

DOI: <http://dx.doi.org/10.5281/zenodo.5092126>

Polyploidy promotes Harderian glands function under photo-oxidative stress in desert rodents

Ouanassa Saadi-Brenkia^{1,2*}, Saida Lounis^{1,2}, Nadia Haniche²¹ Department of Biology, University of Boumerdes Faculty of Sciences, Avenue de l'indépendance, 35000 Boumerdes, Algeria² Laboratory of Biology and Physiology of Organisms, Neurobiology, Scientific and Technical University Houari Boumediene, 16111 Algiers, Algeria

* Corresponding author: Phone: +213662543556, E-mail: saadianissa@yahoo.fr

Received: 25 April 2021; Revised submission: 15 June 2021; Accepted: 12 July 2021

<https://jbrodka.com/index.php/ejbr>
Copyright: © The Author(s) 2021. Licensee Joanna Bródka, Poland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>)

ABSTRACT: The presence of higher-ploidy cells within Harderian glands (HG) of desert rodents could be explained as an adaptive response to mitigate the effects of photo-oxidative stress. The principally products of HG are porphyrins, pigmentary accretions which interact with the intense luminosity of the Sahara and, then produce reactive oxygen species. Thus, the gland permanently suffers a physiological oxidative stress, with a great number of signs of degeneration, but without compromising the gland integrity. In this work, we used light and transmission electron microscopy to examine the morphological features of cell ploidy in HG of three species of Gerbillidae. *Psamomys obesus*, *Meriones libicus* and *Gerbillus tarabuli*. The results showed that, the glands of these species are large in size and lobulated. The glandular parenchyma consists of tubulo-alveoli surrounding a lumen into which the secretions are discharged. Frequently cells are binucleated and multinucleated. Transmission electron microscopy reveals the presence of secretory cells with conspicuous nuclei and sometimes with micronuclei. Binuclear cells are created by acytokinetic mitosis. No cell membranes within the cytoplasm are observed. Our results provide morphological evidences, that HG of desert rodents employ polyploidy as cellular adaptive response to extreme arid environment.

Keywords: Harderian glands; Polyploidy; Cellular stress; Micronuclei; Electron microscopy; Desert rodents.

1. INTRODUCTION

Endopolyploidy (polyploidy), the condition in which the number of genome copies has been increased through endoreduplication in the cells of certain tissues and organs. Polyploidy have been reported to be more frequent in extreme environments, including the subarctic regions, high elevations and xeric environments [1, 2]. In animals, in contrast to plants, endopolyploidy primarily occurs in highly specialized cell types with high metabolic output [3]. Even fewer examples of endopolyploidy in animals in relation to environmental stresses have been documented. It is assumed, that, endoreduplication has a recognized role in driving body size [4] or in maintaining tissue and organ growth in response to exogenous stresses, such as regeneration of damaged liver and cardiomyocytes [5, 6]. As reported by Hessen [7] polyploidy could also be promoted by solar radiations. This has clearly been verified for terrestrial plants [8] and for invertebrates [9] as well as freshwater fish [10]. In light of the well-established link between solar

radiations and polyploidy, an initial investigation at morphological level of this association is examined in HG of small mammals, desert rodents.

Harderian glands are large retro- orbital glands. In desert rodents as in all rodents, they are particularly well developed. Those ocular glands have tubuloalveolar endpieces (tubular alveoli) and their main products are lipids and porphyrins. But from different species of rodents are markedly distinct in their histological structures [11]. In Gerbillinae subfamily, they appear different phenotypically from species to species [12, 13]. By light and electron microscopy, basophilic pyramidal and acidophilic columnar cells have been described in the secretory epithelium of the gerbil and meriones. However, in *Psammomys* basophilic pyramidal cells are totally absent [14, 13]. Previous works on hamster [15-17] have shown that porphyrins accumulation in the gland produce photoreaction. As a consequence, the generation of reactive oxygen species (ROS) inducing an increase of oxidative stress which gives rise to cellular damage. This physiological stress has also been studied in our models (Unpublished data). Thus, in this study, we aimed to reveal morphological and cytological traits of polyploidy considered as an adaptive response in desert rodents HG to enduring photo-oxidative stress.

2. MATERIALS AND METHODS

We used 10 adult males of each species. They were trapped in the arid zone of Beni-Abbes (wilaya of Bechar), 1250 km south west of Algiers. The species selected for this study are belonging to the Gerbillinae subfamily, which contains 14 genera [17]. From the genus *Gerbillus*, *Gerbillus tarabuli* (Thomas 1902) nocturnal, granivore species has been taken. *Meriones lybicus* (Lichtenstein 1823) was chosen from genus *Meriones*, it's granivore and partly nocturnal species and *Psammomys obesus* (Cretzschmar 1828) diurnal herbivore rodent being part from *Psammomys* genus. The animals were cared for in accordance with the criteria outlined in the "Guide for the Care and Use of Experimental Animals" following approval by the Institutional Animal Care Committee of the Algerian Higher Education and Scientific Research. The permits and ethical rules were achieved according to the Executive Decree No. 10-90 completing the Executive Decree No. 04-82 of the Algerian Government, establishing the terms and approval modalities of animal welfare in animal facilities.

2.1. Histological studies

The HG were removed and fixed in 10% formaldehyde solution. The tissue was washed and then dehydrated in ascending series of ethanol alcohol, cleared in xylene, embedded in paraffin wax. Sections were cut at 5-6 μm thickness and stained with haematoxylin and eosin, Van Gieson stain. The sections were then viewed under light microscopy (Zeiss Axioplan) and photographed with a high-resolution optics microscope camera (Premiere, MA88-500).

2.2. Ultrastructural studies

The HG of the studied species were excised and small pieces were prefixed in glutaraldehyde-paraformaldehyde (pH 7.4) at 4°C and then, the tissue samples were Post fixed in 1% OsO_4 for 2 h; they were dehydrated in ascending series of ethyl alcohol, cleared in propylene oxide, infiltrated, and embedded in epoxy resin until it polymerizes. Samples were cut with ultramicrotome (LKB Bromma, 8800 Ultratome III). Semithin sections (1 μm) were stained with toluidine blue and examined by light microscopy Zeiss and photographed with a high-resolution optics microscope camera. Ultrathin sections (20–50 nm) were collected on uncoated 200 mesh copper grids were double-stained with Reynolds' lead citrate and ethanolic uranyl acetate [18] and examined with a Zeiss EM-109 transmission electron microscope (Zeiss, Germany) operating at 80 kV.

3. RESULTS

The HG of the investigated desert rodents species, *Psammomys obesus*, *Meriones lybicus* and *Gerbillus tarabuli* have all been shown to be polyploid.

3.1. Gross anatomy

The HGs of the studied species were similar being large in size and lobulated in appearance. Significant difference was seen in the color, brown in *Psammomys*, pink in *Meriones* and yellow in gerbil (Fig. 1).

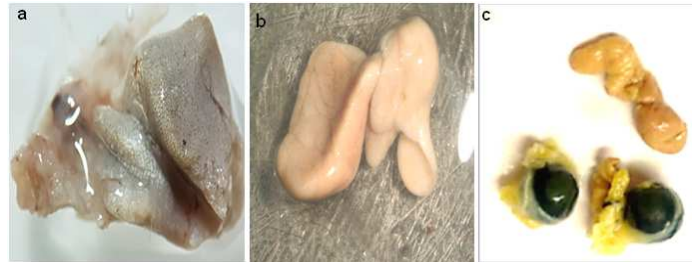


Figure 1. Macroscopic view of desert rodent's Harderian glands (HG). Note the coloration and the lobulation of the gland in (a) *Psammomys obesus*, (b) *Meriones lybicus* and (c) *Gerbillus tarabuli*.

3.2. Light microscopy

At low magnification, the HG have the same basic histology in all three species of Gerbillinae, they are multilobular glands. They consist of tubuloalveolar units. The lumen of the glands is large and surrounded by secretory columnar cells (Fig. 2a). Often filled with porphyrins and cellular debris (Fig. 2b). Thus, observed at higher magnifications, the glandular cells constitute a diversified population of mononuclear, binuclear and multinuclear cells. So, the nuclei are enlarged and irregular, and the cell body again enlarged, this may indicate their polyploidy (Fig. 3). However, some interspecific differences were detected. As seen, in the number of cellular types in glandular epithelium and in the pigmentation intensity in interstitial tissue.

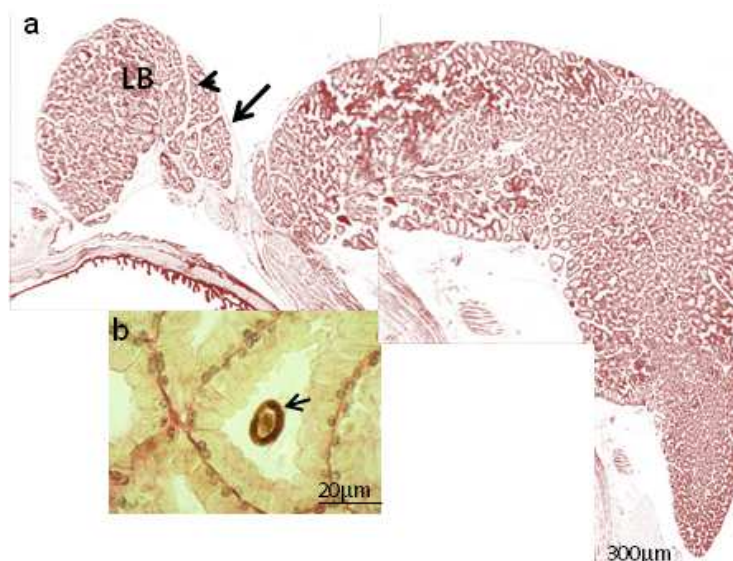


Figure 2. A low power view (a) of a longitudinal section through the gerbil Harderian gland showing the large size, the lobulation and the tubulo-alveolar endpieces. Lobe (LB), lobule (arrowhead), capsule (arrow); H/E stain. (b) Showing porphyrins (arrow) in the lumen of glandular unit. Van Gieson stain.

A uniform glandular epithelium containing one type of columnar cells, and heavily pigmented connective tissue were revealed in sand rat (Fig. 3a). While in the gerbil, we have noted a pseudostratified epithelium with basophilic pyramidal cells and columnar cells; then, in the interstitial tissue the melanin is sparse (Fig. 3b). The same features were revealed in meriones but the basophilic pyramidal cells are scarce in the tubulo-alveoli and sometimes totally absent from glandular units (Fig. 3c).

3.3. Electron microscopy analysis

Multinucleated cells have been well detected among various morphological markers of polyploidy in desert rodent's Harderian glands. Indeed no cell membranes within the cytoplasm of cells with two or more nuclei (Fig. 4). ultrastructural analysis confirms the light microscope findings. Thus, enlarged and irregular nuclei were present. And additionally, micronuclei were often observed in glandular cells (Fig. 5). AS nuclei, micronuclei were surrounded by a double membrane and contained a mixture of euchromatin and heterochromatin.

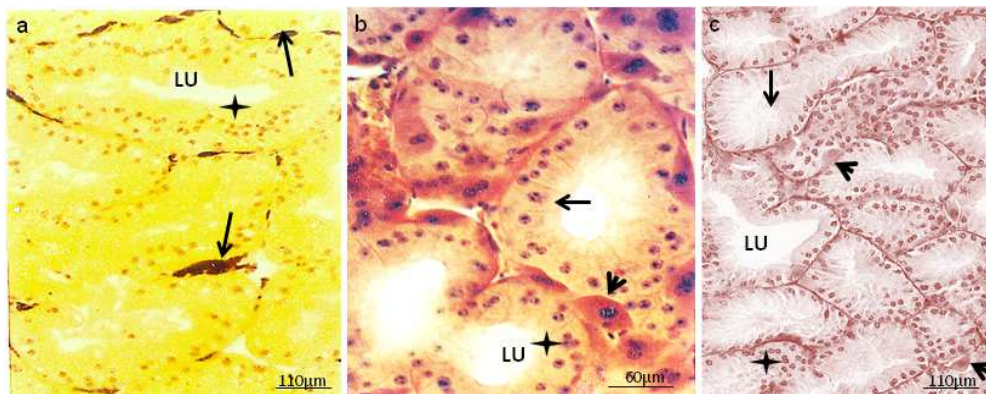


Figure 3. Histological organization of desert rodents Harderian glands. (a) Sand rat's HG. Columnar uniform epithelium, melanin (arrow) nuclear fast red/pic stain. (b) gerbil's HG, pseudostratified epithelium with columnar (arrow) and pyramidal (arrowhead) cells. Van Gieson stain. (c) merione's HG, pyramidal cells are rare (arrowhead). H/E stain. Note that most cells are bi or multinucleated (asterisk) in the three species, lumen (LU).

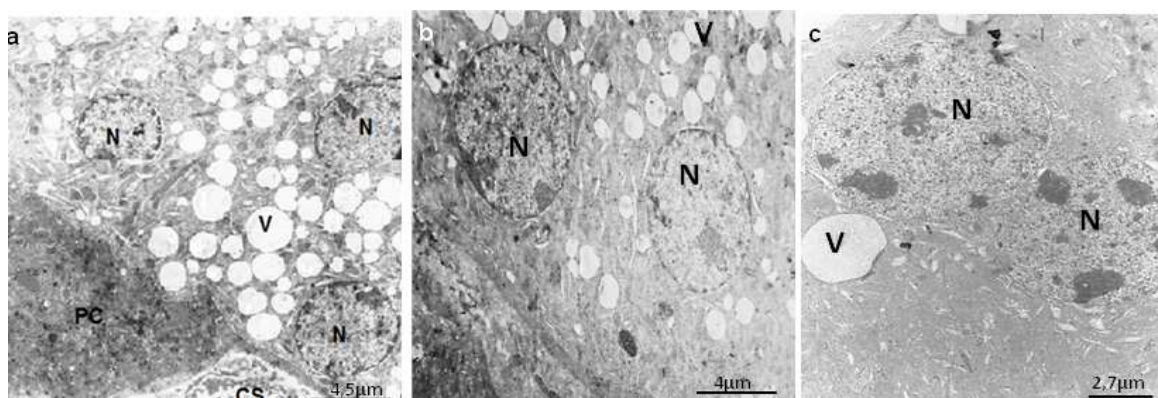


Figure 4. Ultrastructural evidence for multinucleated cells in Harderian glands of Gerbillidae. (N) nucleus, (V) vacuole, (CS) capillary, (PC) portion of pyramidal basophilic cell.

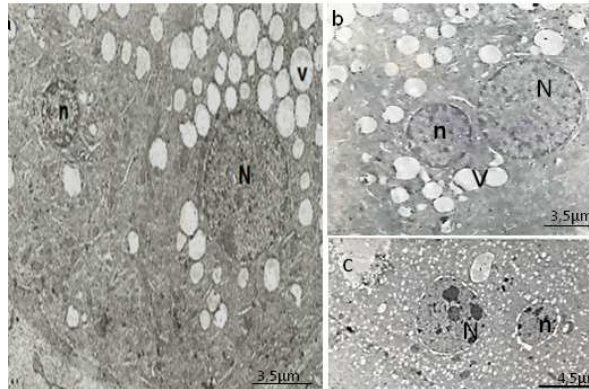


Figure 5. Electron micrographs of HG cells. Some cells contain nucleus (N) and micronucleus (n), vacuoles (V).

4. DISCUSSION

Polyploidy frequently encountered in plants whereas in animals, it's limited to certain tissues in mammals, such as liver cells, megakaryocytes and giant trophoblast cells in the placenta. Our results show that polyploidy is a common feature of HG cells in desert rodents. It seems that it makes them tolerant against photo-oxidative stress. Earlier reports have shown that, environmental fluctuations and stressors constantly challenge organisms in the wild. Organisms thus use cellular mechanisms to adapt to and to survive environmental fluctuations.

The results of the current study showed interspecific difference concerning the coloration of the glands. Melanin has already been described in some species of rodents HG [20-25]. In accordance with Costin and Hearing [26] melanin in HG, may have a photo protective function and acts as a physiological redox buffer. A large organ and cells size are revealed in all studies species. It seems to be the main characteristics of the polyploidy. According to Frawley and Orr-Weaver [27] a better adaptability of individuals and increased organ and cell sizes are usually associated with polyploidy. Additionally, Wendel [28] and Comai et al. [29] have reported that, polyploidy does have immediate phenotypic effects, such as increased cell size and organ size, and sometimes greater vigor and biomass, and new phenotypic and molecular variation can arise shortly after polyploid formation. Nuclear abnormalities are detected in secretory cells, such as enlarged nuclei, irregularities in shape and presence of micronuclei. Previous data [30-33] have revealed that, the size of nuclei has often been reported to increase according to ploidy level. Fenech [34] have stated that, micronuclei and other nuclear anomalies such as nucleoplasmic bridges and nuclear buds are biomarkers of genotoxic events and chromosomal instability. Numerous reports indicate that micronuclei are fragments of chromosomes or whole chromosomes that are left out of daughter nuclei during division. These displaced chromosomes or chromosome fragments are eventually surrounded by a nuclear membrane and, except for their smaller size, are morphologically similar to nuclei after conventional nuclear staining. These data are consistent with our findings. So, it is confirmed that chromosomes within micronuclei are brutally damaged or pulverized [35-37]. It was shown by Beedanagari et al. [38] that increased incidence of micronuclei formation serves as a good biomarker for genotoxic damage. In our results the genetic and genotoxic effects of solar radiations were evidenced by the significant presence of micronuclei in secretory cells of HG.

The current study showed that, desert rodents HG displayed phenotypic variations which can be clearly linked to species-specific differences in lifestyle. The common occurrence of polyploidy in secretory cells of HG may be protective in several ways, given that more DNA templates could help to sustain genome integrity under damaging UV-B and promote the genetic pathways that produce melanin which is active in the stress response

and UV-B absorption. Further interdisciplinary approaches are recommended to establish the link between polyploidy, genomic instability, phenotypic variations and lifestyle in desert rodents HG. It appears that, HG of Gerbillinae may be a suitable model for biomedical researches.

Authors' Contributions: All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by OSB and NH. The first draft of the manuscript was written by OSB and SL revised the manuscript. All authors read and approved the final manuscript.

Conflict of Interest: The author has no conflict of interest to declare.

Ethics Approval: Agreement of the university ethical committee "Association Algérienne des Sciences en Experimentation Animale" AASEA (Agreement Number 45/DGLPAG/DVA.SDA.14).

Funding: This work was supported by the Algerian Ministry of Higher Education and scientific research. Program (No F002 2008 0002).

REFERENCES

1. Arrigo N, Barker MS. Rarely successful polyploids and their legacy in plant genomes. *Curr Opin Plant Biol.* 2012; 15: 140-146.
2. Lexer C, Fay MF. Adaptation to environmental stress: a rare or frequent driver of speciation? *J Evol Biol.* 2005; 18: 893-900.
3. Edgar BA, Or-Weaver TL. Endoreplication cell cycles: more for less. *Cell.* 2001; 105(3): 297-306.
4. Flemming AJ, Shen ZZ, Cunha A, Emmons SW, Leroi AM. Somatic polyploidization and cellular proliferation drive body size evolution in nematodes. *Proc Natl Acad Sci USA.* 2000; 97: 5285-5290.
5. Lee HO, Davidson JM, Duronio RJ. Endoreplication: polyploidy with purpose. *Genes Dev.* 2009; 23(21): 2461-2477.
6. Gentric G, Mailliet V, Paradis V, Couton D, L'Hermitte A, Panasyuk G, et al. Oxidative stress promotes pathologic polyploidization in nonalcoholic fatty liver disease. *J Clin Invest.* 2015; 125(3): 981-992.
7. Hessen D. Solar radiation and life, In: *Solar Radiation and Human Health.* Bjertness E. (ed.). *Norweg Acad Sci Lett.* 2008; 123-137.
8. Brochmann C, Brysting AK, Alsos IG, Borgen L, Grundt HH, Scheen AC, et al. Polyploidy in arctic plants. *Biol J Linn Soc.* 2004; 82: 521-536.
9. Rothschild LJ. The influence of UV radiation on protistan evolution. *J Euk Micro.* 1999; 46: 548-555.
10. Le Comber SC, Smith C. Polyploidy in fishes: patterns and processes. *Biol J Linn Soc.* 2004; 82: 431-442.
11. Payne AP. The Harderian gland: a tercentennial review. *J Anat.* 1994; 185: 1-49.
12. Djeridane Y. The harderian gland and its excretory duct in the Wistar rat. A histological and ultrastructural study. *J Anat.* 1994; 184(3): 553-566.
13. Saadi-Brenkia O, Haniche N, Bendjelloul M. Light and electron microscopic studies of the *Gerbillus tarabuli* (Thomas, 1902) Harderian gland. *Zoolog Sci.* 2013; 30(1): 53-59.
14. Djeridane Y. The Harderian gland of desert rodents: a histological and ultrastructural study. *J Anat.* 1992; 180: 465-480.
15. Coto-Montes A, Boga JA, Tomas-Zapico C, Rodríguez-Colunga MJ, Martínez-Fraga J, Tolivia-Cadreja J, et al. Physiological oxidative stress model: Syrian hamster Harderian gland-sex differences in antioxidant enzymes. *Free Radic Biol Med.* 2001; 30: 785-792.

16. Tomas-Zapico C, Martinez-Fraga J, Rodriguez-Colunga MJ, Tolivia D, Hardeland R, Coto-Montes A. Melatonin protects against delta-aminolevulinic acid-induced oxidative damage in male Syrian hamster Harderian glands. *Int J Biochem Cell Biol.* 2002; 34: 544-553.
17. Coto-Montes A, García-Macía M, Caballero B, Sierra V, Rodríguez-Colunga MJ, Reiter RJ, Vega-Naredo I. Analysis of constant tissue remodeling in Syrian hamster Harderian gland: intra-tubular and inter-tubular syncytial masses. *J Anat.* 2013; 222(5): 558-569.
18. Chevret P, Dobigny G. Systematics and evolution of the subfamily Gerbillinae (Mammalia, Rodentia, Muridae). *Mol Phylogenet Evol.* 2005; 35: 674-688.
19. Reynolds ES. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J Cell Biol.* 1963; 17: 208-212.
20. Sakai T, Yohro T. A histological study of the Harderian gland of Mongolian gerbils, *Meriones meridianus*. *Anat Rec.* 1981; 200 (3): 259-270.
21. Johnston HS, Mc Gadey J, Thompson GG, Moore MR, Payne AP. The Harderian gland, its secretory and porphyrin content in the Mongolian gerbil (*Meriones unguiculatus*). *J Anat.* 1983; 137(3): 615-630.
22. Shirama K, Furuya T, Takeo Y, Shimizu K, Maekawa K. Influences of some endocrine glands and of hormone replacement on the porphyrins of the Harderian glands of mice. *J Endocrinol.* 1981; 91(2): 305-311.
23. Krause WJ, McMenamin PG. Morphological observations on the harderian gland of the North American opossum (*Didelphis virginiana*). *Anat Embryol.* 1992; 186(2): 145-152.
24. Djeridane Y. Comparative histological and ultrastructural studies of the Harderian gland of rodents. *Microsc Res Tech.* 1996; 34(1): 28-38.
25. Sabry I, Al Azemi M, Al Ghaith L. The Harderian gland of the Cheesman's gerbil (*Gerbillus cheesmani*) of the Kuwaiti desert. *Eur J Morphol.* 2000; 38: 97-108.
26. Costin GE, Hearing VJ. Human skin pigmentation: melanocytes modulate skin color in response to stress. *FASEB J.* 2007; 21(4): 976-994.
27. Frawley LE, Orr-Weaver T L. Polyploidy. *Current Biology* 2015; 25(9): R353-R358.
28. Wendel J F. Genome evolution in polyploids. *Plant Mol. Biol.* 2000; 42: 225-249.
29. Comai L, Tyagi AP, Winter K, Holmes-Davis R, Reynolds SH, Stevens Y, Byers B. Phenotypic instability and rapid gene silencing in newly formed Arabidopsis allotetraploids. *Plant Cell.* 2000; 12: 1551-1567.
30. Woodhouse M, Burkart-Waco D, Comai L. Polyploidy. *Nature Education.* 2009; 2(1): 1.
31. Barkla BJ, Rhodes T, Tran KT, Wijesinghe C, Larkin JC, Dassanayake M. Making epidermal bladder cells bigger: developmental- and salinity-induced endopolyploidy in a model halophyte. *Plant Physiol.* 2018; 177: 615-632.
32. Van de Peer Y, Meyer A. Large-scale gene and ancient genome duplications. In: *The evolution of the genome* (ed. Gregory TR). 2005; 330-363.
33. Miettinen TP, Caldez MJ, Kaldis P, Björklund M. Cell size control – a mechanism for maintaining fitness and function. *Bioessays.* 2017; 39: 1700058.
34. Fenech M. Micronuclei and their association with sperm abnormalities, infertility, pregnancy loss, pre-eclampsia and intra-uterine growth restriction in humans. *Mutagenesis.* 2011; 26: 63-67.
35. Terradas M, Martín M, Genescà A. Impaired nuclear functions in micronuclei results in genome instability and chromothripsis. *Arch Toxicol.* 2016; 90: 2657-2667.
36. Crasta K, Ganem NJ, Dagher R, Lantermann AB, Ivanova EV, Pan Y, Nezi L, et al. DNA breaks and chromosome pulverization from errors in mitosis. *Nature.* 2012; 482: 53-58.

37. Zhang CZ, Spektor A, Cornils H, Francis JM, Jackson EK, Liu S, et al. Chromothripsis from DNA damage in micronuclei. *Nature*. 2015; 522: 179-184.
38. Beedanagari S, Vulimiri SV, Bhatia S, Mahadevan B. Genotoxicity biomarkers. *Biomark Toxicol*. 2014; 729-742.