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Research Article

The Transplacental Passage of Gadolinium and the Ultrastructural Modifications in Placenta Tissues of Rats

Marwa Mhamdi[1,2]*, Nedra Badri[1,2], Ridha Ben Ali[3], Adrian Florea[2], Horea Matei[2], Tekaia Walid-Habib[4], Samira Maghraoui[1] and Leila Tekaya[1]

[1]Laboratory of Physiology, Faculty of Medicine of Tunis, University of Tunis El Manar, Tunis, Tunisia

[2]Department of Cell and Molecular Biology, Faculty of Medicine, "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania

[3]Experimental Medicine Unit, Faculty of Medicine of Tunis, University of Tunis El Manar, Tunis, Tunisia

[4]Department of Stomatology, Faculty of Medicine Dentistry of Monastir, University of Monastir-Monastir, Tunisia

***Corresponding Author**: Marwa Mhamdi, Laboratory of Physiology, Faculty of Medicine of Tunis, University of Tunis El Manar, Tunis, Tunisia.

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Abstract

The placenta is a complex organ and essential for fetal growth and development [1]. It allows survival of the blastocyst and connects physically and biologically the developing embryo to the uterine wall. The aim of our study is to determine the site of gadolinium sequestration in the placenta tissues and the ultrastructural changes induced by the administration of this chemical element with intraperitoneally way to pregnant rats. The investigation of the figures obtained by Transmission Electron Microscopy (TEM) revealed the presence of lysosomes loaded by dense electron deposits at the level of the placental lysosomes (fetus and maternal side), thus modifications which relate to the noting structure as examples; altered mitochondria, dilated endoplasmic reticulum and cytoplasmic vacuolization. On the other hand no deposit was detected in the placenta of control rats. It can be concluded that lysosomes play a crucial role in the concentration of gadolinium in insoluble form and prevent its invasion into the internal environment.

Keywords: *Pregnancy; Placenta; TEM; Sequestration; Gadolinium*

Introduction

Toxicity is a problem in all sectors, especially in biology, which is manifested by the introduction of certain mineral elements into the living organism in an indirect way: by the release of certain toxic elements into the soil, water, or in a direct manner according to different routes of administration such as; oral, parenteral or pulmonary was poorly understood for a long time, our choice was gadolinium. The interest of this particular choice is essentially related to their physico-chemical properties and essentially its paramagnetic effect put it used in medical and industrial applications. Indeed, this element is widely used in medicine as a contrast agent in some imaging [2-6], it is also used in the most modern industries such as the manufacture of cathode ray tubes for televisions, batteries, CD-ROM, computer memories [7]. Frequent use of this element would therefore increase the risk of contamination to humans whether introduced naturally or accidentally. This is why the study of their fate in the placenta would be interest.

Objective of the Study

The objective of this study was to determine the behavior of gadolinium in the placenta tissues of a pregnant rat treated with gadolinium and to determine the cell organelle that is responsible in the sequestration phenomenon as well as some ultrastructural changes induced by the introduction of this metal. To solve these aims we have used Transmission Electron Microscopy.

Materials and Methods

We used in this study 16 pregnant female Wistar rats, weighing around 250g. Each cage contained two females and one male to make the coupling. A vaginal smears test was done to check the pregnancy gestation. These rats were divided into two groups (M1, M2). Beginning with the 16th day of gestation, the female rats underwent daily injections as follows:

- The first group (M1) of 8 rats received 4 intraperitoneal injections of 1 ml containing 4 mg of soluble solution of gadolinium, in order to accumulate a total dose of 64 mg/kg of body weight.
- The second group (M2) of 8 control rats received physiological serum in the same experimental conditions.

Twenty-four hours after the last injection, all rats were anesthetized, the placenta was removed and all rats were killed. The rats used in this study were maintained and treated according to internal institutional rules, based on the principles of ethics in animal experimentation.

Method of sampling

Histological and ultrastructural studies were performed using regular techniques of conventional Transmission Electron Microscopy: placenta samples measuring 1 mm3 were fixed by immersion in a 3% glutaraldehyde solution in sodium cacodylate buffer for 24 hours at 4°C. After rinsing in the same buffer, the fragments were post-fixed in 1% osmium tetroxide for two days at an ambient temperature of 25°C. All fragments were dehydrated in ethanol baths of increasing concentration and in two baths of propylene oxide, then embedded in Resin Epoxy (Epon) and incubated for 48h at 45°C and for 24h at 60°C. Semithin sections of 100 to 150 nm thicknesses were obtained. Tissues areas selected on semithin sections were then cut to obtain ultrathin sections that were collected on 300 mesh copper grids. These cuts were contrasted with uranyl acetate and lead citrate and examined with the transmission electron microscopy.

Results

Maternal side of placenta

The investigations of maternal side of placenta from pregnant rats treated with gadolinium during four days, showed the presence of many electron-dense surcharges within the lysosomes of maternal tissues. Changes in the architectural histology noticed by altered mitochondria (Figure 1). The TEM of control placenta sections showed a normal histological aspect and no loaded lysosome has been observed in maternal side of placenta (Figure 2).

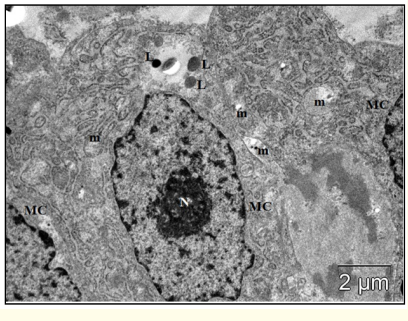

Figure 1: *(Magnification X 20000) Ultrastructural micrograph of Maternal cells of placenta (MC) with their round euchromatic nucleus (N) from gadolinium-treated rats showed the presence of loaded lysosomes (L) and altered mitochondria (m).*

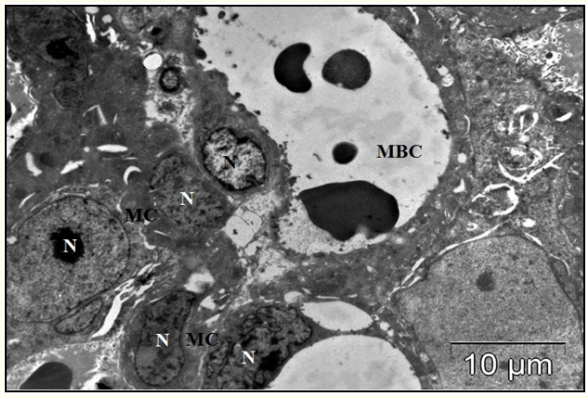

Figure 2: *(Magnification X 5000) Normal ultrastructural of Maternal cells of placenta (MC) with their round euchromatic nucleus (N) of control rats and showed maternal blood capillary (MBC).*

Fetus side of placenta: (Syncytiotrophoblast and Cytotrophoblast)

Syncytiotrophoblast

The ultrastructural study of rats placenta given by intraperitoneal way, a dose of 64 mg of gadolinium /Kg of body weight, showed the presence of electron dense granules in the lysosomes of syncytiotrophoblast tissue. In addition, a cytoplasmic vacuolisations associated to suffering mitochondria were also seen (Figure 3). The investigations of the various territories of placenta from pregnant rats which received saline solution under the same experimental conditions didn't showed any intralysosomal inclusions (Figure 4).

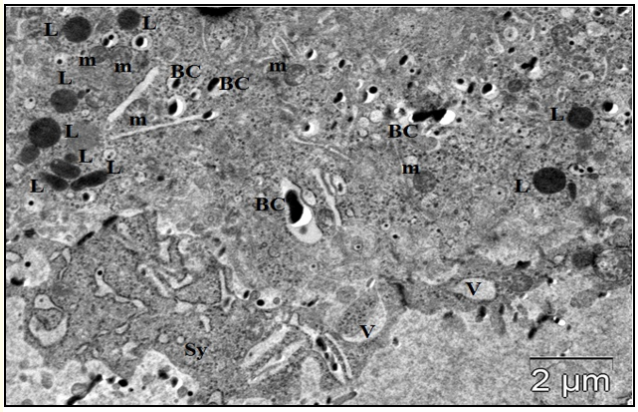

Figure 3: *(Magnification X 20000) Ultrastructural study of Syncytiotrophoblast (Sy) of rats placenta given by intraperitoneal way the gadolinium, showed the presence of electron dense granules in the lysosomes (L) of syncytiotrophoblast tissue, blood capillary (BC), a cytoplasmic vacuolisations (V) associated to suffering mitochondria (m).*

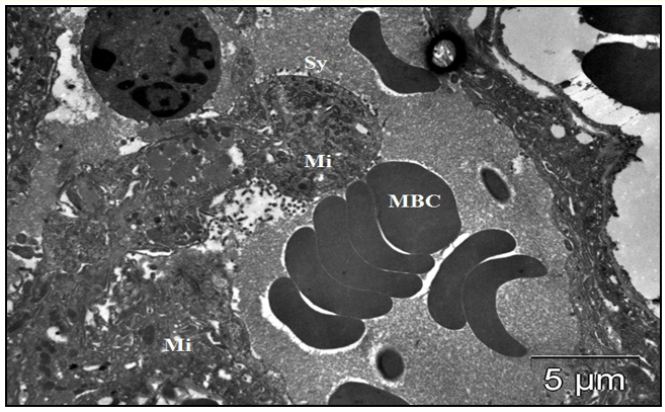

Figure 4: *(Magnification X 5000) The investigations of the various territories of placenta from pregnant rats which received saline solution showed a normal structure of Syncytiotrophoblast (Sy) with their microvilli (Mi) and maternal blood capillary (MBC).*

Cytotrophoblast

The ultrastructural observations of ultrathin sections of placenta tissue of treated rats identified the presence of numerous abnormal lysosomes in the cytoplasm of cytotrophoblast cells charged with an electron-dense material. In addition, there are mitochondrial suffering with no visible crystals well as rough endoplasmic reticulum profiles were also altered in the cytotrophoblast tissue (Figure 5). No loaded lysosomes in the different territories sections of cytotrophoblast control rats and absence of histological destruction were also detected (Figure 6).

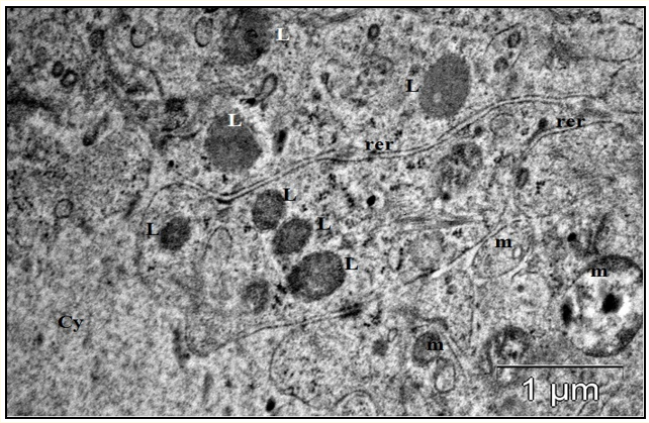

Figure 5: *(Magnification X 20000) The ultrastructural observations of ultrathin sections of cytotrophoblast (Cy) tissue, showed the presence of abnormal lysosomes (L) charged with an electron-dense material, altered mitochondria (m), a rough endoplasmic reticulum (rer) were altered.*

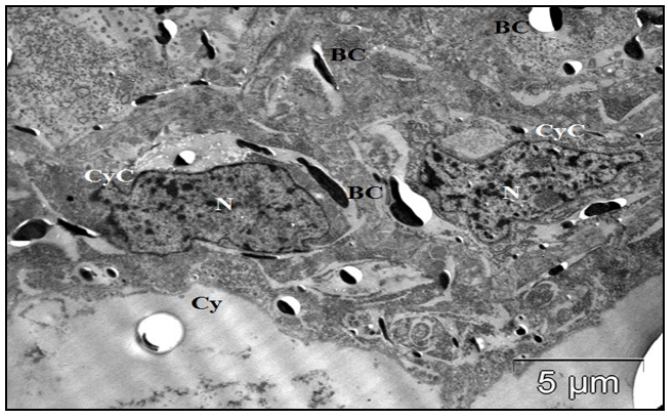

Figure 6: *(Magnification X 6000) The ultrastructural observation of cytotrophoblast (Cy) section from control rats showed Cytotrophoblast with their characteristic cells (CyC), nucleus (N) and blood capillary (BC).*

Discussion

Previous studies

Since the introduction of new observation and analysis techniques such as Transmission Electron Microscopy (TEM), Electron Probe Microanalysis (MASE) and Analytical Ionic Microanalysis (MIA) in the field of biology, a second lysosome function has emerged that of concentrating certain mineral elements. Indeed, for the first time in 1974, Galle demonstrated the role of the lysosome in the concentration of mineral elements following the subcutaneous administration of a soluble solution of uranium or gold, which precipitate within cell lysosomes of renal proximal in insoluble form [8]. Remember that the primary role of this cellular organelle is the degradation of organic matter [9].

Subsequently, this phenomenon of precipitation has been generalized for other elements such as the so-called noble ones, namely the gold that precipitates within the renal proximal cells [8,10,11], bone marrow macrophages in patients with rheumatic fever [12]. Gold was also found in the steroidogenic cells of the adrenal cortex [13,14], testicular Leydig interstitial cells [13,14] and liver cells [15]. Palladium and platinum precipitate within lysosomes of renal proximal cells [11] and nickel has been localized within the lysosomes of tumor cells in culture [11]. All these elements precipitate within the lysosome with sulfur probably in the form of an insoluble sulphate salt, under the action of an intralysosomal enzyme: arylsulfatase.

For other heavy metals, for example, chromium has been found in lysosomes of renal proximal cells [16] group III-A elements such as gallium found in lysosomes of epithelial cell mammary gland as well as lysosomes of tumor cells of the bone marrow [17]. Aluminum, a light element of the same family as gallium, was found in lysosomes of parathyroid cells [18], liver cells [19] and renal cells [20] and indium has been localized in lysosomes of renal proximal cells [20] as well as in testicular cells [21-23].

As for lanthanides, still called rare earths such as cerium, it has been found in the lysosomes of hepatic and renal cells [24,25], in the basement membrane of the renal proximal tubule as well as in alveolar macrophages and in liver cells [26-28]. Hafnium and zirconium have been found in the lymph nodes [11,29]. Lanthanum and samarium were localized in lysosomes of hepatic cells, spinal macrophages and splenics, as well as lysosomes of hepatic and renal cells [11,30,31]. As for gadolinium, it precipitates within the lysosomes of liver cells, those of the macrophages of the spleen, as well as within the lysosomes of the pulmonary alveoli, the lysosomes of the mammary glandular cells and those of the basement membrane cells of the renal proximal tubule [3,28].

Apart from the so-called noble elements all these aforementioned elements seem to use another intralysosomal enzyme which is acid phosphatase, precipitating with phosphorus very probably forming a phosphate salt insoluble in the lysosome.

Contribution of this work and discussion

The ultrastructural study of placental tissue showed the presence of dense electrolyte granulations within lysosomes of both the maternal and the fetal face cells of the placenta. Structural changes affecting both mitochondria and rough endoplasmic reticulum, cytoplasmic vacuolization have been observed. Note the total absence of inclusions in the lysosomes of the different territory of placenta from the control rats.

In addition, to the presence of gadolinium in the lysosomes of placenta of treated rats during a week, our ultrastructural results provide additional information on the consequences of gadolinium administration on the cellular structure of placental tissue since gadolinium appears to cause intracellular lesions manifested primarily by important mitochondrial alterations.

Our results are reminiscent of those previously performed by our team showing that gadolinium precipitates in lysosomes of liver cells [26-28,30,32], lysosomes of alveolar macrophages [26,27,30], bone marrow [11,26,27,30] and mammary glandular cells [28].

Our studies confirm other studies using either lanthanum found in spinal macrophages, splenics as well as lysosomes in liver cells [11,26,31] and renal proximal cells again the cerium found within the zona pellucida [33].

Our microscopic observations are in agreement with other studies showing that so-called noble metals such as palladium, which has been found in lysosomes of renal proximal cells [11] or platinum which precipitates within the lysosomes of tumor fibroblastic cells in culture [34] renal proximal and nickel observed in lysosomes of cultured tumor cells [11,35].

Our results confirm other studies showing that the lead gold of so-called noble metals has also been concentrate in the lysosomes of certain endocrine cells such as the adrenal glands [13,14], the thyroid gland [13] or the testes [11,13,14]. The liver was the seat of accumulation of this element [11].

The direct demonstration with TEM that these deposits are formed by phosphorus and gadolinium is impossible as long as this technique has the objective of a just an ultrastructural study, but the presence of gadolinium is in the cells is indicated by observation of deposits in the lysosomes of the treated rats corroborated with and their absence in the lysosomes of the control rats and following the comparison with previous work. We could therefore assume that the deposits observed in a large variety of cells are most likely gadolinium associated with phosphorus [36,37].

Conclusion

Our work demonstrated that gadolinium was probably selectively concentrated in lysosomes on the fetal side of the placenta, as well as in lysosomes on the maternal side of the placenta, in addition, a visible modification in tissue and cells involving cytoplasmic vacuolization, expansion of the rough endoplasmic reticulum and mitochondrial damage. Our experimental results also reported cases of death, malformations, weight loss and abortions, which led to a transplacental passage of the administered element, thus to a placental barrier failure.

Bibliography

1. B Deloison., *et al*. "Placental MRI: Physiology and pathology". *Gynécologie Obstétrique and Fertilité* (2013).

2. Tong CY., *et al*. "Measurement of the extraction efficiency and distribution volume for Gd-DTPA in normal and diseased canine myocardium". *Magnetic Resonance in Medicine* 30.3 (1993): 337-346.

3. Pereira RS., *et al*. "The determination of myocardial viability using Gd-DTPA in canine model of acute myocardial ischemia and reperfusion". *Journal of Magnetic Resonance* 36.5 (1996): 684-693.

4. Bellamy DD., *et al*. "Gd-DTPA bolus tracking in the myocardium using T1 Fast acquisition relaxation maping (T1 FARM)". *Magnetic Resonance in Medicine* 46.3 (2001): 555-564.

5. Thornhill RE., *et al*. "Examining a canine model of stunned myocardium with Gd-DTPA-enhanced MRI". *Magnetic Resonance in Medicine* 45.5 (2001): 864-871.

6. Meade TJ., *et al*. "New magnetic resonance contrast agents as biochemical reporters". *Current Opinion in Neurobiology* 13.5 (2003): 597-602.

7. Pereira RS., *et al*. "The use of Gd-DTPA as a marker of myocardial viability in reperfused acute myocardial infraction". *The International Journal of Cardiovascular Imaging* 17.5 (2001): 395-404.

8. Galle P. "Rôle des lysosomes et des mitochondries dans les phénomènes de concentration et d'élimination d'éléments minéraux (uranium et or) par le rein". *Journal of Microscopy* 19 (1974):17-33.

9. De Duve C and Wattiaux R. "Functions of lysosomes". *Annual Review of Physiology* 28 (1966): 435-492.

10. Rodrigues LE and Galle P. "Lysosomotropisme et action antiinflammatoire des sels d'or". *Revue du Rhumatisme* 52.79 (1985): 470-482.

11. Berry JP. "The role of lysosomes in the selective concentration of mineral elements. A microanalytical study". *Cellular and Molecular Biology* 42.3 (1996): 395-411.

12. Galle P and Villiaumey J. "Concentration de l'or par les macrophages de la moelle osseuse au cours d'affections rhumatismales soumises à la chrysothérapie". *Revue du Rhumatisme* 49.4 (1982): 273-279.

13. L Manoubi., *et al*. "Subcellular localization of gold in suprarenal testicle and thyroid glands after injection of allochrysine in the rats". *Cellular and Molecular Biology* 40.4 (1994): 483-487.

14. Tekaya-El Manoubi L., *et al*. "Rôle des glandes endocrines dans la concentration sélective des sels d'or. Etude microanalytique". *La revue maghrébine d'endocrinologie- diabète et Reproduction* 9.1-2 (2004): 33-37.

15. Berry JP., *et al*. "Phagocytosis by cells of the pulmonary alveoli. Transformation of crystalline particles". *American Journal of Pathology* 93.1 (1978): 27-44.

16. Berry JP., *et al*. "Chromium concentrationby proximal renal tubule cells: An ultrastructural, microanalytical and cytochemical study". *Journal of Histochemistry and Cytochemistry* 26.8 (1978): 651-657.

17. Berry JP., *et al*. "Role of lysosomes in gallium concentration by mammalian tissues". *Biology of the Cell* 51.1 (1984a): 43-52.

18. Galle P., *et al*. "Concentration abnormal d'aluminium dans les lysosomes d'un adénome parathyroïdien primitive". *Annales de Pathologie* 7.1 (1987a): 65-68.

19. Galle P., *et al*. "Ultrastructural localization of aluminum in hepatocytes of hemodialyzed patients". *Annales de Pathologie* 7.3 (1987b): 163-170.

20. Galle P., *et al*. "Subcellular localization of aluminum and indium in the rat kidney". *Applied Surface Science* 231 (2004): 475-478.

21. Maghraoui S., *et al*. "Histological and ultrastructural study of the intracellular behavior of indium in the testicular tissues". *Microscopy Research and Technique* 74.6 (2011): 546-550.

22. Maghraoui S., *et al*. "Microscopy and microanalysis study of indium in behavior in the intestinal mucosa the liver, the kidney and the testicle". *Journal of Electron Microscopy Technique* 60.2 (2011): 183-190.

23. S Maghraoui., *et al*. "Role of parietal and principal gastric mucosa cells in the phenomen of concentration of aluminium and indium". *Microscopy Research and Technique* 75.2 (2012): 182-188.

24. Cézard C., *et al*. "Cérium et lanthanides-éditions techniques-Encycl. Méd. Chir. (Paris, France)". *Toxicologie-Pathologie professionnelle* 16002 C20. (1992): 5p.

25. L Manoubi., *et al*. "Subcellular localization of cerium in intestinal mucosa, liver, kidney, suprarenal and testicle glands, after cerium administration in the rat". *Journal of Trace and Microprobe Techniques* 16.2 (1998): 209-219.

26. Hocine N., *et al*. "Intracellular localization of gadolinium, lanthanum and terbium. Three rare earths. A microanalytical study using ion microanalysis and electron microprobe. Metal ion in biology and medicine". Edition Ph. Collery L.A. Poirier, N.A. Littlefield., J. C. Etienne. John Libbey. Eurotext. Paris (1994a): 193-198.

27. Hocine N., *et al*. "Mapping and localization of gadolinium. A study using ion microanalysis and electron microprobe". *Les editions de physique* (1994b): 841-842.

28. Ayadi A., *et al*. "Rôle des lysosomes des cellules duodénales et hépatiques dans le phénomène d'accumulation du gadolinium". *Comptes Rendus Biologies* 331.5 (2008): 357-362.

29. Berry JP and Galle P. "Preferential localization of hafnium in nodular lymphatic cells. Study by electron microprobe". *Journal of Submicroscopic Cytology and Pathology* 24.1 (1992): 15-18.

30. Hocine N., *et al*. "Subcellular localization of gadolinium injected as soluble salt in rats: A microanalytical study". *Cellular and Molecular Biology* 41.2 (1995): 271-278.

31. L Manoubi-Tekaya., *et al*. "Rôles des lysosomes dans le comportement intracellulaire d'éléments minéraux: cas du Lanthane". *La Tunisie médicale* 78.3 (2000): 195-200.

32. Ayadi A., *et al*. "Lysosomes of the liver and the duodenal enterocytes, a site of handling of two rare earths. Ultrastructural and microanalytical study". *Biological Trace Element Research* 124.1 (2008b): 40-51.

33. C Blandine., *et al*. "Ultrastructural interactions and genotoxicity assay of cerium dioxide nanoparticles on mouse oocytes". *International Journal of Molecular Sciences* 14.11 (2013): 21613-21628.

34. Berry JP., *et al*. "Localization of gallium in tumor cells. Electron microscopy, electron probe microanalysis and analytical ion microscopy". *International Journal of Nuclear Medicine and Biology* 10.4 (1983): 199-204.

35. Berry JP and Lespinats G. "Cis DDP in combination with selenium and sulfur. Subcellular effect in kidney cells. Electron microprobe study". *Journal of Submicroscopic Cytology and Pathology* 20.1 (1988):59-65.

36. Berry JP., *et al*. "Distribution du gallium dans différents tissus de mammifères - Etude en microanalyse par émission ionique secondaire et par spectrométrie de rayons X". *Journal de Physique, Colloque* 45 (1984b): 537-540.

37. Samira M., *et al*. "Comparison of the intracellular behavior of gold (Au) and indium (In) in testicle after their parental administration". *Microscopy* 62.3 (2013): 397-403.

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