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Viability of ex-vivo myography as a diagnostic tool for rectus abdominis muscle electrical activity collected at Cesarean section within a diameter cohort study

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Abstract

Background: *Ex-vivo* myography enables the assessment of muscle electrical activity response. This study explored the viability of determining the physiological responses in muscles without tendon, as rectus abdominis muscle (RAM), through *ex-vivo* myography to assess its potential as a diagnostic tool.

Results: All tested RAM samples (five different samples) show patterns of electrical activity. A positive response was observed in 100% of the programmed stimulation. RAM 3 showed greater weight (0.47 g), length (1.66 cm), and width (0.77 cm) compared to RAM 1, RAM 2, RAM 4 and RAM 5 with more sustained electrical activity over time, a higher percentage of fatigue was analyzed at half the time of the electrical activity. The order of electrical activity (Mn) was RAM 3 > RAM 5 > RAM 1 > RAM 4 > RAM 2. No electrical activity was recorded in the Sham group.

Conclusions: This study shows that it is feasible to assess the physiological responses of striated muscle without tendon as RAM, obtained at C-section, under *ex vivo* myography. These results could be recorded, properly analyzed, and demonstrated its potential as a diagnostic tool for rectus abdominis muscle electrical activity.

Keywords: Contractility, Rectus abdominis muscle, C-section, Myography, Skeletal muscle

Background

The Rectus Abdominis Muscle (RAM), a striated muscle without tendon, extending along the abdomen to the pubic symphysis, undergoes physiological adaptations during pregnancy [1–5]. As a striated muscle, the main focus of research has been on contractile muscle cells [6]. RAM has synergistic contractile activity with the pelvic floor



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muscles in labor and urinary continence mechanisms [5, 7, 8]. The importance of the RAM analysis may be underscored by the fact that some complications during the pregnancy, such as gestational diabetes mellitus (GDM), cause serious morphological changes [9–11] leading to atrophy and, as a consequence, urinary incontinence (UI) up 2 years after C-section [1, 9, 12–14].

The challenge of identifying such abnormalities of RAM contractility is overcome by its accessibility during C-sections allowing detailed studies of the muscle contractility [15–17]. For this analysis, the *ex-vivo* myography of RAM samples may clarify the knowledge of the pathophysiology of diseases and disorders that affect the RAM [17–22]. Although it is widely known that the *ex-vivo* myography is used to record muscle contractility through the myogram, a tracing graphic recording [18, 19, 23], that requires tendon attachments through the myograph [24–26]. Currently, there is no information available on the *ex-vivo* electrical activity in skeletal muscle, such as RAM without tendon. Therefore, efforts are needed to be made for the success of the RAM analysis through myography that will serve as an important tool for female muscle functional evaluation, its disorders and the pathophysiological mechanisms involved [26, 27].

The main goal of this study was to determine the viability of detecting electrical activity through *ex-vivo* myography in RAM samples without tendon collected during C-section as a physiological response to assess its potential as a diagnostic tool, for continuous electric stimulation, the fatigue and loss of tissue viability.

Results

Baseline maternal demographics, RAM sample characteristics and the programmed parameters of *ex-vivo* myography are summarized in Table 1. Of the five patients with RAM samples collected, all are from term pregnancy, three are primiparous, one has obesity plus chronic hypertension and pre-eclampsia and all are continent and normoglycemic.

A100% of RAM samples without tendons responded positively to programmed electrical stimulus allowing to record and to analyze the muscle functionality (Fig. 1, Table 1). All these 5 RAM samples presented a serial peak of contractile activity with two different patterns, viz, three samples (RAM 1, 4, and 5) showed contractility activity characterized by high and low peaks consecutively, and two samples (RAM 2 and 3) showed contractility activity with only by high peaks (Fig. 2A–E, Table 1). Both record patterns decreased in force at half (22.5 min) of the experiments and all samples remained viable during 1 h and 15 min experiments. The elastic ribbon (inert material) from the sham group showed no activity, as expected (see Fig. 2F). The response to the programmed electrical stimulation in the five RAM samples was measured considering the same major and minor peak values, and 100% of response in the *ex-vivo* myography. RAM 3 showed higher parameters of weight, width and length, average peak heights greater than 17.5 ± 12.5 compared to the other RAM samples, initial peak greater than 20.5 mN. However, a higher percentage of fatigue or loss of viability in RAM 3 was observed with a decrease in muscle contraction strength at 22.5 min compared with the other samples (Figs. 2C and 3).

RAM 2 showed lower average peak heights and had an initial peak height of 10.8 mN and a lower peak of fatigue or loss of viability at 22.5 min (see Figs. 2, 3 and Table 1).

Table 1 Baseline maternal characteristics of ex vivo myograph—programmed parameters and RAM activity

Experimental group		Sham group					
Clinical characteristics of pregnant woman							
Age	In years	24	27	25	31	24	
Parity		Primiparous	Second-pregnancy	Primiparous	Primiparous	Second-pregnancy	
Gestational week	In weeks	38.2	38.4	38.6	37	38.3	
Obesity	Yes (Y) or No (N)	N	Y	N	N	N	
Chronic arterial hypertension	Yes (Y) or No (N)	N	Y	N	N	N	
Preeclampsia	Yes (Y) or No (N)	N	Y	N	N	N	
Pregnancy specific urinary incontinence	Yes (Y) or No (N)	N	N	N	N	N	
Gestational diabetes mellitus	Yes (Y) or No (N)	N	N	N	N	N	
RAM activity	Description	Response					Krebs solution and elastic ribbon
		RAM 1	RAM2	RAM 3	RAM 4	RAM 5	
Total number of major peak	Major peaks/5 min	248	248	248	248	248	–
Total number minor peak	Minor-peaks/5 min	248	248	248	248	248	–
Response rate	Compared to previously programmed (%)	100.0	100.0	100.0	100.0	100.0	–
Contraction force (mN)		8.50 ± 0.67	6.67 ± 5.03	17.5 ± 12.5	6.7 ± 0.4	15.9 ± 17.6	–
Characteristics RAM samples							
Weight (grams)		0.30 ± 0.09					
Width (cm)		0.51 ± 0.09					
Length (cm)		1.50 ± 0.19					

Figure 3 shows muscle fatigue or loss of viability of five RAM (RAM 1–5) samples, respectively. RAM 1 (6.40 mN), RAM 2 (1.56 mN), RAM 3 (8.23mN), RAM 4 (7.23mN), RAM 5 (4.42mN) which is at half the time of the electrical activity.

Discussion

This study shows that fresh striated muscle without tendon reacted to electrical stimuli and stays viable showing a 100% response to a programmed electrical stimulation. This experimental model provides information on the performance and functionality of striated muscle without tendons from pregnant women, opening a promising field that

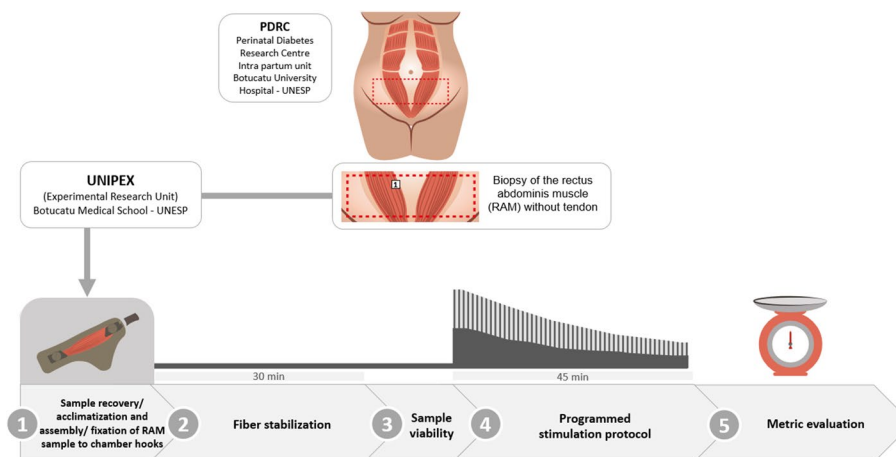


Fig. 1 Four steps of the experiment performed from the RAM without tendon

would allow making inferences, analyses, evaluations, and measurements, using their tissues through the *ex vivo* myography method in humans.

It is reported that women with GDM showed altered muscle morphology and 3D ultrasound which may influence the functionality of RAM with consequences for UI [14, 28]. We are aware of other modalities for studying contractility, such as electromyography, ultrasound, morphology, etc. However, myography may be considered an approach to detect the contractile activity in muscles without tendon not only in UI due to GDM but also to allow testing of the effects of *ex-vivo* drug application to human and animal tissues. The electrical stimulation and other parameters used in this study were based on previous literature on different muscles with tendons [24, 25]. This study shows that RAM reacted to electrical stimulation with contraction force and fatigue. These results may, however, be affected by parameters that may interfere with the contraction force of RAM samples, such as sample size, width, and length, and the region of the RAM collection, which varies from superficial, medial, and deep RAM [29], the presence of fat, fascia, the muscular collagen concentration, and the presence of slow and fast fibers percentage.

The electrical activity of RAM biopsies after 11.8 Hz electrical stimulation (force ~ 10 mN) was lower than other striated muscles, such as the extensor digitorum muscle longus (EDL) which showed a force ~ 80 mN [24] and the gastrocnemius muscle with a force ~ 62 mN [30–32]. Thus, the absence of a tendon may have a great impact on the lower observed contraction force in RAM. As verified in RAM 2, a lower contraction force may be linked to the concentration of collagen which is largely found in striated muscles during pregnancy [10, 14]. RAM 3 showed a higher initial peak, greater weight, width, and length, probably related to a greater number of motor units and sarcomeres recruited during muscle contraction likely capable of performing a stronger contraction. Since RAM3 was collected before birth its larger force may relate to less muscle fiber damage compared to RAM 2 collected after birth which presented a lower initial peak, weight, and length [31–34].

A selection of best possible parameters to describe the RAM *ex-vivo* myography technique may include: (i) the two types of contraction, (ii) the peak contraction to verify

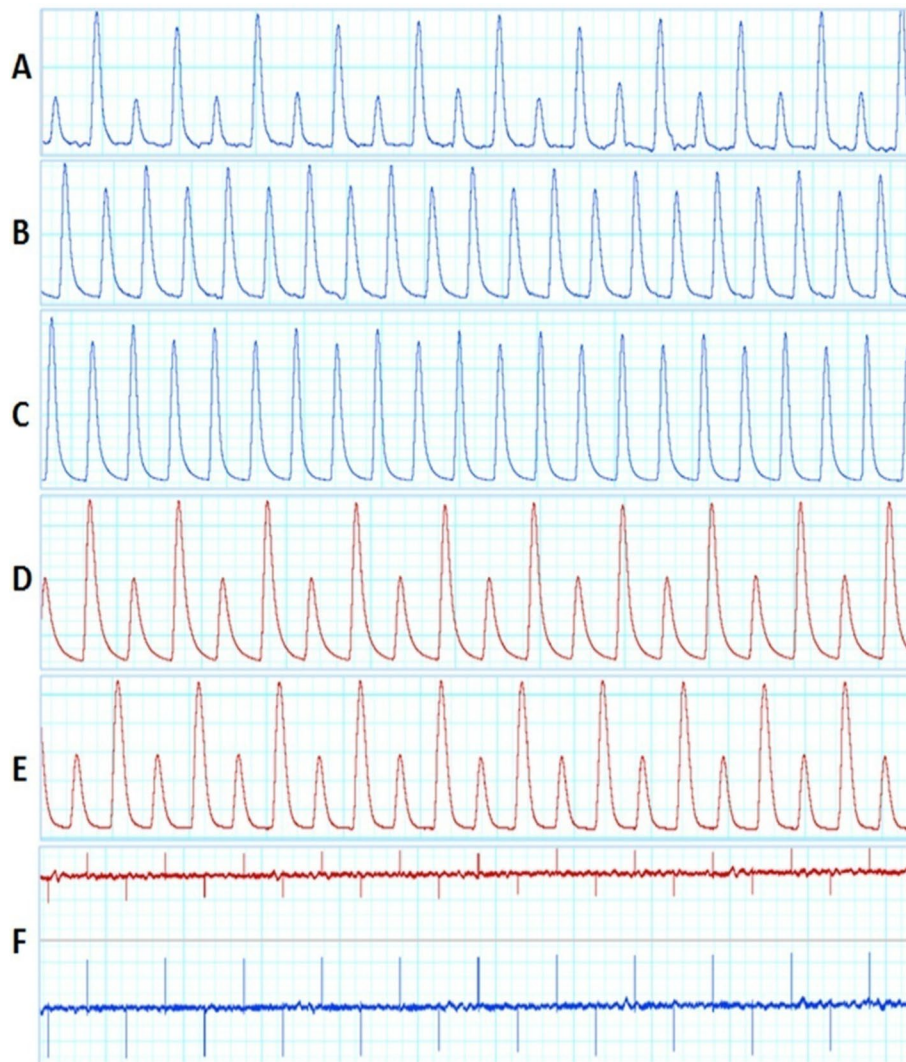


Fig. 2 Response to the programmed electrical stimulation in five RAM samples. The myograph shows the behavior of elastic ribbon (sham) under the same electrical stimulation as RAMs; **A–E** represents RAM 1–5 and **F** represents the sham group

contraction strength and relaxation time, (iii) the loss of strength characterizing muscle fatigue, and (iv) loss of muscle viability by having less processing time from collection to experiment that can be graphically manifested by a rising trend of the waves, showing loss of sustainability of contractility of the muscle fiber. It is critical to consider the region of the RAM sample collection, which varies from superficial, medial and deep RAM [29]. It is known that RAM regions act in series [34] and the differences in the force of contraction can be verified by evaluating its electrical activity by separated. In addition, the absence of a tendon may have a great impact on the lower observed contraction force.

The functionality of the RAM to the electrical stimulus is characterized by a larger peak and a smaller peak. This response may be related to the response of the total number of slow and fast fibers captured by the myograph transducer. Previous studies

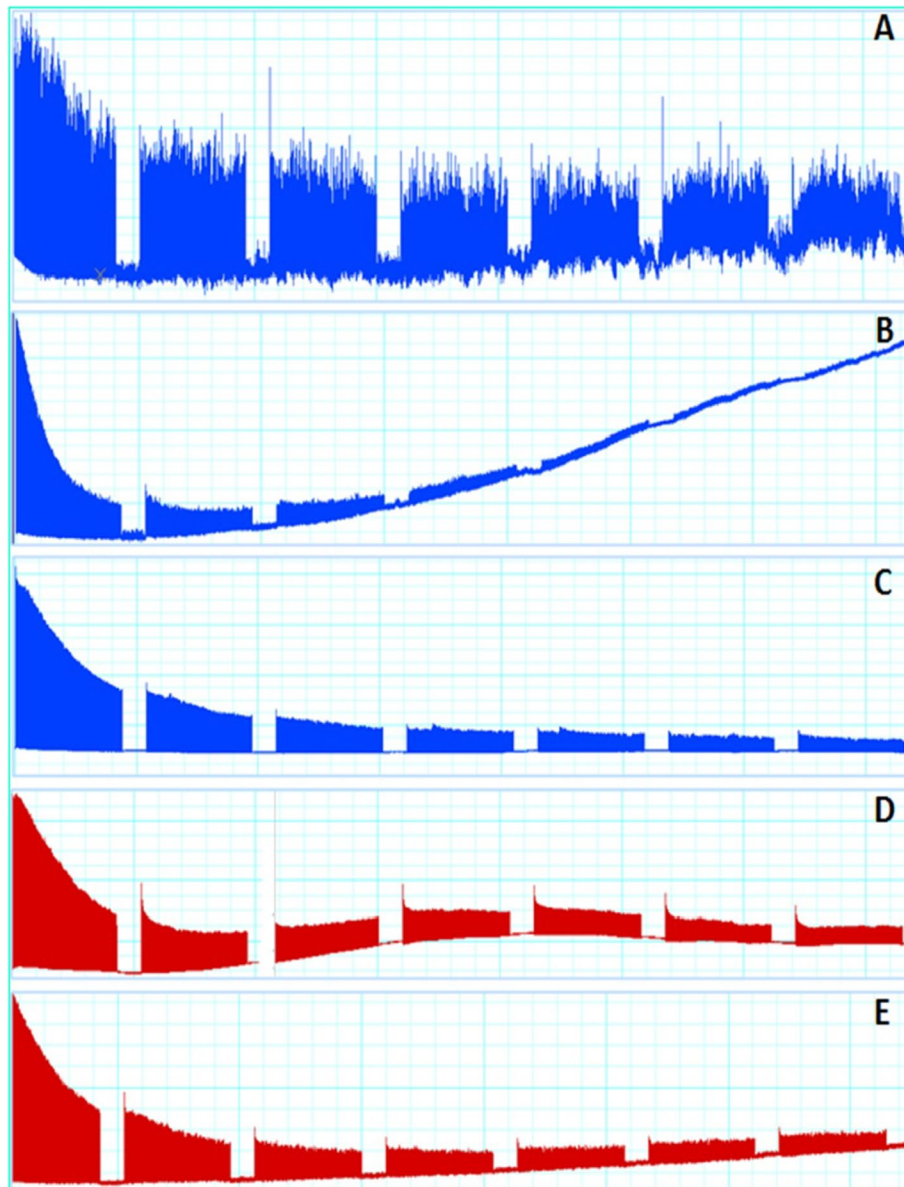


Fig. 3 Fatigue and loss of the viability of five RAM. **A–E** Samples at half the time of the electrical activity

confirmed that the composition and amount of fiber types slow and fast are key factors in muscles' functionality [35]. The limitations of this study are the reduced number of samples analyzed, and the collection procedure and characteristics of the RAM biopsies obtained through an invasive surgical procedure. RAM may vary in size, weight, length, fiber composition, and the presence of fasciae.

Conclusions

This pilot study demonstrates the viability of *ex-vivo* myography in striated muscles without tendons. The results obtained in this study are the first description of the RAM response under electrical stimulation, such as contraction force and fatigue, supporting the potential of RAM biopsy obtained at C-section as a tissue that can be used in *ex-vivo*

myography. This experimental model provides information on the performance and functionality of striated muscle without tendons in pregnant women. Myography is an approach that might be relevant in detecting the contractile activity in muscles without tendon not only in UI due to GDM but also to allow testing of the effects of *ex-vivo* drug application on human and animal tissues. The *ex-vivo* myography of fresh RAM samples is an approach suggested to be included in the full analysis of hyperglycemic myopathy as an underlying mechanism of long-term UI due to GDM.

Materials and methods

Recruitment

The pregnant women were recruited at the Perinatal Diabetes Research Center (PDRC) of Botucatu Medical School-UNESP, Brazil, between 24 and 28th weeks of gestation. The objectives and importance of the research were explained to the pregnant woman. Written informed consent to include in the study was obtained. The RAM samples were collected during C-section.

Study population and groups

This viability study constitutes part of a Diamater cohort study protocol [13]. This current study followed the CONSORT-2010 statement for the extension to randomized pilot and viability trials [36]. This study was approved by the Institutional Research Bureau of Botucatu Medical School-UNESP (protocol n° CAAE: 82225617.0.0000.5411). Three primiparous and two second-birth pregnant women over 18 years of age were included. The recruitment was carried out from August 2019 to March 2021. Two groups were used in this study, the study group (RAM biopsy) and the sham group (elastic ribbon).

RAM samples collection

The RAM biopsy collection was performed by the medical staff after surgical suture of the myometrium, visceral and parietal peritoneum during C-section with medical and or

obstetric indication, within a maximum period of 10 min after fetal extraction. In non-emergency C-sections, RAM samples could be obtained before childbirth. Immediately after resection, a RAM sample of 1 cm was collected, the fat and connective tissue were removed, and the muscle samples were placed in a Falcon tube containing 5 mL Krebs solution (118.5 μ M NaCl, 30 μ M KCl, 290 μ M NaHCO₃, 9 μ M MgSO₄, 9 μ M KH₂PO₄, 20 μ g CaCl₂, 5.5 g D-glucose, 300 μ M L-arginine, pH 7.4) and kept at 4 °C until analysis. The time between sample collection and the beginning of *ex-vivo* myography was a maximum of 30 min.

Equipment

This study used a DMT-myograph system (model 820MS DMT-Danish Myo Technology[®], Ann Arbor, Michigan USA) to assess the electrical activity. The chamber contains hooks, where the muscles are mounted to the edges, one end of the chamber, contains a force transducer that converts kinetic energy (muscle responses, under electrical stimulation) into an electrical signal that can be recorded and analyzed with

PowerLab equipment (PowerLab Data Acquisition System, AD Instruments, São Paulo, Brazil) and LabChart software (LabChart 8 for Windows, AD Instruments). The equipment was calibrated according to the manufacturer's recommendations and as per the training obtained at the DTM Company (Ann Arbor, Michigan, USA).

Muscle mounting method

The settings adjusted in the stimulation equipment were performed based on previous literature [24, 25, 37] and performed using other muscles with tendons and tissues [24, 38, 39].

Sample recovery/acclimatization and assembly/fixation of RAM sample to chamber hooks (1.st step)

An isolated organ bath was performed in Krebs solution with carbogenic gas for 15 min, previously heated to 37 °C, to recover the mechanical properties of the muscle. The RAM fragment was mounted vertically, following the arrangement of the fibers, in the myograph chamber filled with 4 mL of Krebs solution with continuous gas flow and minimal bubbling, which provides essential gases to maintain muscle in conditions that simulate the organic environment.

Fiber stabilization (2nd step)

the Krebs solution was changed twice at 15 min intervals, with a fixed tension force of 10 mN applied at each Krebs change. To carry out the 3rd and 4th stages, electrical stimulation was performed using platinum electrodes, placed parallel to the longitudinal axis, at 0.8 cm from the muscle tissue and integrated into the myographic cells, which provide electrical stimuli through the electrodes, by a series of predefined quadratic waves, due to its characteristic digital pulse (0–1). These conditions allow the observation of minimum and maximum points (dual pulse wave, gradient, ramp, sine, and triangle), controlled by MyoD analog output software (Danish Myo Technology®, Michigan, USA), with voltage-controlled (20 V) computer software and constant current management. To capture muscle responses with greater sensitivity and precision, muscle stimulation was performed with stimulation equipment (Grass Model S48, Danish Myo Technology®, Michigan, USA);

Sample viability (3rd step)

to verify the viability, electrical stimulation was performed with three stimuli of biphasic wave force of 10 mN, the voltage of 20 V, and pulse width of 25 mS. Approximate run time of 2 min.

Programmed stimulation protocol (4th step)

the application of the continuous alternating current stimulation protocol was performed with a fixed pulse train. Pulse voltage (volts): 20 V, pulse width (milliseconds): 25 ms, pulse interval (milliseconds): 60 ms, power: 10mN, pulse frequency (Hertz): 11.8 Hz, pause between pulse trains (milliseconds): 500 ms, pulse trains in the group: 500, repeat group: 10 times, pause between groups of trains (milliseconds): 60000 ms, total run time: 45 min.

Metric evaluation (5th step)

After the stimulation, the equipment was turned off and the parameters of the muscle sample, length(cm), width(cm) and weight(g) were determined. In the analysis of the results, the average of three measurements performed to normalize the results was considered. A digital caliper (STARFER[®], Vargem Grande do Sul, SP, Brazil) was used to measure muscle length and a digital analytical balance (model SHIMADZU[®] ATX224K ern ABT220-4NM, Kyoto, Japan) was used to weigh the fragment. Muscle weight was recorded in grams (dry weight).

Analysis of muscle biomechanics response under electrical stimulation of RAM***Frequency per quadrant***

A quadrant with a standard time interval was used for all analyses. A 10:1 speed was taken and the interval time was 5 min. All the highest and lowest peaks are counted in this 5 min time interval. These results corroborated the contraction frequency in relation to the electrical stimulus used to confirm whether the muscle contracted or not according to the programmed training (electrical stimulus performed by the equipment as set up in the software).

Peak height and time to peak

The speed was decreased between the biggest and the smallest peaks so they became more evident. The highest and lowest peak height values were recorded and the strength and response to the stimulus (muscle function) were calculated.

Force

The speed was increased to detect the descent of the graph (decreased strength which probably characterizes fatigue or viability loss).

Datapad

The values of the highest and lowest peaks were extracted from the datapad, this way we were able to assess when the peak started to be lowest (decrease in strength and fatigue).

Pulse calculation

According to the equipment set up there are 2 repetitions (two stimuli) in 1 s (2 stimuli/second), each repetition has 1 ms of duration separated by 500 ms which is the interval time named "rest". Finally, the overall electrical pulses were calculated, it would be 2 stimuli per second multiplied by the period of time analyzed.

Statistical analysis

This is a descriptive study to document the viability of the *ex-vivo* myograph technique in RAM samples of pregnant women. Therefore, there were no comparisons, and basic statistical analyzes of mean, median, and standard deviation were applied to properly assess the data for each sample.

Abbreviations

RAM	Rectus abdominis muscle
C-section	Cesarean section
UI	Urinary incontinence
GDM	Gestational diabetes mellitus
EDL	Digitorum muscle longus
PDR	Perinatal diabetes research center
DMT	Danish Myo technology
cm	Centimeters
g	Grams

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Author contributions

MVCR, AMPB and IMPC conceived the project and were responsible for funding, data curation, formal analysis and project administration; LS for methodology, investigation, validation, writing review and editing; DR, PSR, JFA, SLF, RLSH, SV, SMBC, JF, EMAE, CNFC and FB sample collection, methodology and investigation; DR wrote the original draft; PSR, JFA, SLF, RLSH writing review and editing; SV, SMBC, JF, EMAE and FB: literature research and revision of the manuscript; DIAMATER Study Group designated as authors qualify for authorship as a fundamental part of this cohort study. All authors have agree to be accountable for all aspects of the work. All authors read and approved the final manuscript.

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Availability of data and materials

Contact the corresponding author for data requests.

Declarations

Ethics approval and consent to participate

This study was approved by the Institutional Ethical Committee of Botucatu Medical School, São Paulo State University (UNESP), Botucatu, São Paulo State, Brazil (CAAE: 82225617.0.0000.5411). The informed consent to participate document from all patients included in this study was obtained by following the ethical guidelines (Resolution 466/2012) of the Brazilian Health Council. All methods were performed in accordance with the relevant guidelines and regulations.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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