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MORPHOLOGICAL CHARACTERISTICS OF THE SUBCUTANEOUS TISSUE OF THE LEG REGION IN HUMAN FETUS

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Abstract

Introduction. In recent decades, a new type of adipose tissue has been identified. Because of their external structure and location, these adipocytes are called beige adipocytes. It is believed that beige adipocytes originate from brown adipocytes, which are located in depots of white adipose tissue. In the literature, there is a single piece of information on the peculiarities of the formation and topography of adipose tissue in human fetuses of various ages.
The objective of the study was to assess the peculiarities of the structure and distribution of adipose tissue of the lower leg in human fetuses of 5-8 months.
Materials and methods. Microscopic examination was carried out on preparations of the leg region of 18 human fetuses 136.0-310.0 mm parietal-coccygeal length (PCL) without external signs of anatomical deviations or anomalies in the development of bone, fascial-muscular and vascular-nervous structures of the lower extremities.

Résumé

Caractéristiques morphologiques du tissu sous-cutané de la région de la jambe chez le fœtus humain

Introduction. Au cours des dernières décennies, un nouveau type de tissu adipeux a été identifié. En raison de leur structure externe et de leur emplacement, ces adipocytes sont appelés adipocytes beiges. On pense que les adipocytes beiges proviennent d'adipocytes bruns, qui sont situés dans des dépôts de tissu adipeux blanc. Dans la littérature, il existe une seule information sur les particularités de la formation et de la topographie du tissu adipeux chez les fœtus humains d'âges divers.
L'objectif de l'étude a été d'évaluer les particularités de la structure et de la distribution du tissu adipeux de la jambe inférieure chez les fœtus humains de 5 à 8 mois.
Matériels et méthodes. Un examen microscopique a été effectué sur des préparations de la région de la jambe de 18 fœtus humains de 136,0 à 310,0 mm de

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Results. The composition of adipose tissue in the examined human fetuses of different gestational periods is heterogeneous and is represented by unilocular and multilocular cells united in clusters of different shapes. During the fetal stage, the number and ratio between the types of fat cells change.
Conclusions. Adipocytes are located singly in 5-month-old human fetuses, in 6-month-old fetuses they form elongated clusters, and from the end of the 7th to the beginning of the 8th month of the fetal period of ontogenesis, adipocytes form plaque-like structures located in several rows. The lowest number of fat cells is observed in 5-month-old human fetuses, among which the percentage of multilocular cells is 85.3±0.92%. In 6-month-old human fetuses, the ratio of multilocular cells to unilocular cells is 93.0±0.12%; in 7-month-old fetuses – 72.7±0.16%; in fetuses of 8 months of gestation – 57.8±0.17%.

Keywords: multilocular cell, unilocular cell, beige adipocyte, adipose tissue, fetus.

longueur pariéto-coccygienne sans signes externes de déviations anatomiques ou d'anomalies dans le développement des structures osseuses, fascio-musculaires et vasculo-nerveuses du membres inférieurs.
Résultats. La composition du tissu adipeux chez les fœtus humains examinés de différentes périodes de gestation est hétérogène et est représentée par des cellules uniloculaires et multiloculaires réunies en grappes de formes différentes. Au cours de la phase fœtale, le nombre et le rapport entre les types de cellules graisseuses changent.
Conclusions. Les adipocytes sont localisés individuellement chez les fœtus humains de 5 mois, chez les fœtus de 6 mois, ils forment des amas allongés, et de la fin du 7e au début du 8e mois de la période fœtale d'ontogenèse, les adipocytes forment une plaque ressemblant à des structures situées sur plusieurs rangées. Le plus faible nombre de cellules graisseuses est observé chez les fœtus humains âgés de 5 mois, parmi lesquels le pourcentage de cellules multiloculaires est de 85,3±0,92%. Chez les fœtus humains âgés de 6 mois, le rapport des cellules multiloculaires aux cellules uniloculaires est de 93,0±0,12% ; chez les fœtus de 7 mois – 72,7± 0,16%; chez les fœtus de 8 mois de gestation – 57,8±0,17%.

Mots-clés: cellule multiloculaire, cellule uniloculaire, adipocyte beige, tissu adipeux, fœtus.

Introduction

The study of the structural organization of white and brown adipocytes, as the main types of adipose tissue, remains a subject of morphological research. The main location of brown adipose tissue in human fetuses is in the interscapular and cervical areas, the perirenal tissue, less often in the front wall of the abdominal cavity. White adipose tissue, in turn, occupies all other typical places. White adipose tissue stores excess energy in the form of triglycerides, while brown adipose tissue specializes in dissipating energy through heat production[1]. However, in recent decades, a third new type of adipose tissue has been identified. Due to their external structure and location, these fat cells were called beige adipocytes[2-3]. It is believed that beige adipocytes originate from brown adipocytes, which are located in depots of white adipose tissue. Literature sources[4-5] provide information on the morpho-functional and biochemical characteristics of brown and white adipose tissue. White adipocytes are characterized by the presence of one large lipid droplet, which occupies 90% of the cell space and consists of triglycerides. There are only a few thin elongated mitochondria in white adipocytes. Brown adipocytes contain triglycerides in numerous smaller vacuoles. Brown adipose tissue is well vascularized and contains a significant number of large spherical mitochondria with lamellar cristae. Beige adipocytes, like brown adipocytes, exhibit thermogenic properties in addition to similar morphological and biochemical characteristics, including multilocular lipid droplets and dense mitochondrial cristae[6].

Beige adipocytes have characteristics of both brown and white fat cells. Beige adipocytes develop in the subcutaneous tissue from a separate subset of preadipocytes or are formed as a result of the differentiation of existing white adipocytes. To identify the type of adipose tissue, their structural differences are taken into account, in particular the size of cells and the number of lipid droplets in the cytoplasm, as well as the presence of the uncoupling protein UCP1[7-9].

Some researchers[10] indicate that classic brown and beige adipocytes share the same progenitor cells with skeletal muscle cells.

The research of the origin and potential of beige adipocytes continues[11-14]. Brown and beige human adipocytes are characterized by high plasticity and sensitivity to environmental conditions, which expands the field of their further study[15,16]. Under normal conditions, beige adipocytes function like white adipocytes. However, a certain stimulus, such as prolonged exposure to cold or caloric restriction, can turn beige adipocytes into brown. This process was called «browning»[17-19].

The study of the development and metabolism of various types of adipose tissue and their relationship with other organs, and in particular with skeletal muscles, liver, other organs, and structures of the cardiovascular and central nervous systems, will help deepen knowledge about the mechanisms of metabolic syndrome and improve approaches to its prevention and treatment[20,21]. However, in the literature, the information about the peculiarities of the structural organization and formation of the topography of adipose tissue in human fetuses of different ages is scarce[22,23]. This study is a continuation of our research on the morphology of structures of the leg region of human fetuses[24].

The objective of the study was to evaluate the peculiarities of the microscopic structure and distribution of adipose tissue of the lower leg in human fetuses of 5-8 months.

Materials and methods

The research was carried out on preparations of leg regions of 18 human fetuses 136.0-310.0 mm in parietal-coccygeal length (PCL) without external signs of anatomical deviations or anomalies in the development of bone, fascial-muscular and vascular-nervous structures of the lower extremities in accordance with the contract of cooperation between the Chernivtsi regional communal medical institution «Chernivetske oblasne patholohoanatomichne biuro» and the institution of higher education Bukovinian State Medical University, Ukraine.

The morphological study was carried out in compliance with the basic bioethical provisions of the Council of Europe Convention on Human Rights and Biomedicine (dated 04.04.1997), the Helsinki Declaration of the World Medical Association on the Ethical Principles of Scientific Medical Research with Human Participation (1964–2013), the Order of the Ministry of Health of Ukraine Nº 690 dated September 23, 2009, and taking into account the methodological recommendations of the Ministry of Health of Ukraine "Procedure for extracting biological objects from dead persons whose bodies are subject to forensic examination and pathological anatomical research, for scientific purposes" (2018). The Commission on Biomedical Ethics of Bukovinian State Medical University has not revealed any violations of bioethical and moral norms during the scientific study.

A microscopic study was performed using the staining of histological sections with hematoxylin and eosin, and for better contrast of the protein elements of the structures – a histochemical study of protein with bromophenol blue according to the Mikel Calvo method. Usually, this technique is used to establish the ratio between amino and carboxyl groups in proteins. However, this method was also effective for identifying the types of fat cells (uni- or multilocular), in particular, their protein membranes (cellular and intracellular), which surround the locations of fat droplets. To obtain reliable results, a certain list of procedures was followed[25].

The percentage of multilocular cells was calculated on digital copies of optical images in the environment of the computer program ImageJ 1.53t (2022)[26] with subsequent statistical processing of quantitative data.

The statistical analysis of the study results was performed using open-source software «PAST» (PAleontological STatistics Version 4.9 2022)[27]. In particular, the average percentage of multilocular cells and its statistical error were calculated, the confidence intervals of the percentage at the level of p=0.05, the difference between the average percentages was evaluated using Fisher's transformation using the probability value «p». Differences at the p≤0.05 level were considered statistically significant.

Results and discussion

During the histological study of the structure of the leg region of human fetuses of 5-8 months, we paid attention to the peculiarities of the microscopic structure and the location of adipose tissue.

Thus, in fetuses 136.0-165.0 mm PCL of adipose tissue at the level of the upper third of the leg is represented by single cells that are located around blood vessels and nerves (Fig. 1).

Only loose connective tissue is located between the dermis and the muscles of the leg, which will later form the fascia of the leg. In fetuses of this age, flat plaque-like clusters of a small number of unilocular and multilocular adipocytes, mostly located near blood vessels, were found at the level of the middle and lower thirds of the leg. The percentage of multilocular adipocytes is 85.3±0.92% (confidence interval 70.8-94.4% at p=0.05). Other adipocytes were defined as unilocular. The aforementioned clusters of fat cells are separated from neighboring structures by loose connective tissue **(Fig. 2).**

In fetuses of 185.0-230.0 mm PCL, the subcutaneous tissue of the leg region has the appearance

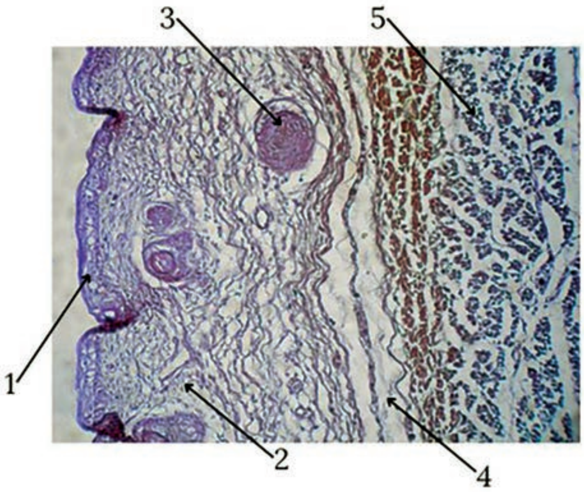

Fig. 1. Structures of the upper third of the leg region of a fetus 150.0 mm PCL. Staining of the histological section with bromophenol blue according to the Mikel Calvo method. 4x planachromat lens. Without glasses: 1 – epidermis; 2 – dermis; 3 – hair follicle; 4 – loose connective tissue; 5 – muscle tissue.

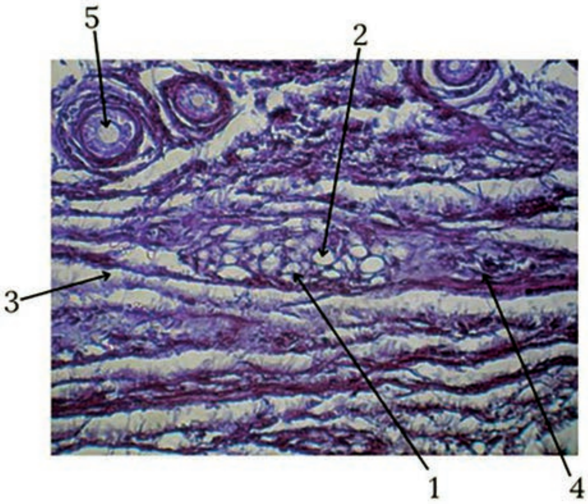

Fig. 2. Structures of the lower third of the leg region of a fetus 165.0 mm PCL. Staining of the histological section with bromophenol blue according to the Mikel Calvo method. 10x planachromat lens. Without glasses: 1 – multilocular adipocyte; 2 – unilocular adipocyte; 3 – loose connective tissue; 4 – blood vessel; 5 – hair follicle.

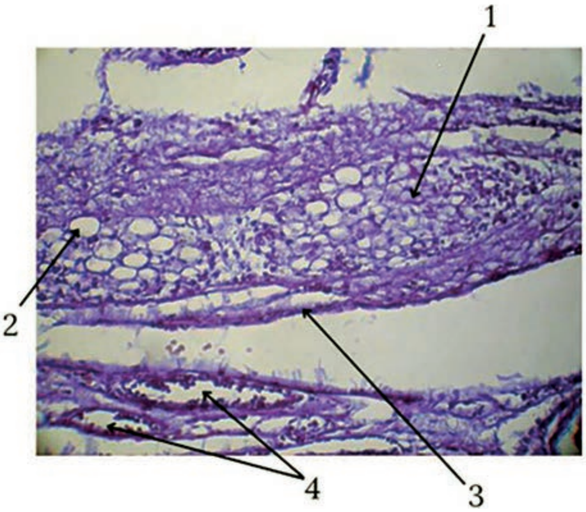

Fig. 3. Structures of the middle third of the leg region of the fetus 230.0 mm PCL. Staining of the histological section with bromophenol blue according to the Mikel Calvo method. 20x planachromat lens. Without glasses: 1 – multilocular adipocyte; 2 – unilocular adipocyte; 3 – loose connective tissue; 4 – blood vessels.

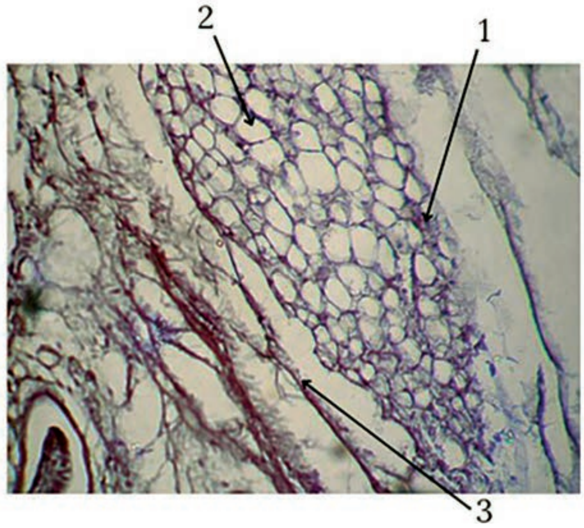

Fig. 4. Structures of the upper third of the leg region of the fetus 250.0 mm PCL. Staining of the histological section with bromophenol blue according to the Mikel Calvo method. 20x planachromat lens. Without glasses: 1 – multilocular adipocyte; 2 – unilocular adipocyte; 3 – loose connective tissue.

of elongated flat plaque-like clusters located in one row. Adipose tissue is well vascularized and clearly separated from neighboring structures (**Fig. 3**). In the quantitative ratio, multilocular adipocytes prevail, 93±0.12% (confidence interval 88.7-96.0%, p=0.05). At the same time, the probability of discrepancy in the percentage of multilocular adipocytes in fetuses of the previous age group according to Fisher's angular transformation was not sufficient (p=0.260), which is confirmed by the crossing of confidence intervals for the compared gestational periods.

In fetuses of 240.0-260.0 mm PCL, adipose tissue is represented by clusters of cells, which in their shape resemble a plaque or a flat island. Such accumulations are located in one row immediately under the loose connective tissue that forms the fascia of the lower leg. Adipose tissue in fetuses of this age group also consists of two types of cells: 1) larger in

Fig. 5. Structures of the middle third of the leg region of the fetus 260.0 mm PCL. Staining of the histological section with hematoxylin and eosin. 20x planachromat lens. Without glasses: 1 – branching of multilocular adipocytes; 2 – unilocular adipocyte.

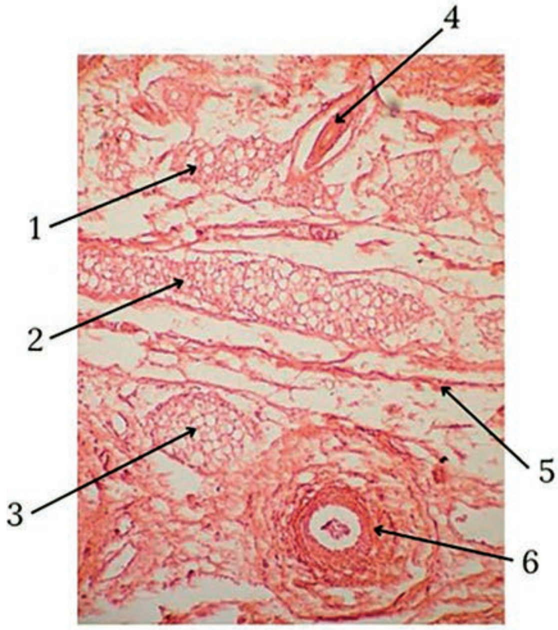

Fig. 6. Structures of the middle third of the leg region of a fetus 275.0 mm PCL. Staining of the histological section with hematoxylin and eosin. 4x planachromat lens. Without glasses: 1 – surface layer of adipocyte cluster; 2 – middle layer of adipocyte clusters; 3 – deep layer of adipocyte clusters; 4 – hair follicle; 5 – loose connective tissue; 6 – blood vessel.

size – unilocular adipocytes with one large lipid droplet and a nucleus shifted to the periphery; 2) smaller in size – multilocular adipocytes with multiple lipid droplets and a nucleus in the center (**Fig. 4**).

Multilocular cells predominate in the peripheral parts of the plaque, and unilocular cells in their central part. The percentage of multilocular fat cells is 72.7±0.16% (confidence interval 67.8-77.8%, p=0.05). The probability of discrepancy in the percentage of multilocular adipocytes in fetuses of 185.0-230.0 mm PCL and 240.0-260.0 mm PCL according to Fisher's angular transformation was high (p=0.001), which is also confirmed by the absence of overlapping confidence intervals for the compared terms gestation.

In individual plaque-like clusters of lipocytes, strands of multilocular cells branch outward from them towards the epidermis (**Fig. 5**). It is most likely that these strands are the source of the formation of new rows of plaque-like accumulations of lipocytes.

For the first time in fetuses 270.0-291.0 mm PCL, adipocyte islands are located in two or more rows. In particular, in a fetus of 275.0 mm PCL, the subcutaneous tissue is represented by three layers of adipose cell clusters: superficial, medium, and deep, which differ among themselves in terms of shape, number of cells, and percentage ratio. Surface clusters of adipocytes have not yet formed into one continuous plaque, and consist of a small number of cells located fragmentarily around blood vessels or hair follicles. The percentage of multilocular cells is 56.8±0.22%. The middle layer of adipocytes is represented by more fat cells and forms well-defined plaque-like structures. Multilocular cells make up 52.5±0.21% of the total number of cells in this layer. The deep layer of fat cell clusters takes on a rounded shape and is localized near blood vessels. The percentage of multilocular cells is 63.7±0.24%. Due to the increase in the size and number of unilocular adipocytes, deeper islands have a more rounded shape and are separated from each other and neighboring structures (**Fig. 6**).

The total number of fat cells increases in fetuses of this age group (**Fig. 7**). The percentage of multilocular fat cells was 57.8±0.17% (confidence interval 51.8-63.3%, p=0.05). The probability of discrepancy of the percentage of multilocular adipocytes in human fetuses at 7 and 8 months of gestation according to Fisher's angular transformation was high (p=0.009), which is confirmed by the lack of overlap of the applied confidence intervals for the compared gestational periods.

According to existing data, unilocular cells containing one large fat drop[4-6] can be identified as white adipose tissue, and multilocular cells with multiple fat drops can be identified as brown adipose tissue. Human brown adipose tissue can consist of a combination of multilocular and unilocular adipocytes. In our opinion, it is better to name such fabric differently. In particular, the research results obtained by us agree with the opinion of some authors[2,3] about the

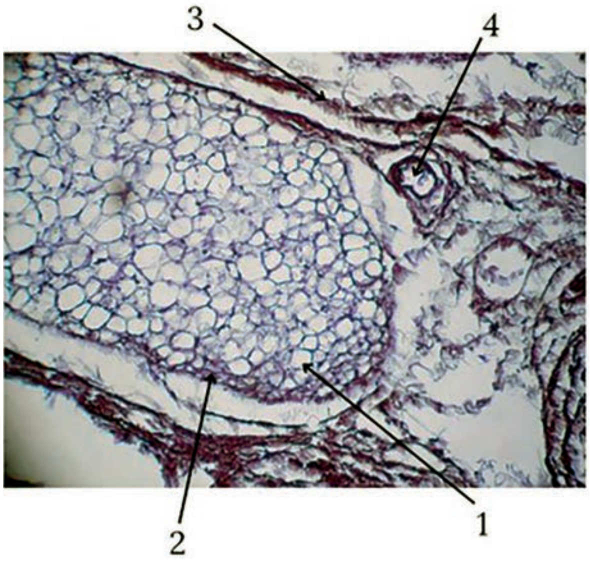

Fig. 7. Structures of the middle third of the lower leg of a fetus of 280.0 mm PCL. Staining of the histological section with bromophenol blue according to the Mikel Calvo method. 10x planachromat lens. Without glasses: 1 – multilocular adipocyte; 2 – unilocular adipocyte; 3 – loose connective tissue; 4 – blood vessel.

existence of a third type of fat cells (beige adipocytes), which morphologically resemble brown adipose tissue, but are localized in areas of traditional accumulation of white adipose tissue.

There is an opinion that brown and beige adipocytes develop from different embryonic progenitors[7-10]. Brown adipocytes are known to originate from Myf5-positive cells. The exact origin of beige fat cells is still debated[12-22]. There are two theories: the first suggests that they arise from white adipocyte precursors and remain beige adipocytes in response to environmental stimuli such as exposure to cold; and according to another theory, mature white adipocytes can transdifferentiate due to the action of certain stimuli in beige. It is possible that both propositions are correct and that, depending on the environment, genetic background, and location of adipose tissue, the origin of the beige cells may be different, or there is another source of their origin.

Since, in this study, the detected areas of migration of multilocular cells towards the epidermis, where later fat cells will turn into unilocular cells, it can be assumed that multilocular cells are the source of the development of unilocular cells.

For the first time, we have established that the areas around blood vessels are the primary place of formation of fat cell clusters, which confirms the clearness of the connections between these structures. It is known that adipose tissue is one of the most vascularized tissues in the body. There is no doubt that the interaction between adipocytes and vascular cells is critical to ensure the optimal delivery of oxygen, nutrients, hormones, and other biologically active molecules to fat cells. Adipocyte progenitors can also secrete several angiogenic factors, including VEGFA, HGF, fibroblast growth factor 1 (FGF1), and FGF2[16].

Conclusions

The composition of adipose tissue in human fetuses of different gestational periods is heterogeneous and is represented by unilocular and multilocular cells. During the fetal period of development, the number and ratio between the types of fat cells change. Five-month-old fetuses have the lowest number of fat cells, among which the percentage of multilocular cells is 85.3±0.92%. In 6-month-old human fetuses, the ratio of multilocular cells to unilocular cells is 93.0±0.12%; in 7-month-old fetuses 72.7±0.16%; in fetuses of 8 months of gestation 57.8±0.17%.

The subcutaneous tissue in 5-month-old fetuses is better expressed in the lower and middle thirds of the leg. The first accumulations of fat cells appear around blood vessels, and this trend is also observed during the formation of adipocyte layers in older fetuses.

In 5-month-old fetuses, adipocytes are located singly, in 6-month-old fetuses, they form elongated clusters, and from the end of the 7th to the beginning of the 8th month of the fetal period of ontogenesis, adipocytes form plaque-like clusters located in several rows.

The distribution of adipocytes in the plaque-like clusters is uneven: multilocular cells are located on the periphery, and unilocular cells are located in the center.

In all studied fetuses, clusters of adipocytes are well separated from adjacent structures by loose connective tissue.

Author Contributions:

T.V.K. is responsible for data acquisition, anatomical investigations, data analyzing, design, and writing the manuscript. I.S.D. and T.V.K. are responsible for the conception, review, and edited the manuscript. T.V.P. and I.G.B. aided in the design, reviewed, and edited the manuscript. All authors contributed equally to the present work. All authors contributed to the critical revision of the article for valuable intellectual content. All the authors have read and agreed with the final version of the article.

Compliance with Ethics Requirements:

"The authors declare no conflict of interest regarding this article"

"The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1964, as revised in 2013, as well as the national law"

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