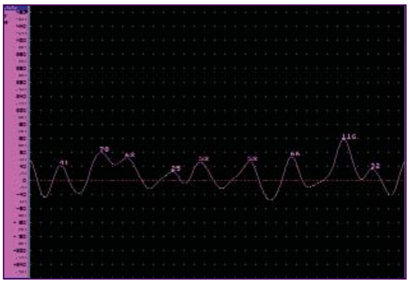

Fig. 4.
Pattern of bradygastria at online EGG recording.

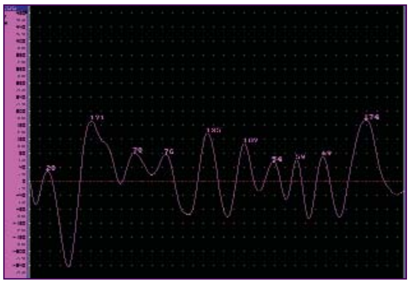

Fig. 6.
Pattern of tachygastria at online EGG recording.

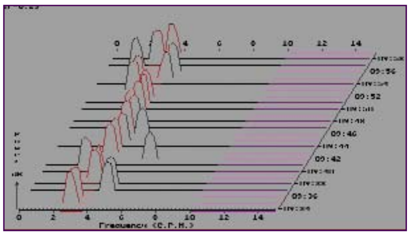

Fig. 3.
Record of EGG with a prevailing rhythm of three cycles/min (60% running spectrum percent activity).

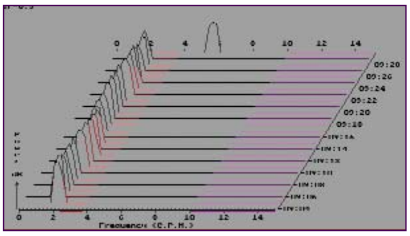

Fig. 5.
Record of EGG with a prevailing bradygastria (96% running spectrum percent activity).

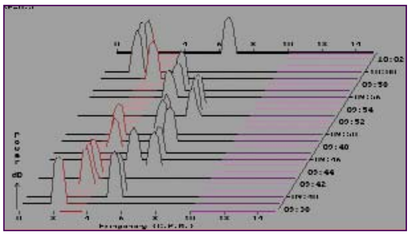

Fig. 7.
Record of EGG with a prevailing tachygastria (60% running spectrum percent activity).

## ETHICS

The Project was approved by the Institutional Review Board of Animal Care Committee of the Institute of Experimental Biopharmaceutics, Academy of Sciences of the Czech Republic, Protocol Number 149/2006. Animals were held and treated in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes [15].

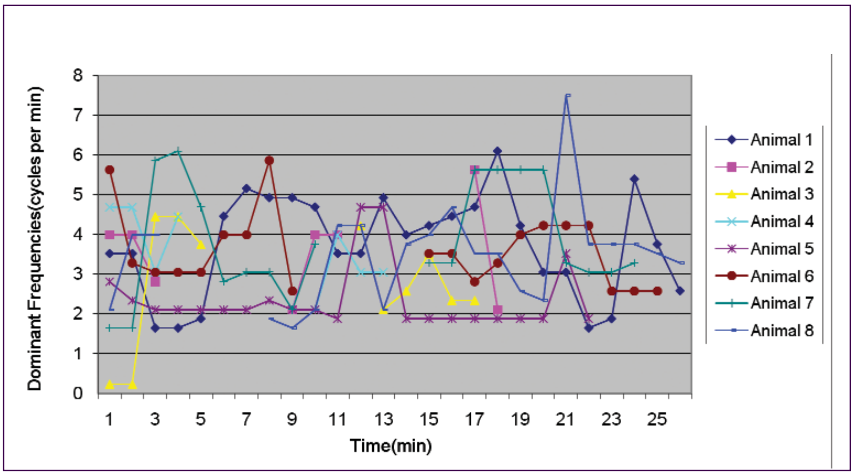


Fig. 8.
Electrogastrography analysis. Dominant frequencies (cycles/min) in experimental pigs. Basic EGG recording at fasting condition under general anaesthesia.

## RESULTS

EGG recording was successfully accomplished in all animals. All low, medium and high frequencies of gastric slow waves were found in particular animals (Fig. 2–7). Overall tabular data and dominant frequencies of gastric slow waves are listed in tables 1 and 2. The normal dominant frequency in experimental pigs was 3.3 ± 0.5 cycles/min (i.e. basic measurement in fasting animals under general anaesthesia). Mean dominant frequencies of gastric slow waves after volume challenge were significantly higher compared with basic measurement (p = 0.014) and recording after itopride (p = 0.030). The difference be-

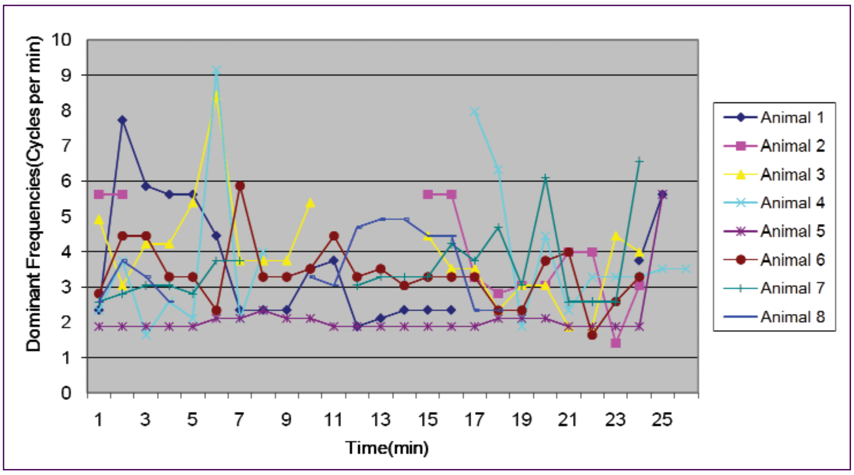


Fig. 9.
Electrogastrography analysis. Dominant frequencies (cycles/min) in experimental pigs. EGG recording after intragastric administration of 100 mg itopride.

tween basic measurement and recording after itopride was not significant, however the power of the performed test was below the desired level (0.800). Consistent data were obtained in all but one animal (# 4), see Fig. 8–10. There was no significant correlation between values found in particular animals at basic, itopride and volume challenge measurements (using Pearson correlation). There was a non-significant trend for correlation between the animals' weight and the mean dominant frequency of gastric slow waves after volume challenge (r = 0.613; p = 0.106).

## DISCUSSION

The aim of this study was to introduce EGG in experimental pigs. Assessable recordings were obtained in all animals. The normal dominant frequency that we found in pigs in this project (3.3 ± 0.5 cycles/min) is fully comparable with those in adult humans [29]. Thus, our original assumption was proved - the young adult pig is a suitable model for experimental EGG. We have not found any study on EGG in pigs in the available literature, so we compare our re-

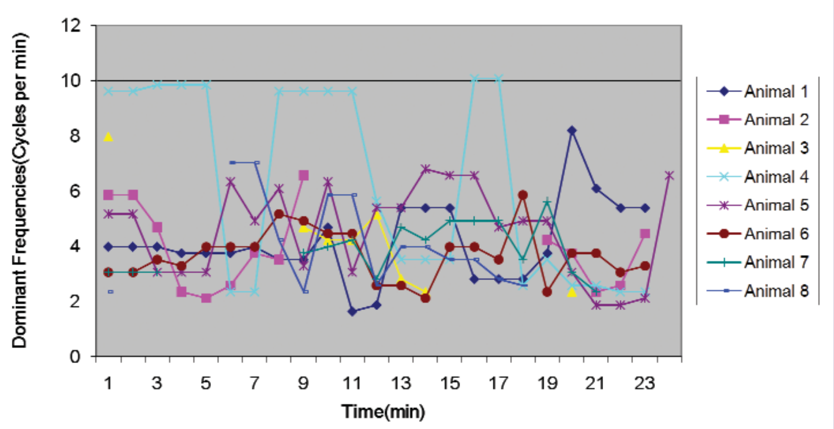


Fig. 10.
Electrogastrography analysis. Dominant frequencies (cycles/min) in experimental pigs. EGG recording after volume challenge of 360 mL water.

**Table. 1.** Electrogastrography in experimental pigs. Overall tabular data.
Running spectral analysis (based on Fourier transform) was used for evaluation.

| Animal (number) | Weight (kg) | Measurement | Electrogastrography | | |
|---|---|---|---|---|---|
| | | | Gastric slow waves < 2.8 cpm (%) | Gastric slow waves 2.8–3.8 cpm (%) | Gastric slow waves > 3.8 cpm (%) |
| 1 | 28 | basic<br>itopride<br>volume | 18.5*<br>50<br>8.7 | 29.6*<br>5.6<br>21.7 | 51.9*<br>44.4<br>69.6 |
| 2 | 29.5 | basic<br>itopride<br>volume | 22,2<br>7.7<br>20 | 11.1<br>38.5<br>20 | 66.7<br>53.8<br>60 |
| 3 | 30 | basic<br>itopride<br>volume | 41.7<br>14.3<br>22.2 | 16.7<br>23.8<br>22.2 | 41.7<br>61.9<br>55.6 |
| 4 | 34 | basic<br>itopride<br>volume | 11.1<br>33.3<br>16.7 | 33.3<br>33.3<br>33.3 | 55.6<br>33.3<br>41.7 |
| 5 | 31.5 | basic<br>itopride<br>volume | 78.3<br>96.2<br>16 | 13<br>0<br>24 | 8.7<br>3.8<br>60 |
| 6 | 28 | basic<br>itopride<br>volume | 4.8<br>16<br>8.3 | 57.1<br>60<br>41.7 | 38.1<br>24<br>50 |
| 7 | 28 | basic<br>itopride<br>volume | 15<br>0<br>11.8 | 45<br>65<br>35.3 | 40<br>35<br>52.9 |
| 8 | 35 | basic<br>itopride<br>volume | 30.4<br>14.3<br>20 | 21.7<br>42.9<br>33.3 | 47.8<br>42.9<br>46.7 |

Notes:
*raw data were not edited due to a technical fault
cpm – cycles per minute
basic – basic electrogastrography recording under general anaesthesia (see text for details)
itopride – electrogastrography recording after intragastric administration of 100 mg itopride
volume – electrogastrography recording after volume challenge (360 mL of water per animal)

**Table. 2.** Electrogastrography in experimental pigs.
Dominant frequencies of gastric slow waves (given in cycles per minute).

| Character | Number of animals | Mean | Standard deviation | Confidence interval of mean | Median | Inter-quartile range |
|---|---|---|---|---|---|---|
| basic | 8 | 3.31 | 0.54 | 0.50 | 3.57 | 2.93<br>3.62 |
| itopride | 8 | 3.49 | 0.59 | 0.49 | 3.64 | 3.45<br>3.84 |
| volume | 8 | 4.35 | 0.82 | 0.69 | 4.14 | 3.89<br>4.41 |

Notes:
basic – basic electrogastrography recording under general anaesthesia (see text for details)
itopride – electrogastrography recording after intragastric administration of 100 mg itopride
volume – electrogastrography recording after volume challenge (360 mL of water per animal)

sults mostly with studies performed on humans.

However, our initial experience must be evaluated and interpreted with caution. We are fully aware of possible limitations of this study. First of all, the number of investigated animals is small and we did not study day-to-day reproducibility of EGG in these pigs. In humans, data on reproducibility of EGG are inconsistent [1,17,19,24]. In dogs, the reproducibility of EGG was acceptable [2,22].

All our EGG recordings were performed in young adult female pigs. There was a non-significant trend for correlation between the animals' weight and the mean dominant frequency of gastric slow waves after volume challenge. Age, sex (including menstrual cycle in women) and body-mass index might influence EGG in humans [30,32,35].

All recordings had to be performed under general anaesthesia in pigs, otherwise EGG would not be practicable. In humans, both general and epidural anaesthesia may affect the myoelectrical activity of the stomach [14,27,28].

The second measurements were carried out immediately after intragastric administration of 100 mg itopride (i.e. ~ 3 mg/kg; corresponding to the maximum single dose for man). There was no obvious change in dominant frequency during subsequent EGG recording. However, it is possible that the dose was not big enough and/or the time was not sufficient to allow the itopride to exert its prokinetic effect. Itopride has linear kinetics. In humans, the maximum plasmatic concentration (t-max) of itopride is reached at about 45 min after peroral administration (half-time of elimination is six hours). Gastric emptying and start of intestinal absorption of itopride might be delayed under general anaesthesia [31,36]. Iwanaga et al [18] studied the gastroprokinetic effect of itopride in conscious dogs. Itopride at a dose of 3 mg/kg did not affect gastrointestinal motility. With 10 mg/kg itopride, the contractile force of the gastric antrum was increased (doubled) within 5 min after intra-duodenal administration of itopride [18].

We decided on a volume challenge of 360 mL water that is comparable with 500 mL usually used in adult humans. The mean dominant frequency after volume challenge was significantly higher compared with basic measurement and recording after itopride. Several studies performed previously in humans have shown that volume overload after drinking water generally affects both dominant frequency (to tachygastria) and dominant power (characterised by an increase in amplitude) which has been attributed to factors such as 1. gastric distension; 2. gastric displacement; 3. slow wave changes and/or 4. neurohumoral mechanisms [8,10,16,20,26].

Last but not least, listing the possible limits of our study, we did not evaluate the power ratio of particular measurements at this stage. Nevertheless, our initial results are promising and stimulating for future research.

## CONCLUSIONS

EGG in experimental pigs is feasible. Consistent data were obtained in all but one animal. Mean dominant frequency in pigs is comparable with that found in humans. This study provides a basis for further preclinical projects.

### Conflicts of interests

The authors state that there are no conflicts of interest.

### Acknowledgement

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